Journal of Allergy

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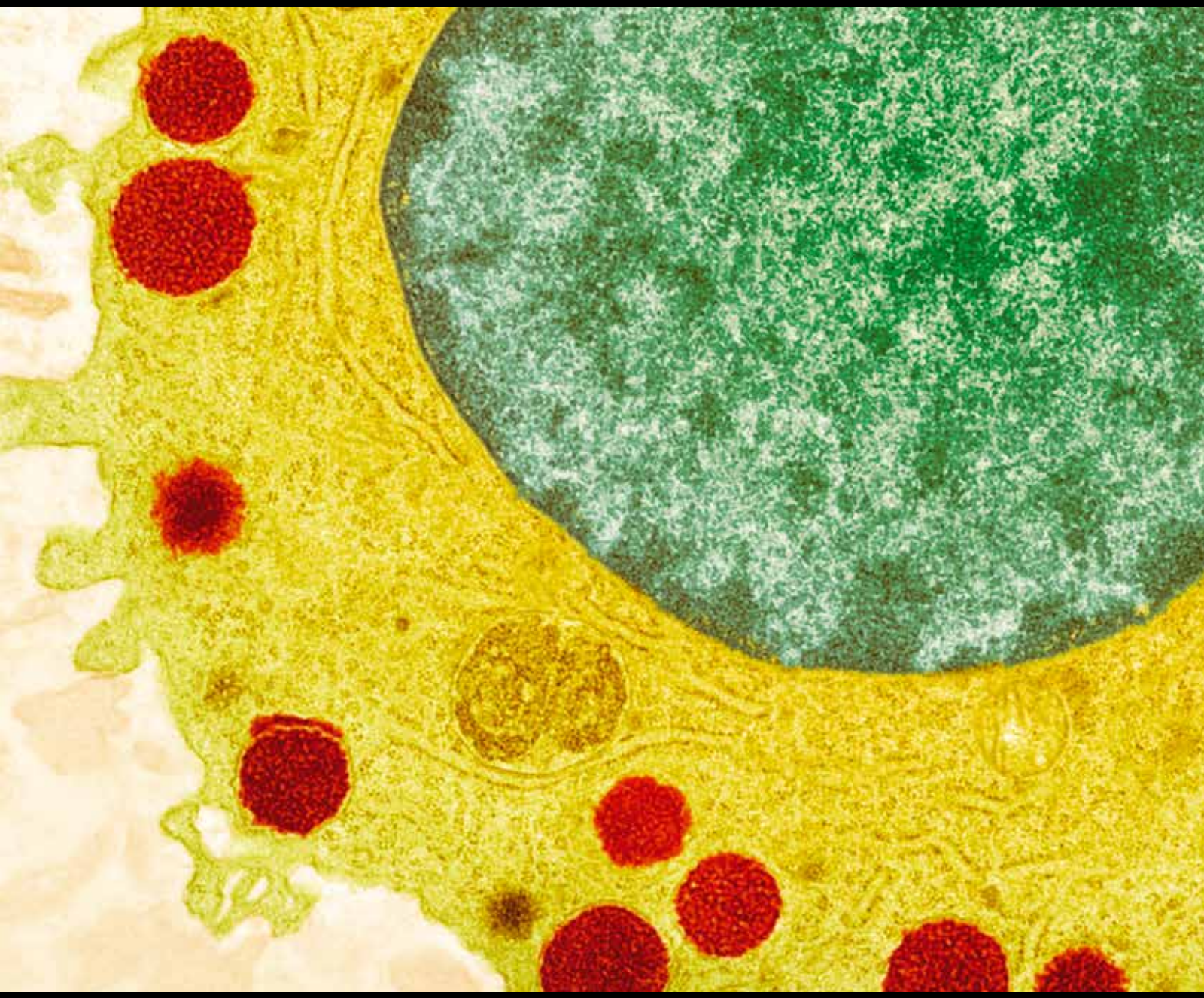
Guest Editors: Ralph Mösges, Carlos E. Baena-Cagnani, and Desiderio Passali



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Editorial Board

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Editorial

Nonpharmacological Treatment of Rhinoconjunctivitis and Rhinosinusitis

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Infectious and allergic diseases of the upper airways are among the most common illnesses of all age groups. Numerous guidelines have been issued for the evidence-based treatment of these diseases. Therapy involves local or systemic application of well-characterized pharmacologically active medications such as glucocorticosteroids, antihistamines, leukotriene-receptor antagonists, alpha-adrenergic receptor agonists, mast-cell stabilizers, and a monoclonal antibody targeting specific immunoglobulin E. In spite of the fact that the various therapeutic options have proven their efficacy and effectiveness in a myriad of well-designed clinical trials, many patients express their discontent with current therapies. In several surveys a majority of patients stated that they would prefer nonpharmacological "nonchemical" treatment over what is currently prescribed or recommended by their physicians. This desire stands in clear contrast to a systematic review conducted some years ago proving that there was practically no evidence at that time for the efficacy of alternative forms of treatment of allergic diseases of the upper airways.

Ever since, several meta-analyses, systematic reviews, and well-designed clinical trials have been published, bearing witness to the scientific basis of some nonpharmacologic options like nasal irrigation in the treatment of different pathologies of the upper airways, namely, for rhinosinusitis and for allergic rhinoconjunctivitis.

In this special issue, we want to highlight some new approaches that until lately have found less public attention in this domain.

In their article on the clinical efficacy of a spray containing hyaluronic acid and dexpanthenol after surgery in the nasal cavity, I. Gouteva et al. demonstrate beneficial effects on wound healing for two substances that have a long history of local application in the nose but formerly were used separately.

The so-called "extremolyte" ectoine is a substance, which was introduced a few years ago as a "natural" treatment of allergic and inflammatory pathologies of the skin and the mucosal tissues. Five articles in this issue illuminate the mechanism of action and the potential benefits of a nasal spray containing ectoine in diseases of various aetiologies like rhinitis sicca or allergic rhinitis.

A form of local treatment of the nasal mucosa that is proximate to ectoine is the nasal spray containing liposomes. Its use in allergic rhinitis has been well established. A. Eitenmüller and coauthors present in their article for the first time data on ectoine in chronic rhinosinusitis.

For patients who are sceptical about using any sort of active ingredients in their nasal spray, the application of mere water in the form of thermal water inhalations could be an alternative. S. Keller et al. have conducted a meta-analysis which demonstrates some benefits of this least-invasive local therapy.

There has been much controversy over the last decade on the meaningfulness of probiotics in preventing allergies. New forms now come as treatment of chronic diseases of the upper airways. M. F. Kramer and his coauthor M. D. Heath present

the latest state of knowledge of this nutritional therapeutic approach.

Another much debated therapy in the field of allergic diseases is acupuncture. Most investigations have previously focussed on pollen-allergic patients. It is the merit of Hauswald and coauthors to have conducted a randomized controlled clinical trial using acupuncture as an alternative to treatment with the state-of-the-art antihistamine loratadine in house dust allergic patients.

It has been the intention of the editors of this special issue to round up our knowledge on alternative therapies for upper airways diseases beyond the fields of herbal medicine and homeopathy. The number of articles that have been submitted but more so their quality was a positive surprise.

We are very grateful to the publishers who have had the courage of disseminating unusual ideas. Ghada Ali and Marie-Josefine Joisten were the driving spirits behind the scenes and without their continuous commitment this special issue would not have seen the light of the day. We are deeply obliged and thankful for their endeavours.

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Clinical Study

Clinical Efficacy of a Spray Containing Hyaluronic Acid and Dexpanthenol after Surgery in the Nasal Cavity (Septoplasty, Simple Ethmoid Sinus Surgery, and Turbinate Surgery)

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Background. This prospective, controlled, parallel-group observational study investigated the efficacy of a spray containing hyaluronic acid and dexpanthenol to optimise regular treatment after nasal cavity surgery in 49 patients with chronic rhinosinusitis. *Methods*. The control group received standard therapy. Mucosal regeneration was determined using rhinoscopy sum score (RSS). Pre- and postoperative nasal patency was tested using anterior rhinomanometry. The participants were questioned about their symptoms. *Results*. Regarding all RSS parameters (dryness, dried nasal mucus, fibrin deposition, and obstruction), mucosal regeneration achieved good final results in both groups, tending to a better improvement through the spray application, without statistically significant differences during the whole assessment period, the mean values being 7.04, 5.00, 3.66, and 3.00 (intervention group) and 7.09, 5.14, 4.36, and 3.33 (control group). No statistically significant benefit was identified for nasal breathing, foreign body sensation, and average rhinomanometric volume flow, which improved by 12.31% (control group) and 11.24% (nasal spray group). *Conclusion*. The investigational product may have additional benefit on postoperative mucosal regeneration compared to standard cleaning procedures alone. However, no statistically significant advantage could be observed in this observational study. Double-blind, controlled studies with larger populations will be necessary to evaluate the efficacy of this treatment modality.

1. Introduction

Ventilation, mucociliary transport, and the epithelial barrier are considerably impaired initially following surgical procedures of the nasal cavity.

Injury of the nasal mucosa involved not only with the procedure but also prior to surgery caused by various pathologies usually results in the reduction of the protective secretion film and damage to the highly sensitive cilia [1]. Rapid wound healing from rhinosurgical procedures therefore reduces the risk of new infections considerably.

Although minimally invasive endoscopic technology and instruments enable functional endoscopic paranasal surgery that is gentle to the mucosa, the final results remain dependent upon proper wound healing of the nasal or paranasal mucosa without extreme scarring. Large-scale crusting, mucosal changes, ventilation disorders due to excessive secretion and edema, secondary hemorrhaging, or the development of synechia with possible reobstruction are critical factors that can lead to postoperative complications.

Besides frequent check-ups and wound debridement on the part of the treating physician, meticulous postoperative care of the mucosa using nasal irrigation, inhalation, sprays, and ointments on the part of the patient complements local treatment approaches and the measures taken to prevent adhesion up to the full and proper healing of the wound. In this connection, no established gold standard exists.

At present, investigations are available on a broad spectrum of topical nasal preparations for the postoperative care of the mucosa. To date, however, studies on the combination of hyaluronic acid (HA) and dexpanthenol as the main components of a nasal spray have not yet been carried out.

Hyaluronic acid belongs to the group of glycosaminoglycans and is an omnipresent macromolecule in the interstitium of vertebrates. It is involved in the modulation of various physiological processes (including morphogenesis, regeneration, wound healing, and tumor invasion [2]) and also controls signaling pathways, ergo cell behavior and interactions [2–4].

If tissue continuity is disrupted because of injury, a relatively uniform inflammatory response is induced in the body to break down necrotic tissue, eliminate pathogenic microorganisms, and restore initial integrity through tissue proliferation and repair [5, 6]. As a fundamental component of the extracellular space, hyaluronic acid functions as a framework for wound healing. In addition, it performs other various functions during the regeneration process. Its involvement and specific interaction in subprocesses are complex and to some extent still unknown for the individual steps to be attributed to a specific property.

As a response to tissue injury in the skin, the unusually high hyaluronic acid level influences tissue hydration during the subsequent inflammatory process. This is relevant with regard to cell proliferation and migration, as the pronounced hygroscopy of the polymer increases the moisture content of the tissue locally, which weakens cell adhesion mechanisms in the extracellular matrix and permits temporary separation for the purpose of cell migration and proliferation [7].

Scarless regeneration in human fetal wounds is attributed to unusual hyaluronic acid abundance in the matrix during embryonic development [8, 9].

Diverse biological effects of hyaluronic acid are related to its molecular size. High molecular sized polymers have antiangiogenic and immunosuppressive functions, thereby reflecting intact tissue, while smaller units are distress signals and potent inducers of inflammation, angiogenesis, and mobilization of immune cells [10–12]. Hyaluronic acid and its degradation products originating from the wound healing process are able to regulate tissue or cellular reactions, most notably the promotion of fibroblast proliferation and angiogenesis [2, 13].

The unique viscoelasticity and mucoadhesive capability of hyaluronic acid [14, 15] together with its high immunological and toxicological product safety have led to its versatile use in a number of application forms for various dermatological [16–22], pharmaceutical [3, 14, 23–32], and tissue engineering [33, 34] purposes, or during surgical procedures as well as for postoperative treatment [35–56].

In support of the therapeutic potential of sodium hyaluronate, hysan *Pflegespray* also contains dexpanthenol, which is a long-established active substance having excellent skin tolerance and penetration capacity [57] and a particularly positive impact on the mucociliary clearance of the respiratory epithelium [58, 59].

In the skin, dexpanthenol (provitamin B5) metabolizes to pantothenic acid (vitamin B5), which is essential for the normal function of the epithelial cells, especially during the energy-intensive early phase of epithelial regeneration (within the first 4 days) [60].

Particularly as a topical dermatological preparation for treating wound healing disorders, dermatoses, scars, extensive burn wounds, or skin transplantations [57, 60–62] as well as for treating wounds following nose surgery, the long-established anti-inflammatory and epithelium-protective effect [63] of dexpanthenol has been used for decades in clinical routine [63–66]. Various studies have scientifically confirmed the effectiveness of its preservative-free nasal ointment (predominantly) or spray application forms in treating rhinitis sicca anterior or after nasal and paranasal surgery [63–69]. It also improves the tolerability of rhinological preparations containing preservatives [1, 66, 69]. The local application of dexpanthenol in acute and chronic rhinitis is a part of routine standard therapy [66].

Corresponding to clinical experience, external therapy with dexpanthenol preparations is normally considered very well tolerated, having a minimum risk of skin irritations or sensitization [70].

Even though hyaluronic acid and dexpanthenol have long been clinically proven to be antiadhesive and mucosal conditioning substances separately, no study has yet investigated the possibility of a more intensive, wound-healing promotive effect based on the synergy of their set combination in a nasal spray. This was the reason that this dual-center, clinical trial examined a CE-labelled medical device (nasal spray) which was used for its intended purpose of regenerating damaged nasal mucosa; the study was carried out in strict accordance with the definition of nonintervention [71].

2. Patients and Methods

2.1. Patients. Included in the study were patients who suffered from chronic rhinosinusitis and who had undergone the following surgical procedures of the nasal cavity: septoplasty, simple ethmoid surgery, turbinate surgery, pansinus surgery, and maxillary sinus surgery.

The total population consisted of 49 patients. Of these, 27 patients were assigned to the intervention group. The other 22 patients comprised the control group which received customary conditioning preparations that were not documented.

2.2. Design. This trial was carried out as a prospective, open-label, observational study in two doctor's offices from 11 September 2008 to 13 September 2011. Investigators collected test results and the patients' subjective assessments at a minimum of five check-up visits, the initial examination, three intermediate examinations, and one final examination, and documented the data in the observation form.

At the initial examination, the patient was thoroughly informed about the planned noninterventional study, indications for surgery, and preoperative rhinomanometry. Patients were not randomised to receive the study medication. The choice of the appropriate postsurgical care was based on the investigator's judgement of the patients' clinical condition after surgery and the patients' willingness to apply the spray regularly instead of using the alternative nasal pipetting or ointments.

All participating patients signed a data privacy declaration form, giving their consent to allow their data in

pseudonymous form to be recorded and forwarded to the sponsor or competent authorities.

This observational study examines how wound healing is influenced after the first check-up and after removal of packing material, if inserted. This was not documented in the observation form and was not considered in the results.

Furthermore, the adjuvant postoperative administration of antibiotics, antiphlogistics, or analgesics as concomitant medication, if necessary, was recorded.

A repeated anterior rhinomanometry (at visit 1 in the 1st postoperative week) and anterior rhinoscopy (optional endoscopy) (at all other visits in accordance with the observation schedule) were conducted for documentation purposes, for monitoring the final results of surgical treatment with respect to nasal patency, and for the visual assessment of nasal mucosa conditions. In addition, the patients were questioned about their subjective perceptions with respect to nasal breathing and foreign body sensation, tolerability of the nasal spray, and any noticeable problems or complaints in connection with the preparation used.

This paper was compiled in accordance with the STROBE (strengthening the reporting of observational studies in epidemiology) statement.

2.3. Ethical Aspects and Professional Regulations. The investigational preparation and the control medication were both CE-certified. According to the Medical Devices Act, this investigation was therefore exempted from requiring approval from the competent federal authority and the competent ethics committee. Investigators in charge of the study received consultation with respect to professional regulations before the study commenced.

2.4. Study Medication. The object of the investigation was the nonprescription "hysan Pflegespray" manufactured by Ursapharm Arzneimittel GmbH, Saarbrücken, Germany. At the beginning of the study (2008), the product was called "Hylocare-Nasenspray." It was renamed "hysan Pflegespray" in 2011. It is a liquid pharmaceutical preparation with a dosing spray applicator for the prophylactic or curative topical treatment of inflammatory conditions. It can be applied as monotherapy as well as a concomitant adjuvant to therapy with decongestant nasal sprays (or drops) for rhinosinusitis.

Hysan Pflegespray is a sterile, preservative-free solution containing 0.25 mg/mL sodium hyaluronate, 2% dexpanthenol, as well as sodium dihydrogen phosphate $\times$ $2H_2O$, sodium monohydrogen phosphate $\times$ $2H_2O$, sorbitol, and water. One bottle contains 10 mL of solution, which corresponds to approximately 70 sprays [72].

2.5. Dosage of Study Medication. One to two puffs of nasal spray to each nostril were to be administered three times, distributed evenly throughout the day. If additional therapy with other nasal sprays was applied, nasal spray was always to be used last, allowing at least 30 minutes to elapse between nasal sprays.

2.6. Conventional Care Preparations. Treating otolaryngologist Nr. 1 administered a proprietary composed solution for pipetting by the patients 3-4 throughout the day in both nasal cavities, with the following ingredients: glucose-monohydrate: 5.0 g, menthol: 0,025 g, Olynth 0.1% nasal drops (active agent: xylometazoline hydrochloride): 5.0 g, eucerinum anhydricum: 7.0 g, and peanut oil: ad. 50.0 g.

Treating otolaryngologist Nr. 2 prescribed a proprietary composed ointment as a standard local postoperative care formulation which was to be applied twice a day. The ingredients of the mixture were hydrocortisone: 0.01 g, vitamin A (retinoic acid): 0.4 g, Bepanthen ointment (active agent 5% Dexpanthenol): 16.0 g, and Otrivin nasal drops 0.1% (active agent: xylometazoline hydrochloride): 1 g.

Both preparations were individually compounded by a pharmacist.

2.7. Recording of Efficacy. The primary variable was changed in the sum score (RSS: rhinoscopy sum score), which was attained from the clinical, objectively recorded endoscopy findings: nasal dryness, dried nasal mucus, fibrin deposition, and nasal obstruction. All variables pertaining to rhinoscopic mucosal findings were evaluated using a 4-point scale as follows: absent = 0, mild = 1, moderate = 2, and severe = 3.

For the secondary variable, the patient's subjective perception of unobstructed nasal breathing and foreign body sensation was rated on a scale from 1 to 3 (1 = good, 2 = average, and 3 = poor).

In order to categorize the initial symptom situation and objectivization of patients' statements on symptoms of nasal obstruction and to record the effectiveness of surgery and the monitoring of the final results of surgery, pre- and postoperative active anterior rhinomanometry were carried out at the initial examination.

The treating physician recorded general efficacy and tolerability in free-text format at the end of the observation period.

2.8. Recording of Safety. In spite of the broad clinical experience with both active ingredients contained in nasal spray, special importance was attached to the documentation of adverse events when collecting data in the present study.

The holder of the marketing authorization for the medical device investigated here was obliged to report the severity or intensity of adverse events to the Bundesinstitut für Arzneimittel und Medizinprodukte (The Federal Institute for Drugs and Medical Devices, Pharmacovigilance Division). The treating physician was also able to document his/her own or the others comments or noticeable signs relating to the patient's general condition, product use, or general remarks about the course of treatment in free-text format on the observation forms under "Other notes."

3. Analysis

3.1. Handling of Documentation Errors and Analysis Problems. Missing entries resulted in incomplete data sets, so individual parameters could not be evaluated. These missing data were

Table 1: Rhinoscopy sum score (RSS), postoperative. Confidence interval 95%.

		RSS week 1	RSS week 2	RSS week 4	RSS week 6
Control group	*N* (valid)	22	21	19	18
	Mean	**7.09**	**5.14**	**4.36**	**3.33**
	Standard deviation	1.77037	1.98206	1.77045	1.97037
Nasal spray group	*N* (valid)	24	24	21	19
	Mean	**7.04**	**5.00**	**3.66**	**3.00**
	Standard deviation	2.23566	1.41421	1.49443	1.63299

generally treated as "missing values" and not taken into consideration in the analysis.

3.2. Statistics. The detailed analysis of the parameters was carried out using SPSS 19 statistics software manufactured by SPSS Inc. Frequencies, mean values, standard deviations, medians, and minimum and maximum values within the treatment groups were given for the various variable forms.

To this purpose, patient data were first entered into separate SPSS databases by two people independent of each other. The monitoring person in charge recognized all occurring discrepancies and illogical values. The merged database underwent a plausibility test. Data were then synchronized and subsequently analyzed in SPSS. A significance level of $\alpha = 0.05$ was defined for all statistical tests.

3.3. Data Analysis. Descriptive statistical methods were applied. The statistical values (number, mean value, minimum, maximum, and standard deviation) for continuous variables such as height, age, and time periods were listed in a table. Discrete variables were categorized in the form of frequency distributions with their percentage-wise relationships to the total sample. Free-text answers were transferred post hoc in the appropriate coding schemes and analyzed as frequency distributions. Clinical parameters of disease progression were evaluated and illustrated in the form of intraindividual differential analyses (first versus last examination). Categorically recorded clinical data were analyzed in the form of contingency analyses (before/after). Subgroup analyses were not defined a priori. Any results yielded using comparative statistical methods were of purely explorative character.

4. Results

4.1. Patients: Demographic Data. Overall, 49 patients participated in the study, 8 of whom were female and 41 male. Patients were aged 15 to 58 years (mean age of the total population was 33.12 years, SD: ±11.04 years).

4.2. Rhino-/Endoscopic Mucosal Findings. A scale from 0 to 3 (0 = none, 1 = mild, 2 = moderate, and 3 = severe) was used to assess all parameters (nasal dryness, dried nasal mucus, fibrin deposition, and development of obstructions). The treating physician thereby documented, added, and averaged the mucosal findings obtained via rhinoscopy/endoscopy during the weeklong application of the nasal spray. The resulting rhinoscopy sum score (RSS) is shown in Table 1 and illustrated in Figure 1. Details for each individual parameter can be found in Table 2.

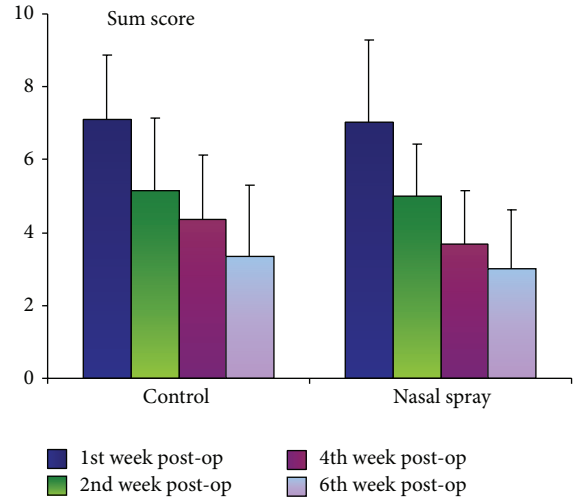

Figure 1: Rhinoscopy sum score (RSS) of the individual groups.

4.3. Patient Evaluation of Nasal Breathing and Foreign Body Sensation. A scale from 1 to 3 (1 = good, 2 = moderate, and 3 = poor) was used for the patients' self-assessment of nasal breathing. The patients rated their subjective perception of a foreign body during the entire postoperative follow-up interval on a scale from 0 to 2 (0 = absent, 1 = moderate, and 2 = severe).

4.4. Pre- and Postoperative Rhinomanometry (8th–10th Postoperative Day). The comparison between pre- and postoperative rhinomanometry shows a similar percentage increase for the mean volume flow in both comparison groups: hysan group 11.24% and control 12.31% (Table 4, Figure 2). Here, the mean preoperative volume flow of 688.13 mL/s (±209.524 mL/s) in the hysan group was slightly above the initial value for the control group at 643.16 mL/s (±188.253 mL/s).

5. Discussion

In spite of gender inhomogeneity, both patient populations were comparable.

Table 2: Individual rhinoscopy findings during the examination period (1st–6th postoperative week).

Post-op week		"Dryness"				"Dried nasal mucus"				"Fibrin deposition"				"Obstruction"			
		1.	2.	4.	6.	1.	2.	4.	6.	1.	2.	4.	6.	1.	2.	4.	6.
Control group	*N* (valid)	22	21	19	18	22	21	19	18	22	21	19	18	22	21	19	18
	MV	**1.64**	**1.24**	**1.21**	**0.83**	**1.95**	**1.33**	**1.16**	**0.89**	**1.86**	**1.33**	**1.11**	**0.89**	**1.64**	**1.24**	**0.89**	**0.72**
	SD	0.581	0.539	0.535	0.514	0.653	0.658	0.501	0.583	0.468	0.483	0.567	0.583	0.581	0.539	0.459	0.575
Nasal spray group	*N* (valid)	24	24	21	19	24	24	21	19	24	24	21	19	24	24	21	19
	MV	**1.67**	**1.25**	**1.00**	**0.84**	**1.92**	**1.29**	**0.95**	**0.74**	**1.83**	**1.42**	**0.90**	**0.74**	**1.63**	**1.04**	**0.81**	**0.68**
	SD	0.702	0.442	0.316	0.375	0.717	0.464	0.384	0.452	0.702	0.504	0.436	0.452	0.576	0.359	0.602	0.478

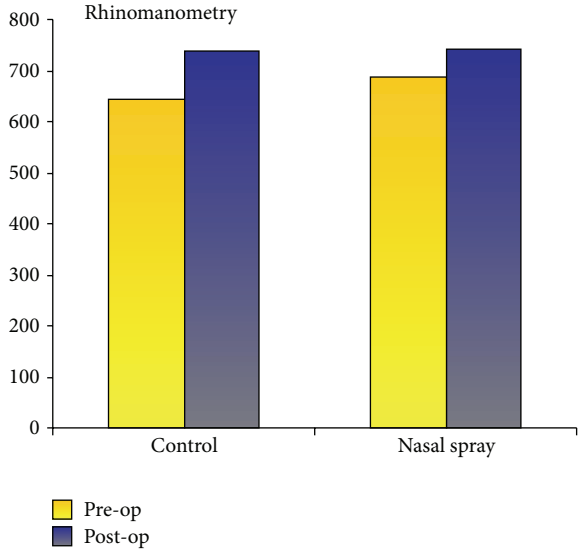

Figure 2: Values for pre- and postoperative rhinomanometry in [mL/s] at 150 Pa, sum left and right nasal cavity. Bar graph.

It was up to the treating physician to decide whether the patient required concomitant medication in the postoperative healing period. No valid conclusion could be made with regard to the possible influence of antibiotics, antiphlogistics, or analgesics on the effect of hysan nasal spray.

In both patient populations, the condition of the endonasal mucosa improved continuously during the follow-up period with respect to the defined objective parameters (nasal dryness, dried nasal mucus, fibrin deposition, and obstruction). The mean RSS values, however, exhibited no significant differences between both populations (see Table 1). Nevertheless, the hysan group showed lower values in the 4th and 6th week (3.66 pts. and 3.00 pts., resp.) compared to the control group (4.36 pts. and 3.33 pts., resp.). A clinical comparison of an isotonic saline spray containing dexpanthenol with a simple saline spray for postoperative treatment over 6 weeks showed comparable efficacy regarding all objective parameters of the endoscopic mucosal analysis and the majority of subjective symptoms as well [59]. Another similarly designed study compared an ointment containing hyaluronic acid (Rhinogen) with a plant-based ointment (H.E.C.). Both preparations, yet again, did not differ significantly with respect to the objective parameters of mucosal dehydration, formation of blood clots, and mucosal lesions [73].

Furthermore, the application of the control substances might have led to falsified results in the control group, because of the potentially positive effects of their active agents on the wound healing process. An enhancing effect on morphological and functional cilia regeneration has been ascribed to retinoic acid [74–77]. Systemic prednisolone administration together with local application of 5% of dexpanthenol ointment had a beneficial effect especially on the late spontaneous wound closure in a standardized animal model [68]. On the other hand, the addition of dexpanthenol (5%) resulted in a statistically significant reduction of the toxicity of α-sympathomimetic decongestants like xylomethazoline [63].

Mean values for the rhinoscopic parameter of nasal dryness were almost identical in both groups of this observational study except in the 4th postoperative week (Table 2). In the control group, the mean value at visit 2 (1.24 pt.) dropped only slightly by visit 3 (1.21 pt.), while it decreased more in the intervention group (from 1.25 to 1.00 pt.: no statistical significance). This fact could be attributed to the intense hydration effect of hyaluronic acid. This explanation was based on the important clinical observations made by Soldati et al. that the application of the hyaluronic acid containing ointment prevented large-scale crusting in the first postoperative week compared to the control substance [73].

The mean values for the parameter dried nasal mucus exhibited almost analogous dynamics (Table 2). Although a greater decrease in the mean value for the degree of dried nasal mucus was observed among hysan users in the 4th and 6th weeks, it did not reach a level of significance. This was probably ascribed to the increased local hydration due to hyaluronic acid, which formed an even, stable, and long-lasting moisture film on the nasal mucosa, thereby serving as a lubricant during the vulnerable regeneration process and as a vehicle for dexpanthenol in the late phase of wound healing phase, allowing its full cilia-protective effect to unfold. Consequently, the improved mucociliary clearance helped gently loosen dried nasal mucus.

The significantly decreased crusting during the 1st and 2nd postoperative weeks after applying a combination solution for mucosal care (that contained isotonic saline, algae extract, hyaluronic acid, panthenol, and Tonimer Gel Spray) may also support this assumption [78]. Another clinical

Table 3: Patient evaluation of nasal breathing and foreign body sensation.

Postoperative week		Nasal breathing				Foreign body sensation
		1.	2.	4.	6.	1–6.
Control group	*N* (valid)	21	21	19	18	21
	Mean value	**1.90**	**1.48**	**1.11**	**1.00**	**0.52**
	SD	0.625	0.512	0.315	0.000	0.512
Nasal spray group	*N* (valid)	24	24	20	19	24
	Mean value	**1.58**	**1.25**	**1.10**	**0.95**	**0.71**
	SD	0.584	0.442	0.447	0.229	0.464

comparison between dexpanthenol seawater spray (Mar plus) and normal saline irrigation resulted in less crusting at the 2nd check-up visit and better mucociliary clearance at the 4th check-up in the intervention group [79].

A clinical reduction in the formation of dried nasal mucus was observed after an 8-week treatment with dexpanthenol in a spray application form in patients with chronic rhinitis sicca as well [65]. Analogous results were shown by Hahn et al. after the four-week application of a dexpanthenol ointment [80].

The mean values for fibrin deposition in the hysan group were somewhat lower in the late phase of wound healing between the 4th and 6th postoperative weeks compared to the prior weeks (Table 2).

The last mucosal findings collected by the treating physician concerned nasal obstruction as observed via rhinoscopy. At all scheduled examinations, the results of both groups were of similar magnitude and without statistically significant differences (Table 2). Notable were the initially rapid drop of the mean value in the hysan group in the 2nd week and the consistent small decline over the remaining three visits. This tendency gave rise to the presumption that the use of the nasal spray greatly reduced nasal mucosal obstruction in the early phase of wound healing and was responsible for lower mean values in general over the entire period of application. In the end, however, no considerably better results were obtained than in the control group.

This correlation could lead one to assume that, due to hyaluronic acid, increased tissue hydration, which according to Kühnel et al. enables the early reduction of dried nasal mucus [81], and the reduced formation of hyperplastic granulation tissue [68, 82], as well as the accelerated reepithelialization due to dexpanthenol [82], resulted synergistically in diminished postoperative nasal obstruction symptoms.

Our study, however, could not clearly verify this theory.

The subjects were asked to categorize their subjective perceptions of free nasal breathing and foreign body sensation on the observation form, since according to definition an observational study is to consider the individual assessments of the product users as an important influencing factor. The results are summarized in Table 3.

The responses from the nasal spray patients at the first two examinations are striking with their low mean values for free nasal breathing, again without significant differences to the control group. The results were almost identical for the last visits in both groups (Table 3). This fact suggests that patients tended to perceive nasal breathing as freer while using hysan spray during early postoperative tissue regeneration. A possible explanation for this could be the film formed by the aerosol of the nasal spray that temporarily covered the mucosal areas and which the patient mistakenly interpreted as mildly impaired nasal breathing in the last two weeks.

Similar results of positive influence of an isotonic seawater spray containing dexpanthenol on the total nasal subjective symptom score and on patient satisfaction, although again without significance, were confirmed by Fooanant et al. [79].

In this connection, conflicting results have been mostly published in the literature. A significant improvement of the patient-reported comfort (ease of breathing, nasal tension, and feeling of dryness) has been observed by Ercan et al. [78]. Soldati et al. also confirmed a significant improvement in respiration among subjects who applied ointment containing hyaluronic acid, with nasal patency being highly significant on the 7th postoperative day and significant on the 14th postoperative day [73]. Kehrl and Sonnemann [65] and Hahn [80] verified positive dynamics in the subjective sensory scale in terms of nasal airway obstruction among dexpanthenol spray users with rhinitis sicca. Significant improvement of nasal obstruction showed a saline aerosol containing hyaluronic acid in the phase of functional regeneration during sinonasal remodeling, as described by Macchi et al. [83].

The mean value for patient's self-assessment of foreign body sensation was in general relatively positive (Table 3). Limitations existed with respect to the time at which this symptom appeared (the parameter was not enquired upon at every scheduled examination but instead globally assessed for the entire postoperative interval). Contrary to expectations, at a value of 0.52 points in the control group it tended to fall into the category "not present," and at 0.71 points, the parameter tended slightly to "moderately pronounced" among hysan users. One reason for this could be the protective film on the mucosa as mentioned earlier that compromised the patients' perception of a foreign body.

Rhinomanometry data confirmed volume enlargement of the nasal cavity by 12.31% in the control group and by 11.24% in the intervention group, the difference not being statistically significant (Table 4). The application of the test substance (period: 8th–10th postoperative day) resulted

Table 4: Values for pre- and postoperative rhinomanometry in [mL/s] at 150 Pa, sum left and right nasal cavity.

		Preoperative rhinomanometry sum left and right at 150 Pa in [mL/s]	Postoperative rhinomanometry sum left and right at 150 Pa in [mL/s]	Delta rhinomanometry	Improvement in %
Control group	*N* (valid)	19	18	16	
	Mean value	**643.16**	**737.67**	**79.19**	**12.31**
	SD	188.253	118.457	179.300	
Nasal spray group	*N* (valid)	23	19	18	
	Mean value	**688.13**	**743.05**	**77.33**	**11.24**
	SD	209.524	140.956	206.292	

in no significant, objectively measured reduction in nasal resistance. Volume enlargement of the nasal cavity relies in fact only on structure-reducing measures which successfully eliminated any obstruction to nasal breathing.

The investigator's impressions concerning the good tolerability and efficacy of the preparation agreed to a large extent with those of the patients and with the literature.

Soldati et al. also reported high acceptance, safety, and tolerability of the ointment containing hyaluronic acid. Worth mentioning, besides the positive organoleptic evaluation relating to the smell and the sensation of cooling upon application, was also the absence of adverse reactions [73]. The study conducted by Fooanant et al. yielded similarly good results for the dexpanthenol spray with respect to effectiveness and patient satisfaction [79]. The positive influence of dexpanthenol preparations on the subjective symptoms in patients with rhinitis sicca [65, 80] and their high acceptance were, yet again, able to confirm the clinically relevant and statistically significant superiority of the substance.

Dropouts were the most common subject appearing in the text field "other doctor's comments." Only one mild irritation was noted, probably due to intolerance of one of the ingredients; no entry, however, was made under the item "adverse events" on the observation form.

6. Conclusion

Surgical procedures of the paranasal sinuses leave behind extensive wounds that are left up to the secondary self-healing process [6, 84]. The aim of postoperative treatment is optimum wound healing with minimal morbidity.

The present limited observational study showed that the nasal spray is a safe preparation for care of the mucosa after rhinosurgical procedures. Its use did not negatively affect postoperative mucosal regeneration, yet no significant improvement of mucosal conditions could be observed either.

The results might have been impaired by the two treating physicians, who might have not always assessed the nasal mucosa conditions identically, by the possible positive influence of the active ingredients of the conventional care preparations on the control group, or by the unbalanced concomitant use of antibiotics and anti-inflammatory medication. Furthermore, the conclusions of the study might have somewhat been affected by the limited participants number, by the lack of randomization and blinding, by the heterogeneity of the initial pathology state among the patients, or by the variety of the surgical procedures that are scarcely comparable.

Additional multicenter, double-blind studies with larger populations, having a comparable degree of pathology and extent of mucosal extirpation in the same surgical procedure along with detailed surveys, are necessary to clarify further aspects of postoperative wound healing processes of the respiratory epithelium and the influence thereof for achieving adequate functional regeneration and better quality of life.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

The Effectiveness of Acupuncture Compared to Loratadine in Patients Allergic to House Dust Mites

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Background. The aim of this work was to evaluate the clinical effectiveness of acupuncture and its impact on the immune system in comparison to loratadine in the treatment of persistent allergic rhinitis caused by house dust mites. *Methods*. In this study, 24 patients suffering from persistent allergic rhinitis induced by house dust mites were treated either with acupuncture ($n = 15$) or with loratadine ($n = 9$). The evaluation of the data was based on the subjective and the objective rhinoconjunctivitis symptom scores, specific and total IgE, and interleukins (IL-4, IL-10, and IFN-γ) as markers for the activity of Th1 or Th2 cells. *Results*. The treatments with acupuncture as well as with loratadine were considered effective in the patients' subjective assessment, whereby the effect of the acupuncture tended to be assessed as more persistent after the end of treatment. A change in the specific or the total IgE was not detectable in either group. The interleukin profile showed the tendency of an increasing IL-10 value in the acupuncture group. The results of the study show that the effectiveness of acupuncture is comparable to that of loratadine. *Conclusion*. Acupuncture is a clinically effective form of therapy in the treatment of patients suffering from persistent allergic rhinitis. The results indicate the probability of an immunomodulatory effect.

1. Introduction

With a prevalence of 20 to 30%, allergic rhinitis is one of the most frequent atopic diseases in Western Europe [1–3]. It leads to a decrease of the patients' quality of life [4] and causes great cost for medication and social benefits [5].

House dust mites, namely, *Dermatophagoides pteronyssinus* and *D. farinae*, are two of the most common persistent allergens. In 30% of all house dust mite allergies, a development of allergic bronchial asthma with coexisting nasal symptoms is expected. Especially in patients suffering from untreated allergic rhinitis, an exacerbation usually follows 5–15 years after the first occurrence of nasal symptoms [6].

Traditional Chinese medicine (TCM), of which acupuncture is a part, gains in importance as an addition to conventional therapies. According to a report of the World Health Organization of 2002 [7] and to clinical studies [8], acupuncture is ranked among the sufficient methods for the treatment of allergic rhinitis and further allergic diseases such as bronchial asthma [9, 10]. Despite the conventional forms of therapy, 64% of patients suffering from persistent allergic rhinitis (PER) desire acupuncture as an alternative form of therapy [11]. Two of the latest multicentre and randomised trials found evidence for a significant improvement of symptoms and the quality of life through acupuncture in patients with allergic rhinitis [1, 12].

Acupuncture, which is described as immunomodulatory therapy [13, 14], has already been examined for its effect and tested for cellular [15] and humoral [10] components of the immune system. In the last decade, numerous findings concerning the key role of CD4+ cells were made. Depending on the subtype, very different cytokines are produced. These can be regarded as prospective markers for the effectiveness of the therapy. Th1 cells mainly express the cytokine interleukin-2

(IL-2) and interferon- (IFN-)γ, which cause a cellular immune response, while Th2 cells release interleukins (IL) 4, 5, 10, and 13, which control the maturation of B lymphocytes to cells producing antibodies and their total IgE [16–19].

Examinations show that IL-10 can be considered as markers in the course of the therapy [20]. In several studies on bronchial asthma [10, 21, 22] and allergic rhinitis [23–25], it was possible to show in animal testing as well as in patients that the cytokine profile of IL-10 can be modulated through acupuncture. At the same time, an improvement of the symptoms could be observed. Only fragmentary data are available on the effect of acupuncture on interleukins 4 (IL-4) and 5 (IL-5) and INF-γ which are involved in the Th1/Th2 equilibrium [10, 24].

It was the objective of this investigation to prove the effectiveness of acupuncture in the treatment of PER by comparing it with the effectiveness of antihistaminic loratadine as well as to gain a better understanding of the mechanisms of action of acupuncture through the examination of the interleukin profile.

2. Material and Methods

Patients in the outpatient department for allergy of the ENT clinic in Dresden suffering from PER were included in this study if a house dust mite allergy was ascertained by means of specific symptoms, skin prick test, and the assessment of the allergen specific IgE. With regard to the skin prick test (Allergopharma Joachim Ganzer KG, Reinbek), a sensitisation to *Dermatophagoides pteronyssinus* or *D. farinae* was then given if the diameter of the wheal measured ≥3 mm after 20 minutes [4]. The CAP-FEIA system of Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden, was used for the allergological in vitro diagnostics for the determination of the total and the specific IgE. A total IgE of more than 100 kU/L as well as a specific IgE of more than 0.70 kU/L (CAP class 2) [5, 26, 27] was used to verify a sensitisation.

The cytokine profile was examined through the interleukins 4 (IL-4) and 10 (IL-10) and IFN-γ using the ELISA technique by Quantikine Immunoassay, Firma R&D Systems, Wiesbaden-Nordenstadt, Germany.

Exclusion criteria were pregnancy, continuous immunotherapy, other therapies influencing the immune system like glucocorticoids or chemotherapeutics, or the use of additional antiallergic medication.

2.1. Study Design. This study included 30 patients. The data of 24 patients could be collected fully until the end of the study and were evaluated accordingly (acupuncture group: $n = 15$ and loratadine group: $n = 9$). The average age of the patients was 16.5 ± 9.8 years. The mean duration of the disease was 7.8 ± 6.1 years. Table 1 shows the mean age of both treatment groups.

2.2. Therapy Groups. The patients were randomly assigned to the different treatment groups. Patients being treated with acupuncture received twelve acupuncture sessions in total, two sessions a week, using the same acupuncture points for every patient. Sterile, disposable needles made of stainless steel (Seirin, Dreieich/Germany) were used for body (0.3 × 0.3 mm in strength) and for face and ear acupuncture (0.2 × 0.15 mm in strength).

To allow for standardisation and comparability, all patients were acupunctured at the same points which were chosen in accordance with the rules of TCM. Needles were inserted unilaterally or bilaterally at the following points: LI 20, Ex-HN 12, Ex-HN 3, BL 1, BL 2, LI 4, LI 11, ear pont 78, and ear point 55 (Figures 1, 2, and 3). In addition, all patients werde acupuntured at LU 20, GB 20, SI 3, and ST 36 (not shown).

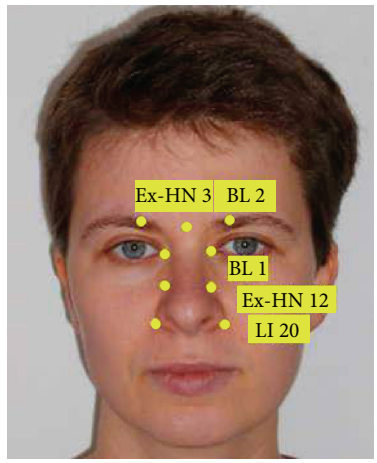

Figure 1: Standardised point chart for facial acupuncture.

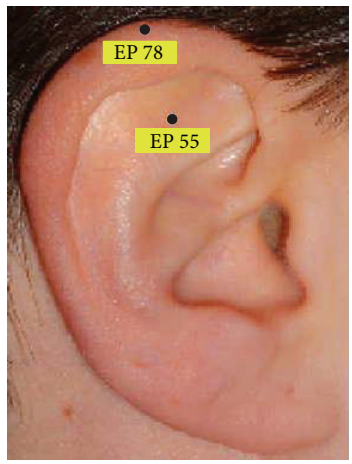

Figure 2: Standardised point chart for ear acupuncture.

Table 1: Demographic structure.

	Acupuncture $n = 15$	Loratadine $n = 9$
Male/female	9/6	3/6
Age (years)	28.1 (±9.9)	24.9 (±9.6)
Duration of disease (years)	7.3 (±6.7)	8.6 (±5.1)

Demographic data: illustration of gender, age, and duration of disease (mean values ± standard deviations) within the groups; *n*: number of patients.

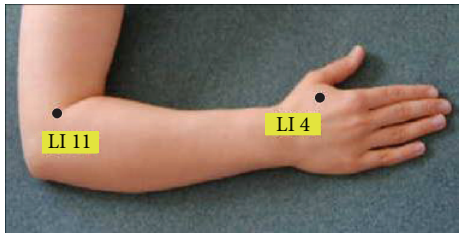

Figure 3: Standardised point chart for "extraordinary" points on the forearm.

The needles were kept in place for 20 minutes. All patients were treated by the same physician throughout the whole study in order to allow for a standardisation.

Patients treated with loratadine took 10 mg of loratadine (Lisino, Essex Pharma GmbH) in the morning of each day over the treatment period of 21 days.

2.3. Course of the Study. The patients were examined three times during this study. The first examination took place before the treatment (t_1), the second at the day after the end of treatment (t_2), and the third after an interval of 10 weeks without therapy (t_3).

At all three points, the clinical examination included anterior rhinoscopy, an estimation of the current nasal symptoms by use of the symptom score, and a blood sample (one tube for heparinised whole blood and one for serum each time).

2.4. Symptom Scores. All symptom scores were recorded on a 5-point scale (FPS).

2.4.1. Objective Symptom Scores. For the anterior rhinoscopy, the condition of the mucosa and the size of the nasal concha were recorded by the physician. Mucosal reddening and swelling of the inferior nasal concha were rated in the following score: 0 = normal, 1 = slightly changed, 2 = moderately changed, 3 = severely changed, and 4 = most severely changed.

2.4.2. Subjective Symptom Scores. While the objective nasal symptoms and findings were recorded three times in the course of the study, subjective symptoms (complaints) were determined retrospectively in the form of patient interviews. Nasal obstruction and secretion were evaluated using the following scale: 0 = free of symptoms, 1 = slight but noticeable symptoms, not interfering with daily activities, 2 = moderate symptoms, hardly interfering with daily activities and sleep, 3 = severe symptoms, clearly interfering with daily activities and sleep, and 4 = most severe symptoms, substantially interfering with daily activities and sleep.

The assessment of sneezing attacks was classified into 3 categories: 0 = no sneezing attacks, 1 = rare sneezing attacks, 1-2 sneezing attacks per day, and 2 = frequent sneezing attacks with more than 3 attacks per day.

2.4.3. Total Symptom Score. All symptom scores were summed up to a total symptom score in order to elucidate the therapeutic effect.

2.5. Subjective Estimation of the Therapeutic Effect. At examinations t_2 and t_3, the subjective state of health was evaluated by comparing the afflictions prior to the therapy with the current ones (1 = improved and 2 = unchanged or worsened).

2.6. Statistics. The data collected were evaluated using the statistical software SPSS Version 21 for Microsoft Windows. The results were given in form of mean ± standard deviation or standard error of the mean. The study was planned as repeated measures design and consequently evaluated by means of an analysis of variance. A significance level of $P < 0.005$ was considered statistically significant.

3. Results

In the acupuncture group, 87% of the patients reported an improvement of their afflictions at the end of therapy (t_1). 13% did not notice a change at all at t_2 and still not at t_3, 10 weeks after the end of therapy. At t_3, 20% did not notice a change in comparison to the beginning of therapy (t_1) anymore. In the loratadine group, 67% of the patients stated an improvement at t_2, while 33% did not detect an improvement. At t_3 none of the patients treated with loratadine noticed an improvement in comparison to t_1 (see Figure 4).

3.1. Total Sum Score. Looking at the subjective and objective symptoms separately, there is no significant difference noticeable, neither in the course of the therapy nor between the groups. The total sum score, however, showed significant changes in the time course of the therapy. Both in the acupuncture and the loratadine group, a significant improvement was gained under therapy. In the ten-week period following the therapy, a significant deterioration which led to the recurrence of the allergic symptoms was shown in the loratadine group, while the significant improvement of the symptoms persisted in the acupuncture group (multivariate tests $P < 0.005$). Comparing both groups, no significance was ascertainable (see Figure 5).

3.2. Allergic Parameter (Total IgE; Specific IgE Dermatophagoides pteronyssinus/D. farinae). Neither the acupuncture nor the loratadine group showed a significant difference in the specific IgE or the total IgE.

3.3. Interleukin Profile (IL-4 and IFN-γ). The intermediate IL-4 level in the acupuncture group slightly increased during therapy, between t_1 and t_2, from 0.182 pg/mL to 0.185 pg/mL. It then decreased to 0.177 pg/mL during the period without treatment, between t_2 and t_3. Contrarily, in the loratadine group, the IL-4 level already decreased during therapy from 0.13 pg/mL (t_1) to 0.112 (t_2) and increased then to 0.126 pg/mL s (t_3).

In the acupuncture group, the serum level of IFN-γ increased between t_1 and t_2 from 4.799 pg/mL to 5.844 pg/mL and decreased between t_2 and t_3 to 4.399 pg/mL. The intermediate IFN-γ level of the loratadine group showed a similar course. After the increase from 5.186 pg/mL to 5.664 pg/mL at the beginning of therapy, the IFN-γ serum level decreased to 5.504 pg/mL (t_3).

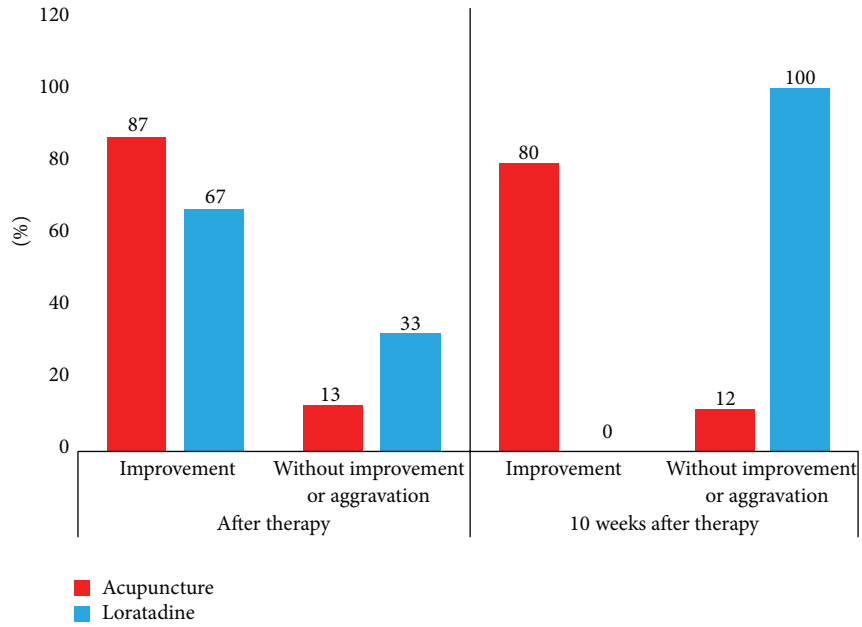

Figure 4: Subjective evaluation of rhinitis symptoms on the day after the end of therapy and after the 10-week therapy-free interval in comparison to the state of health immediately before the beginning of therapy (percentage frequencies in relation to the total patient numbers within the treatment groups).

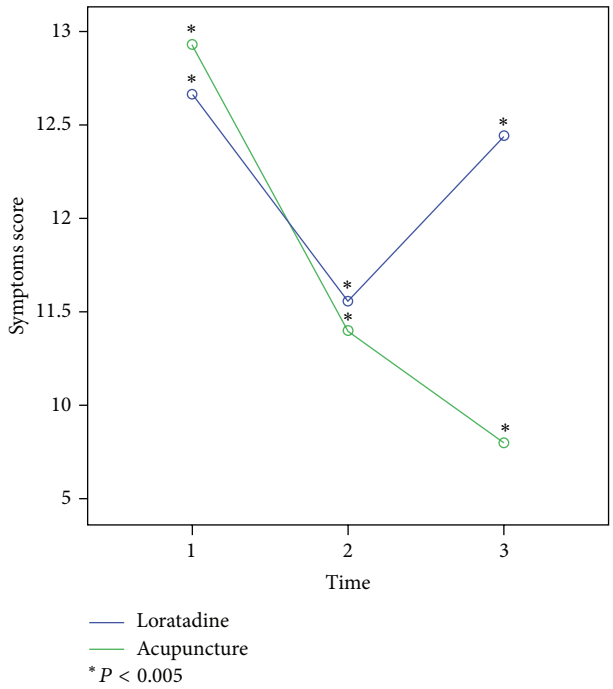

Figure 5: Change in the total symptom scores in both groups during the study period.

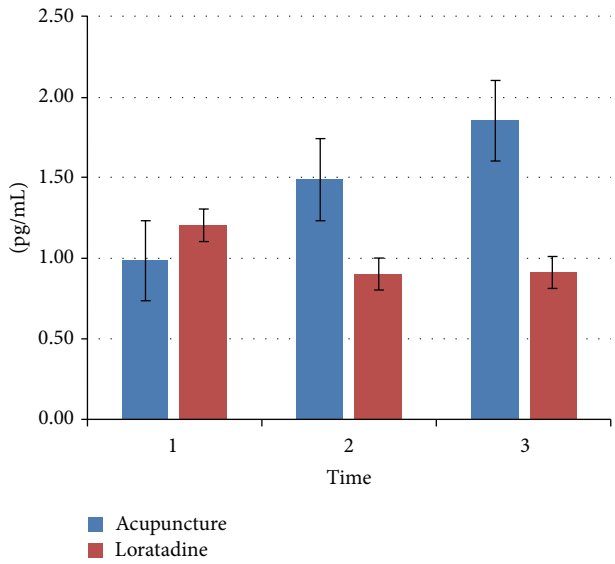

Figure 6: Change in interleukin-10 level in serum; mean values ± standard mean of error at the three examination times.

None of the observed differences of the IL-4 and IFN-γ were significant.

3.4. Interleukin Profile IL-10. For the IL-10 serum level, an increase from 1.001 pg/mL (t_1) to 1.49 pg/mL (t_2) was observed in the acupuncture group. Also, after the end of therapy, an increase to 1.857 pg/mL (t_3) was shown. In the loratadine group, the IL-10 also increased from 1.84 pg/mL (t_1) to 2.013 pg/mL (t_2) but decreased after the end of treatment to 1.909 pg/mL (t_1).

Even though the increase of the IL-10 serum level was not significant, it showed a distinct tendency to increase in the partial eta squared values (Figure 6).

4. Discussion

In order to prove that acupuncture can serve as complementary form of therapy in the treatment of PER, its effectiveness was compared to that of loratadine. It was also examined whether acupuncture has a long-term effect beyond the end of the treatment period. Furthermore, the theory according to which the therapy effect of acupuncture is based on an immunomodulatory effect was checked.

The role of IL-10 in the pathogenesis of allergic diseases during treatment and its level changes in the serum are currently the subject of controversial discussion [20]. It was shown that the IL-10 is able to obstruct the histamine release of activated mast cells [28]. In addition, an increased IL-10 level in the nasal mucosa led to a definite reduction of nasal allergy symptoms of patients with dust mite allergy after nasal provocation [29]. Thus, the IL-10 level could function as a marker for the effectiveness of antiallergic therapy. Some reasearchers suggest that the interleukin levels, especially that of IL-10, change under acupuncture [10, 21, 24].

In our study, we were likewise able to observe that the IL-10 level tends to increase in the acupuncture group. This observation could be an indication for the immunomodulatory effect of acupuncture. Due to the small number of patients, however, it was not possible to show a significance.

Even though there is information that the level of the other cytokines investigated here, namely, IL-4 and IFN-γ, can change during acupuncture [10, 21, 24], this was not observed in our study, which might be due to the small sample size.

In a number of studies, the effectiveness of acupuncture in regard to quality of life and the reduction of medication as well as significant improvements of the clinical symptoms has already been shown [1, 8, 12, 30, 31]. Even though our results did not show any significant differences of the single symptoms, a significant improvement of the symptoms was observed in the total sum scores in both the acupuncture and loratadine groups during the course of the treatment. This outcome correlates with the patients' subjective assessment of their state of health recorded immediately at the end of treatment. The difference between both groups develops within the 10-week period without treatment, between t_2 and t_3. While the patients in the acupuncture group still experienced improvement in symptoms (a significant improvement in comparison to the beginning of therapy), the symptoms of the loratadine group started to increase again after the end of treatment. Nevertheless, this difference between both groups was not significant.

Modern medicine requires an evidence-based, double-blind, and placebo-controlled study design in order to prove effectiveness. This is, however, hardly applicable for acupuncture studies. Especially blinding and placebo control present an unsolved problem. Theoretically, a blinding would be possible for laser acupuncture but a comparability of the effectiveness of needle acupuncture with laser acupuncture has not yet been proven. A placebo treatment with acupuncture needles founders on the circumstance that the so-called sham acupuncture, where acupuncture needles are inserted in the acupuncture points, can still have a physiological impact or an effect on the immune system. As the skin is related to internal organs and body systems by the principle of segmental innervations [32], it is not possible to exclude an impact of the skin irritation on the examined effect.

In our study, we tried to prove the effectiveness of acupuncture in PER through changes in the interleukin level in the serum. However, parameters depending on interleukin can be strongly influenced by factors such as autoimmune diseases, inflammations, or even the weather. It might be for this reason that no significant changes in the interleukin level in the serum could be found. Furthermore, a larger number of participants are necessary to prove significance of the treatment effects. The small number of patients allows in many cases only a statistical tendency to increase.

Despite these limitations, the results at hand make it possible to conclude that acupuncture itself and the acupuncture points used are effective in the treatment of PER. Acupuncture, therefore, presents a suitable alternative for patients with drug intolerance or pregnancy. Further studies with a larger patient collective are necessary to confirm these positive results of the mode of action of acupuncture and to examine the effectiveness of further TCM acupoints.

5. Conclusion

Acupuncture is an effective, well-tolerated form of therapy in the treatment of patients suffering from dust mite allergy with its effect being comparable to loratadine.

Although the theory that the mechanism of action of acupuncture is based on immunomodulation could not be proven significantly, it was possible to show a tendential increase of the IL-10 level in the serum under acupuncture. For a definite assessment of this issue, further studies with larger numbers of patients are necessary.

Acupuncture can function as an effective therapeutic alternative for patients having a contraindication to specific immunotherapy or to a medicinal symptomatic therapy.

Abbreviations

EP: Ear point
PER: Persistent allergic rhinitis
TCM: Traditional Chinese medicine
FPS: Five-point scale.

Consent

The tested subjects were first informed about the studies and their written consents were obtained. The Institutional Review Board considered investigations as safe and in agreement with the principles of the Declaration of Helsinki.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Bettina Hauswald was the principal investigator of the study and contributed to the protocol design together with Christina Dill, Thomas Zahnert, and Yury M. Yarin. Bettina Hauswald and Christina Dill carried out the acupuncture treatment. Yury M. Yarin and Jürgen Boxberger critically revised the paper. Eberhard Kuhlisch performed the statistical analysis under the guidance of Yury M. Yarin. All authors read and approved the final paper.

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Clinical Study

Treatment of Allergic Rhinitis with Ectoine Containing Nasal Spray and Eye Drops in Comparison with Azelastine Containing Nasal Spray and Eye Drops or with Cromoglycic Acid Containing Nasal Spray

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Objectives. Allergic rhinitis is a common disease with increasing prevalence and high impact on economic burden and comorbidities. As treatment with pharmacological drugs is not always satisfactory due to side effects and incomplete efficacy, alternative treatment strategies are needed. Ectoine is an osmolyte with membrane stabilizing and inflammation reducing capacities. Nasal spray and eye drops containing ectoine are promising new treatment regimens for allergic rhinitis sufferers. *Design and Methods*. The current two noninterventional trials evaluated the efficacy and safety of ectoine containing nasal spray and eye drops for treating allergic rhinitis in comparison with either azelastine or cromoglycic acid containing products. Nasal and ocular symptom developments as well as judgment of tolerability and efficacy were assessed both by investigators and patients over a time period of one to two weeks. *Results*. Both trials confirmed that ectoine containing products reduced nasal and ocular symptoms in allergic rhinitis patients. Results clearly demonstrated good safety profiles of the ectoine products comparable to those of azelastine and even better to those of cromoglycate products. *Conclusion*. Ectoine containing nasal spray and eye drops are interesting new treatment strategies for sufferers of allergic rhinitis, combining both good efficacy and absence of side effects.

1. Introduction

Allergic rhinitis is a common disease affecting 10–20% of the population [1]. Since it has great impact on patients' quality of life, school performance, work productivity, and comorbid conditions such as asthma, it is considered as an important health problem. Allergic rhinitis is defined as an allergic reaction (most often IgE-dependent) to offending allergens such as dust mites, insects, animal dander, and pollens. Symptoms include rhinorrhea, nasal obstruction, nasal and nasopharyngeal itching, sneezing, and postnasal drip. Often, allergic rhinitis is accompanied by allergic conjunctivitis with ocular symptoms such as itchy and watery eyes, resulting in the term allergic rhinoconjunctivitis. According to its length of duration, allergic rhinitis is classified into intermittent (symptoms present <4 days a week of <4 weeks) and persistent (symptoms present ≥ 4 days a week and for at least 4 weeks) forms. Symptom severity is used to classify allergic rhinitis into mild or moderate-severe forms.

A number of pharmacological treatments of allergic rhinitis exist, such as, for example, oral and topical antihistamines, leukotriene receptor antagonists, intranasal glucocorticoids, and cromoglycic acid (mast cell stabilizers) [2].

Azelastine is a new-generation antihistamine applied topically as nasal spray or eye drops. It is used as treatment of allergic rhinitis, hay fever, and allergic conjunctivitis. Although azelastine is regarded as effective possible first-line treatment for allergic rhinitis, common side effects, such as bitter taste of the drug and local irritation reactions and rare side effects such as fatigue or headache, can occur [3].

Cromoglycic acid is an antiallergic drug which inhibits the degranulation of mast cells, thereby blocking the release of

inflammatory mediators [4]. Thus, cromoglycic acid prevents the development of allergic reactions rather than reducing acute symptoms and its onset of action is about four to seven days. Due to its short half-life, cromoglycic acid has to be applied at least 4 times a day. Cromoglycic acid is thought to be a safe medication, and adverse events which might occur are usually mild, such as sneezing and sensation of burning. Due to its good safety profile, cromoglycic acid can be prescribed for treating rhinitis in children and pregnant women.

In general, many allergic rhinitis patients are still unsatisfied with the control of symptoms, complain about incomplete relief of symptoms, and suffer from unwanted side effects [5, 6]. Therefore, it is not surprising that increasing interest in the use of alternative and complementary medicine (CAM) for treating rhinitis exists. Thus, it was demonstrated that 40% of the American population uses CAM, 17% of which uses it for treating otorhinolaryngologic diseases [7]. However, so far no general recommendation for the use of CAM can be given by ARIA guidelines as ambiguous study results are available [8].

The present two individual studies compared treatment of allergic rhinitis with ectoine containing nasal spray and eye drops with azelastine containing products (study 1) or treatment with ectoine containing nasal spray with that of cromoglycic acid containing nasal spray (study 2).

Ectoine is a natural amino acid derivate which is produced by bacteria living under extreme harsh environmental conditions where it serves as osmoregulatory compatible solute [9, 10]. Ectoine works via a mechanism called "preferential exclusion" [11, 12]. If it is present together with proteins or lipids, ectoine is expelled from their surfaces, thereby increasing the hydration of the surface and stabilizing lipid layers [13]. Its membrane stabilizing as well as inflammation reducing capacities makes ectoine an interesting candidate for the treatment of allergic rhinitis. These studies served to investigate the efficacy and safety of ectoine containing nasal spray and eye drops in patients with allergic rhinitis.

2. Materials and Methods

The current paper describes two noninterventional studies carried out with ectoine containing nasal spray and eye drops assessing their efficacy in comparison with azelastine nasal spray and eye drops (study 1, NCT02131051) or cromoglycic acid nasal spray (study 2, NCT02131038).

2.1. Medication. The ectoine eye drops contain an isoosmotic solution with 2% ectoine and 0.35% hydroxyethyl cellulose; the ectoine nasal spray is a hypertonic solution with 2% ectoine. Additional ingredients of the eye drops were sodium chloride, sodium dihydrogen phosphate dihydrate, sodium monohydrogen phosphate dihydrate, and water. Additional ingredients of the nasal spray were sodium chloride and water. In study 1, both nasal spray and eye drops were used, whereas only the nasal spray was used in study 2.

Azelastine containing products were used as comparator in study 1. The azelastine eye drops contain 0.5 mg/mL azelastine hydrochloride with one drop administering 0.015 mg azelastine hydrochloride, and the azelastine nasal spray contains 1 mg/mL azelastine hydrochloride with one puff administering 0.14 mg azelastine hydrochloride. Additional ingredients of the eye drops were benzalkonium chloride (preservative), sodium edetate, hypromellose, sorbitol, sodium hydroxide, and water. Additional ingredients of the nasal spray were sodium edetate, hypromellose, citric acid, sodium chloride, sodium hydrogen phosphate, and water.

During study 2, a cromoglycic acid containing nasal spray was used as comparator. The spray contained 20 mg/ml cromoglycic acid corresponding to 2.8 mg sodium cromoglycic acid per puff. In addition, the following ingredients were present in the formulation: benzalkonium chloride (preservative 0.014 mg/puff), sodium edetate, sodium chloride, sodium dihydrogen phosphate, sodium monohydrogen phosphate, sorbitol, and water.

2.2. Treatment and Study Design

2.2.1. Study 1. On day 0 (Visit 1) patients were asked to participate in the study, and upon signing the informed consent form and patient information, they were allocated to one of the study groups, without any washout period. Antiallergic medications used the last two days prior to inclusion were recorded by the physician. Patients were treated either with ectoine containing nasal spray and eye drops or with azelastine containing nasal spray and eye drops. Patients of the ectoine group had to apply one eye drop per eye and one puff of the nasal spray per nostril four times per day. Patients of the azelastine group had to apply one eye drop per eye and one puff of the nasal spray per nostril twice per day. The treatment period was 7 days, and patients were asked to document their symptoms, together with possible comedication and adverse effects daily in patient diaries at the evening. Therefore the patients' assessments started after the products had been applied already. Following treatment, patients came back for Visit 2 (day 7), during which symptom scores were evaluated and tolerability, efficacy and compliance, and possibly comedications, antiallergic and others, were assessed.

In- and Exclusion Criteria. Male or female patients aged 18–70 with proven allergy and acute symptoms in nose and eye (sum nasal score $\geq$ 15 and sum oral score $\geq$ 6) were allowed to take part in the study. Allergy diagnosis was based on positive prick test. Exclusion criteria were pregnant and nursing women, drug addicts and persons unable to give consent to study participation, patients with intolerance against ingredients of any of the study treatments, previous eye or nose surgery, concomitant treatment with antiallergic drugs, and diseases which might influence the output of the study according to the physicians' judgment.

Scoring of Nasal and Ocular Symptoms. Single nasal (nasal obstruction, rhinorrhea, and sneezing) and ocular symptoms (eye itching, tearing, and conjunctivitis) were scored with an 8 point scale ranging from no symptoms (0) to very severe symptoms (8).

Scoring of Efficacy, Tolerability, and Compliance. Efficacy, tolerability, and compliance were judged by using a scale ranging from 0 (very good) to 8 (bad). Thus, a general judgment, of either how well to tolerate or how efficient the products were, had to be given by the patients and documented in the patient diaries. Both scoring values were based on the patients' personal opinion/feeling with the products. Whereas efficacy and tolerability were assessed both by patients and by physicians, compliance was solely judged by physicians.

Statistics. The statistical analysis was carried out with SPSS version 18 and SigmaPlot version 12. Both efficacy and safety analyses were performed on the entire study population. Descriptive statistics were used for a quantitative report of the main study population features. Continuous variables were tested for normal distribution via Kolmogorov-Smirnov test. Further analysis was carried out with the Mann-Whitney U test, Wilcoxon test, or Friedman test. The level of significance was set to $P < 0.05$ in all tests. Unavailable data were treated as "missing values" or substituted by the "last value carried forward" method.

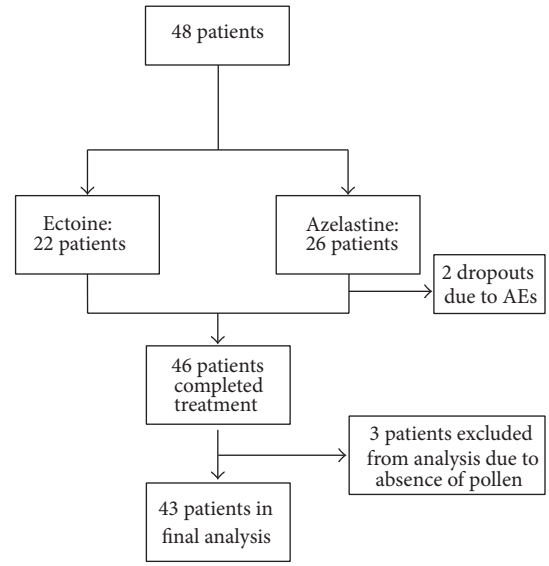

Figure 1: Patient flow during study 1.

2.2.2. Study 2. This study was designed as a crossover study, without any washout period within the first week. Half of the patients received ectoine nasal spray whereas the other half received cromoglycic acid containing nasal spray. After 7 days, patients swapped to the other treatment. Thus, patients who started with one week treatment with ectoine nasal spray received cromoglycic acid containing nasal spray within the second week and vice versa. For simplification reasons, patients starting their treatment with ectoine are termed group A, and patients starting their treatment with cromoglycic acid are termed group B in this paper.

The ectoine nasal spray had to be applied at least 5 times per day, whereas the cromoglycic acid spray had to be applied 4 times a day. Thus, patients had to take the ectoine product at least 5 times a day but could upgrade dosing if they felt that medication was not sufficient. The cromoglycic acid product had to be used according to the instruction for use.

Patients had to attend visits to the investigator on day 0 (V1), day 7 (+2 days) (V2), and day 14 (+2 days) (V3). During those visits, the investigator assessed nasal (nasal obstruction, sneezing, and rhinorrhea) and ocular symptoms (eye itching, tearing, and conjunctivitis) as well as palate itching and turbinate hyperplasia. At the end of the study (V3), efficacy, tolerability, and compliance were determined.

In addition to the investigator's assessment, patients had to document daily their ocular and nasal symptoms as well as their judgment of tolerability and efficacy in a patient diary at the evening. Based on the design the patients scoring started after the study medication had been applied.

In- and Exclusion Criteria. Male or female patients with diagnosed allergy and moderate to severe acute symptoms of nasal obstruction, sneezing, and rhinorrhea were allowed to take part in the study. The diagnosis of the allergy was based on a positive prick test. Exclusion criteria were intolerance against ectoine or cromoglycic acid, pregnancy, previous nose surgeries, or ongoing treatment with additional antiallergic drugs.

Scoring of Nasal, Ocular, and Other Symptoms. Single nasal symptoms (nasal obstruction, rhinorrhea, and sneezing) and ocular symptoms (eye itching, tearing, and conjunctivitis) as well as the symptoms palate itching and turbinate hyperplasia were scored with an 8 point scale ranging from no symptoms (0) to very severe symptoms (8).

Scoring of Efficacy, Tolerability, and Compliance. Efficacy, tolerability, and compliance were judged by using a scale ranging from 0 (very good) to 8 (bad). Thus, a general broad judgment, of how well to tolerate and how efficient the products were, had to be given by the patients and to be documented in the patient diaries. Both scoring values were based on the patients' personal opinion/feeling with the products. Whereas efficacy and tolerability were assessed both by patients and by physicians, compliance was solely judged by physicians.

Pollen Score. In order to reflect the current pollen exposure, data from the online HEXAL pollen calendar were used to grade pollen exposure into mild, moderate, or severe (1, 2, or 3) scores during the course of the study.

Statistics. The statistical analysis was carried out with SPSS version 17 and SigmaPlot version 12. Safety analyses were performed on the entire study population whereas efficacy analysis was performed on all patients who completed the treatment. Continuous variables were tested for normal distribution via Kolmogorov-Smirnov test. Further analysis was carried out with the Mann-Whitney U test, Wilcoxon test, or Friedman test. The level of significance was set to $P < 0.05$ in all tests. Unavailable data were treated as "missing values" or substituted by the "last value carried forward" method.

Table 1: Development of single nasal scores (mean ± SD) during study 1 according to patients' and investigators' assessments.

Symptom	Group	Score d1 (patient)	Score d7 (patient)	P value	Score V1 (investigator)	Score V2 (investigator)	P value
Nasal obstruction	Ectoine	4.14 ± 1.93	3.38 ± 2.20	$P = 0.003$	5.29 ± 1.15	2.86 ± 1.49	$P < 0.001$
	Azelastine	4.38 ± 2.38	3.60 ± 2.37	$P = 0.044$	5.91 ± 1.23	3.0 ± 2.13	$P < 0.001$
Rhinorrhea	Ectoine	3.81 ± 1.86	2.71 ± 1.87	$P = 0.054$	5.19 ± 1.03	2.24 ± 1.58	$P < 0.001$
	Azelastine	3.48 ± 2.11	2.8 ± 2.02	$P = 0.133$	5.45 ± 1.01	2.59 ± 1.89	$P < 0.001$
Sneezing	Ectoine	3.9 ± 1.92	2.9 ± 1.73	$P = 0.475$	6.0 ± 1.48	2.43 ± 1.58	$P < 0.001$
	Azelastine	4.05 ± 1.43	2.45 ± 1.7	$P < 0.001$	5.77 ± 0.92	2.32 ± 2.10	$P < 0.001$
Nasal itching	Ectoine	2.81 ± 1.83	2.05 ± 1.56	$P = 0.068$	4.24 ± 2.32	1.00 ± 1.41	$P = 0.001$
	Azelastine	3.90 ± 1.70	2.25 ± 1.92	$P = 0.002$	4.59 ± 1.99	1.41 ± 1.05	$P < 0.001$

3. Results

Both studies were conducted in accordance with the Declaration of Helsinki. All investigations were carried out with the understanding and consent of all participants.

3.1. Results Study 1. This was a noninterventional trial taking place at two German ear nose throat (ENT) practices starting in June 2010 and being completed in September 2010. Distribution of patients is shown in Figure 1. In total, 48 patients took part in the study, of which 43 were included in the final analysis (31 females and 12 males). Mean age of patients was 35 years, and both groups were comparable in regard to clinical aspects.

3.1.1. Nasal Symptoms. Nasal symptom scores were assessed both as single symptoms and as sum of all nasal symptoms (TNSS). Details of the development of single scores are given in Table 1.

Nasal Obstruction. The mean symptom score of nasal obstruction decreased significantly by 45.95% in the ectoine group and by 49.23% in the azelastine group (V1 to V2, $P < 0.001$ for both groups). The documentation of the patient diaries also reflected a significant decrease by 18.39% in the ectoine group ($P = 0.003$) and by 17.83% in the azelastine group ($P = 0.044$).

Rhinorrhea. A significant decrease in the symptom score was also observed for rhinorrhea from V1 to V2. Mean values decreased by 56.88% in the ectoine group and by 52.50% in the azelastine group ($P < 0.001$ for both groups). The patient documentation showed a clear decrease of the symptom rhinorrhea which, however, was not significant. Values decreased by 28.75% in the ectoine group ($P = 0.054$) and by 19.45% in the azelastine group ($P = 0.133$).

Sneezing. The symptom sneezing decreased significantly from V1 to V2: values decreased by 59.52% in the ectoine group and by 59.84% in the azelastine group ($P < 0.001$ for both groups). The patient documentation also reflected the symptom decrease which was not significant in the ectoine group (25.61%, $P = 0.475$) but significant in the azelastine group (39.47%, $P < 0.001$).

Nasal Itching. Nasal itching decreased significantly from V1 to V2: values decreased by 76.40% in the ectoine group ($P = 0.001$) and by 69.31% in the azelastine group ($P < 0.001$). According to the patient documentation, nasal itching scores decreased by 27.12% in the ectoine group ($P = 0.068$) and by 42.38% ($P = 0.002$) in the azelastine group.

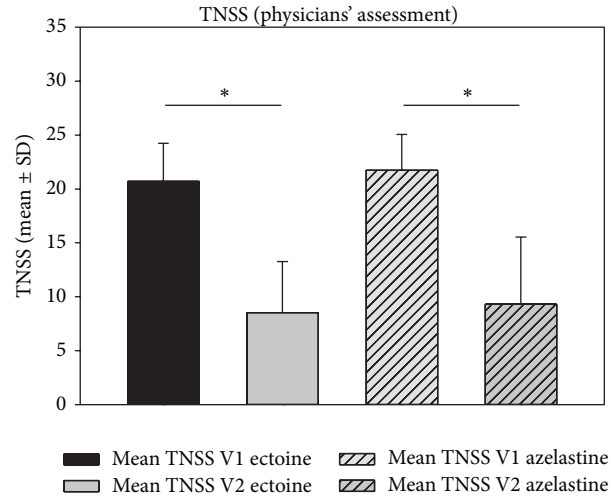

Figure 2: Decrease (mean ± SD) of TNSS from V1 to V2 according to the physicians' assessment. $^{*}P < 0.001$.

3.1.2. Total Nasal Symptom Score (TNSS). The sum of nasal symptom scores (nasal obstruction, rhinorrhea, sneezing, and nasal itching) showed a significant decrease from V1 to V2 (as assessed by physicians): sum scores in the ectoine group decreased from 20.71 ± 3.52 to 8.52 ± 4.74 (decrease of 58.85%; $P < 0.001$) and sum scores in the azelastine group decreased from 21.73 ± 3.34 to 9.32 ± 6.24 (decrease of 57.11%; $P < 0.001$). Data are depicted in Figure 2. According to the patients' assessment (see Figure 3), values decreased by 23.05% in the ectoine group ($P = 0.076$) and by 33.14% in the azelastine group ($P = 0.02$).

3.1.3. Ocular Symptoms. Ocular symptom scores were also assessed as single symptoms and as sum of all ocular symptoms (TOSS). Details of the development of single scores are given in Table 2.

Conjunctivitis. The symptom conjunctivitis clearly decreased from V1 to V2, as reflected by decline of 48.15% in the

Table 2: Development of single ocular symptom scores during study 1 according to patients and investigators' assessments.

Symptom	Group	Score d1 (patient)	Score d7 (patient)	P value	Score V1 (investigator)	Score V2 (investigator)	P value
Conjunctivitis	Ectoine	2.1 ± 1.84	1.38 ± 1.56	$P = 0.218$	2.67 ± 0.97	1.71 ± 1.62	$P = 0.058$
	Azelastine	2.05 ± 1.77	2.35 ± 2.32	$P = 0.885$	3.32 ± 1.73	1.77 ± 1.66	$P = 0.013$
Eye itching	Ectoine	3.24 ± 1.89	2.67 ± 1.91	$P = 0.604$	3.86 ± 1.93	2.0 ± 1.79	$P = 0.008$
	Azelastine	2.9 ± 1.81	2.75 ± 2.1	$P = 0.14$	4.05 ± 1.89	2.18 ± 2.17	$P = 0.002$
Tearing	Ectoine	1.71 ± 1.35	1.62 ± 1.63	$P = 0.886$	2.90 ± 1.3	1.38 ± 1.69	$P = 0.003$
	Azelastine	1.9 ± 1.84	1.55 ± 1.67	$P = 0.357$	2.14 ± 1.67	1.27 ± 1.67	$P = 0.039$

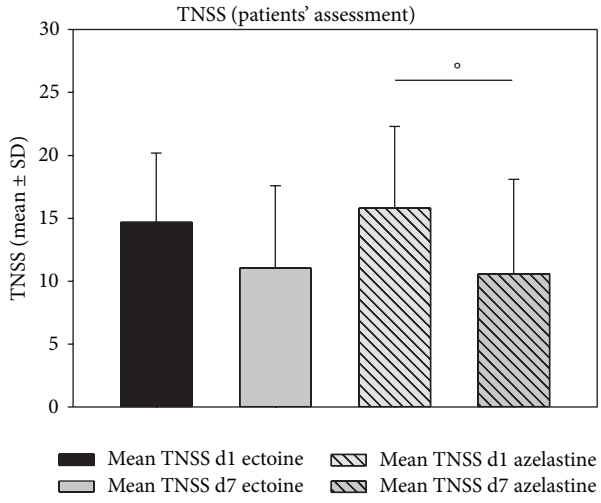

Figure 3: Decrease (mean ± SD) of TNSS from day 1 (d1) to day 7 (d7) according to the patients' assessment. $^{\circ}P = 0.02$.

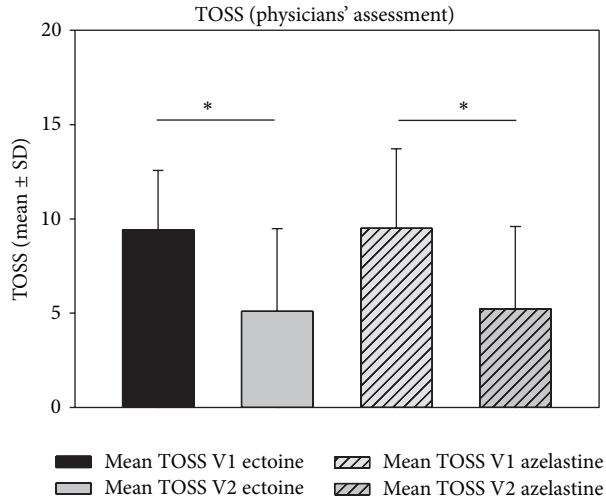

Figure 4: Decrease (mean ± SD) of TOSS from V1 to V2 as assessed by physicians in study 1. $^{*}P < 0.001$.

ectoine group ($P = 0.058$) and of 46.07% in the azelastine group ($P = 0.013$). In the patients documentation, scores of conjunctivitis decreased by 34.09% in the ectoine group ($P = 0.218$) whereas an increase by 14.77% was observed in the azelastine group ($P = 0.885$).

Eye Itching. There was a significant decrease in the symptom scores of eye itching: in the ectoine group, the mean decreased by 48.15% ($P = 0.008$) whereas values of the azelastine group decreased by 46.07% ($P = 0.002$). Corresponding decreases as assessed by the patients were 17.65% in the ectoine group ($P = 0.604$) and 5.33% in the azelastine group ($P = 0.14$).

Tearing. A statistical decrease in the scoring of the symptom tearing was also observed from V1 to V2: in the ectoine group, values decreased by 52.46% ($P = 0.003$) whereas values in the azelastine group decreased by 40.43% ($P = 0.039$). The patient documentation of the symptom tearing also showed a clear decrease of values (5.56% with $P = 0.886$ in the ectoine group and 18.63% with $P = 0.357$ in the azelastine group).

3.1.4. Total Ocular Symptom Score (TOSS). The TOSS (sum of conjunctivitis, eye itching, and tearing) decreased significantly from V1 to V2 in both groups ($P < 0.001$ for ectoine, $P = 0.009$ for azelastine). Starting mean values at V1 were 9.43 ± 3.14 in the ectoine group and 9.5 ± 4.22 in the azelastine group which decreased by 45.96% to 5.10 ± 4.38 in the ectoine group and by 44.98% to 5.23 ± 4.36 in the azelastine group. Decreases of TOSS values as assessed by patients were not significant (Figure 4) (data not shown).

Palate Itching. As for nasal and ocular symptoms, a clear decrease of the symptom palate itching was observed from V1 to V2: in the ectoine group, values decreased from 2.52 ± 2.71 to 1.19 ± 1.72 ($P = 0.024$), and in the azelastine group, values decreased from 3.36 ± 2.68 to 1.5 ± 1.92 ($P = 0.018$). Values of the patients' documentation did only reach statistical significance in the azelastine group: here, the scoring decreased from 3.81 ± 2.5 to 2.15 ± 2.13 ($P < 0.001$). In the ectoine group, values decreased from 1.76 ± 2.1 to 1.67 ± 2.15 ($P = 0.854$).

Correlation of Pollen Count and Nasal Symptoms. In order to normalize the nasal symptoms (nasal constriction, rhinorrhea, and sneezing) to the pollen burden, a quotient from sum score and pollen counts was determined. Values of quotients decreased significantly from 8.97 ± 3.98 to 5.23 ± 3.59 in the ectoine group ($P = 0.002$) and from 9.73 ± 3.59 to 5.76 ± 5.26 in the azelastine group ($P = 0.011$), thus confirming the decrease of nasal symptoms during the pollen season upon treatment.

Efficacy, Tolerability, and Compliance. The physicians' assessment of efficacy of both products was similar at V2, and

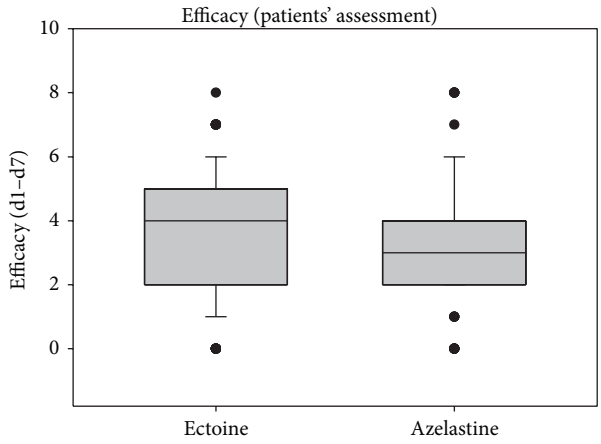

Figure 5: Patients' assessment of efficacy during study 1 from day 1 to day 7. Lines within the box mark the median; the upper and lower ends of the box indicate the 75th and 25th percentiles, respectively. Whiskers above and below the box indicate the 90th and 10th percentiles. Dots (•) represent outlying points.

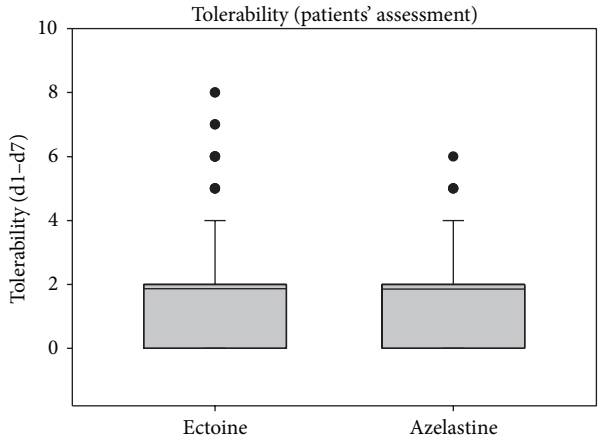

Figure 6: Patients' assessment of tolerability during study 1 from day 1 to day 7. Lines within the box mark the median; the upper and lower ends of the box indicate the 75th and 25th percentiles, respectively. Whiskers above the box indicate the 90th percentile. Dots (•) represent outlying points.

with values of 2.48 (good) in the ectoine group and 2.64 (good-satisfactory) in the azelastine group, there was no significant difference between groups. The general tolerability was assessed as very good to good in both groups (1.33 in the ectoine group and 1.45 in the azelastine group), and the compliance was comparably good (values <1) in both groups.

Values of the patients' assessments of efficacy and tolerability are shown in Figures 5 and 6. The patients' evaluations resulted in comparable values of efficacy and tolerability without statistical differences between treatment groups.

Comparison of Reduction of Symptoms between Groups. In order to calculate if reduction of symptoms from V1 to V2 was different between the treatment groups, differences of mean V1 and V2 values were compared via Mann-Whitney *U* test. As shown in Table 3, there were no statistical differences between the ectoine and the azelastine group, thus confirming that both substances worked comparably well. The same calculation was performed for the patient data. Here, no statistical difference was shown except for the symptom palate itching. Details are shown in Table 4.

Table 3: Correlation of differences of single symptoms (mean values) between V1 and V2 (based on physicians' evaluations) in study 1.

Symptom	Difference means V1-V2 ectoine	Difference means V1-V2 azelastine	*P* value
Nasal obstruction	2.43	2.91	0.546
Rhinorrhea	2.95	2.86	0.882
Sneezing	3.57	3.45	0.787
Nasal itching	3.24	3.18	0.768
Conjunctivitis	0.96	1.55	0.409
Eye itching	1.86	1.87	0.863
Tearing	1.52	0.87	0.254
Palate itching	1.33	1.86	0.426

Table 4: Correlation of differences of single symptoms (mean values) between d1 and d7 (based on patients' evaluations) in study 1.

Symptom	Difference means d1–d7 ectoine	Difference means d1–d7 azelastine	*P* value
Nasal obstruction	0.76	0.78	0.814
Rhinorrhea	1.1	0.68	0.446
Sneezing	1.0	1.6	0.54
Nasal itching	0.76	1.65	0.184
Conjunctivitis	0.72	−0.3	0.42
Eye itching	0.57	0.15	0.73
Tearing	0.09	0.35	0.826
Palate itching	0.09	1.66	0.034

Adverse Events (AEs). In total, 8 AEs occurred during the study (see Table 5). 2 AEs occurred in the ectoine group, whereas 6 AEs occurred in the azelastine group. 2 AEs in the azelastine group led to dropout of the study. No serious adverse event (SAE) occurred during the study.

3.2. Results Study 2. This was a noninterventional trial taking place at a German ear nose throat (ENT) practice starting in May 2009 and being completed in September 2009. Distribution of patients is shown in Figure 7. In total, 50 patients (33 females and 17 males) with an average age of 34 years took part in the study. Both treatment groups were homogeneous from a clinical point of view.

3.2.1. Nasal Symptoms

Nasal Obstruction. Both patient groups started with a comparable mean nasal obstruction score of 5.80 in group A and 5.64 in group B (physician's assessment). The symptom scores

Table 5: Adverse events during study 1.

	N	Description	Outcome
Ectoine group	2	#1: burning of eyes #2: itching of throat during application of products	Recovered
Azelastine group	6	#1–4: burning of eyes ($n = 4$)	1 premature determination of study due to AE
		#5: nausea #6 headache ($n = 1$)	1 premature determination of study due to AE

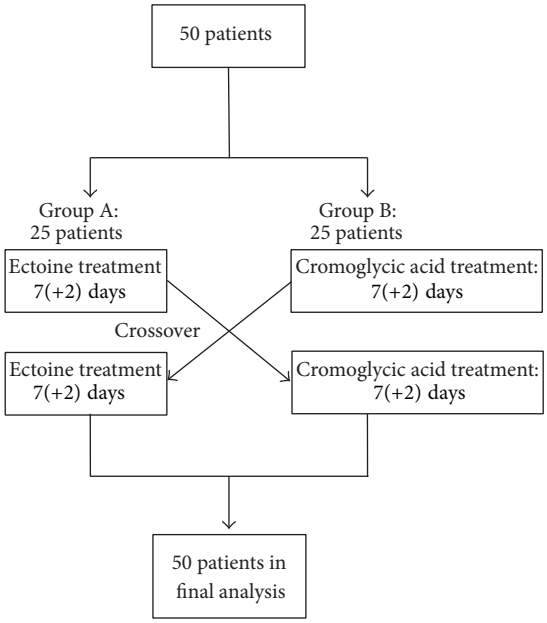

Figure 7: Patient flow during study 2.

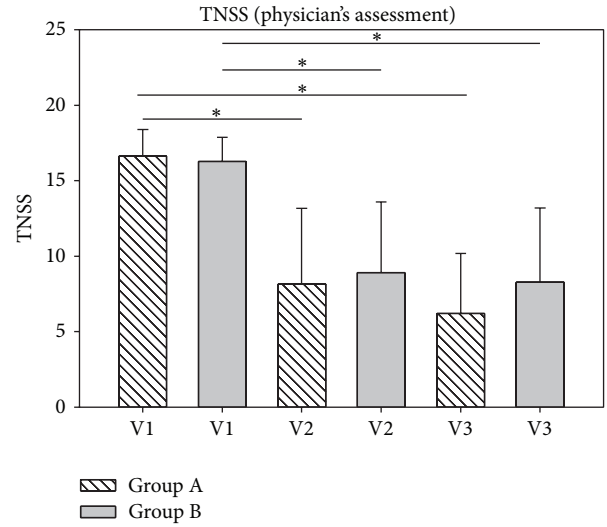

Figure 8: TNSS development according to the physician's assessment. TNSS scores decreased from 16.64 (V1, group A) to 8.16 (V2, group A) and further to 6.20 (V3, group A). In group B, values decreased from 16.28 (V1) to 8.92 (V2) and to 8.28 (V3). $^{*}P < 0.001$.

decreased to 3.2 (group A) and 3.44 (group B) after a week, and a further decrease to 2.52 (group A) and 2.92 (group B) was observed after 2 weeks. Decreases were significant in both groups with P values both for 1 week and for 2 weeks of $P < 0.001$.

Similarly, patient scores of the symptom nasal obstruction decreased from 4.08 (group A) and 3.60 (group B) on day 0 to 2.84 (group A, $P = 0.009$) and 3.24 (group B, $P = 0.464$) on day 7 and further to 2.52 (group A, $P = 0.004$) and 2.56 (group B, $P = 0.041$) on day 14.

Rhinorrhea. The symptom rhinorrhea decreased significantly ($P < 0.001$) for both groups both from V1 to V2 and from V1 to V3 according to the physician's assessment. Values decreased from 5.12 to 2.40 (V2) and further to 1.88 (V3) in group A and from 4.96 to 2.68 (V2) and to 2.76 (V3) in group B.

According to the patients' evaluation, scoring of rhinorrhea decreased from 3.12 to 2.32 (d7, $P = 0.104$) and further to 2.04 (d14, $P = 0.010$) in group A. In group B, values decrease from 3.80 to 3.08 (d7, $P = 0.115$) and further to 2.28 (d14, $P < 0.001$).

Sneezing. The symptom sneezing also decreased significantly ($P < 0.001$) from V1 to V2 and from V1 to V3 in both groups. Baseline scores from group A were 5.72 and decreased to 2.56 (V2) and further to 1.80 (V3), whereas values in group B decreased from 5.68 to 2.80 (V2) and to 2.6 (V3).

According to the patients' evaluation, scoring of the symptom sneezing decreased from 3.16 to 2.44 ($P = 0.20$) on day 7 to 2.12 ($P = 0.265$) on day 14 in group A, whereas values decreased from 4.04 to 2.64 ($P = 0.018$) on day 7 to 2.40 ($P < 0.001$) on day 14 in group B.

3.2.2. Total Nasal Symptom Score (TNSS). To reflect the development of the sum of nasal symptoms, the total nasal score (nasal obstruction, rhinorrhea, and sneezing) was calculated. Results are depicted in Figures 8 and 9. According to the physician's assessment, TNSS scores decreased significantly for both groups both from V1 to V2 ($P < 0.001$) and from V1 to V3 ($P < 0.001$). Scores assessed by patients showed that decreases in TNSS from d1 to d7 were not significant whereas significant decreases in TNSS scores from d1 to d14 were shown both for group A ($P < 0.001$) and group B ($P < 0.001$).

3.2.3. Ocular Symptoms. To investigate the development of ocular symptoms during the treatment period, the single symptoms eye itching, tearing, and conjunctivitis/redness of eyes were assessed both by the investigator and by

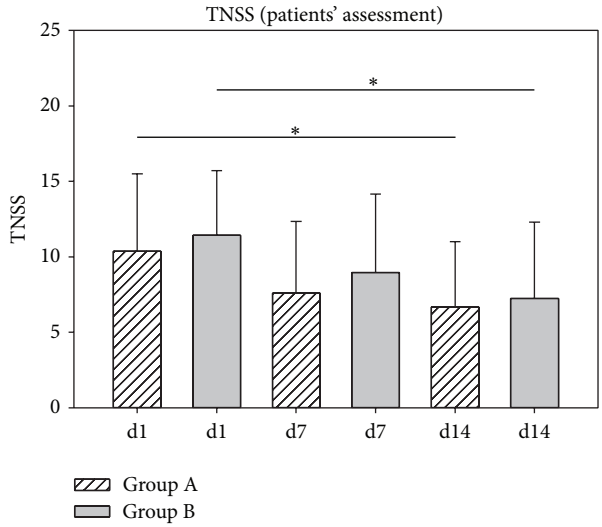

Figure 9: TNSS development according to the patients' assessment. TNSS scores decreased from 10.36 (d1, group A) to 7.60 (d7, group A) and further to 6.68 (d14, group A). In group B, values decreased from 11.44 (d1) to 8.96 (d7) and to 7.24 (d14). $^{*}P < 0.001$.

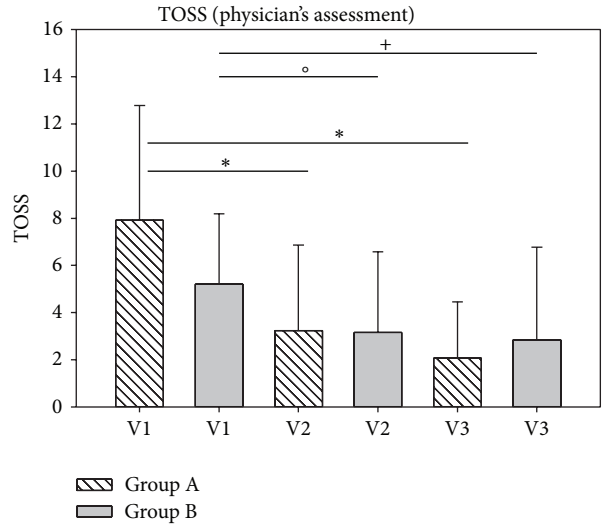

Figure 10: Assessment of sum of ocular symptoms (TOSS) according to physician's assessments in study 2. $^{*}P < 0.001$, $^{\circ}P = 0.008$, and $^{+}P = 0.003$.

the patients. Details of scores are listed in Tables 6 and 7. According to the investigator's assessment, all observed ocular symptoms improved significantly from V1 to V3 in group A, whereas only the symptoms eye itching and tearing improved significantly in group B. The patients' assessment of ocular symptoms showed that the symptoms eye itching and eye redness improved significantly in group A, whereas decreases in symptom scores from day 1 to day 14 were not significant in group B.

3.2.4. Total Ocular Symptom Score (TOSS). The development of the sum of ocular symptoms (eye itching, conjunctivitis, and tearing) as assessed by the investigator is depicted in Figure 10. It could be confirmed that ocular symptoms decreased significantly from V1 to V2 ($P < 0.001$ for group 1; $P = 0.008$ for group B) as well as from V1 to V3 ($P < 0.001$ for group 1; $P = 0.003$ for group B).

The development of total ocular symptom score as assessed by patients is shown in Figure 11. Here, a significant decrease of symptom score was only observed in group A from day 1 to day 14 ($P = 0.026$).

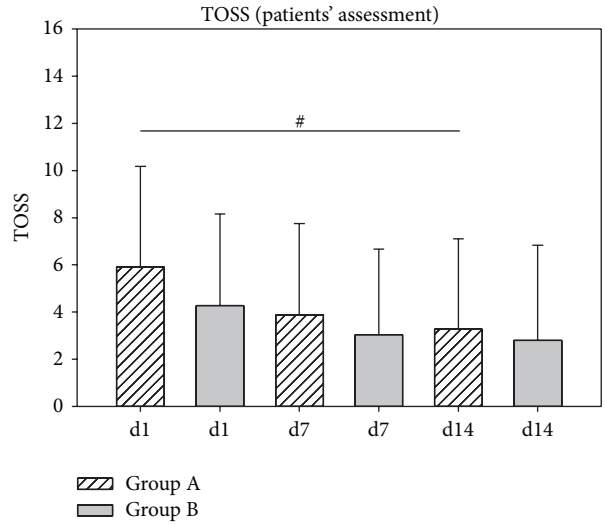

Figure 11: Assessment of sum of ocular symptoms TOSS development according to the patients' assessment during study 2. $^{\#}P = 0.026$.

Palate Itching and Turbinate Hyperplasia. In addition to nasal and ocular symptoms, the development of the symptom palate itching was determined both by the investigator and the patients. As shown in Table 8, significant decreases in the symptom palate itching were observed by the investigator from V1 to V3. In contrast, patients' assessment of this symptom showed only small decreases in this symptom which were not significant.

Additionally, the development of turbinate hyperplasia was determined by the investigator. As shown in Table 8, treatment resulted in a significant improvement of this symptom within the first week of treatment which was still significantly improved after two weeks of treatment. No differences between groups could be determined for this symptom (Table 9).

Correlation of Pollen Count and Nasal Symptoms. In order to rule out that results might be influenced by the existence of pollens, data reflecting the current pollen count were included in the analysis. The quotient of TNSS values and pollen count scores confirmed a significant decrease of TNSS values both from V1 to V2 ($P < 0.001$) and from V1 to V3 ($P < 0.001$) (data not shown).

Efficacy, Tolerability, and Compliance. According to the physicians' judgment, the efficacy of treatment was rated "good to satisfactory" with a score of 2.68 ± 1.89 (group A) and

Table 6: Development of ocular rhinitis symptoms (mean values) during study 2 (physician's assessment).

Symptom	Group	Mean V1	Mean V2	Mean V3	P value V1 versus V2	P value V2 versus V3
Eye itching	A	3.80 ± 2.29	1.68 ± 1.84	1.00 ± 1.38	$P < 0.001$	$P < 0.001$
	B	2.72 ± 2.05	1.60 ± 1.63	1.48 ± 1.78	0.046	0.003
Tearing	A	2.32 ± 1.95	0.76 ± 1.13	0.68 ± 0.95	0.002	$P < 0.001$
	B	1.32 ± 1.35	0.84 ± 1.37	0.64 ± 1.35	0.107	0.006
Conjunctivitis	A	1.80 ± 2.06	0.80 ± 1.19	0.40 ± 0.58	0.086	0.011
	B	1.12 ± 1.13	0.75 ± 1.22	0.72 ± 1.21	0.094	0.383

Table 7: Development of ocular rhinitis symptoms (mean values) during study 2 (patients' assessment).

Symptom	Group	Mean d1	Mean d7	Mean d14	P value d1 versus d7	P value d1 versus d14
Eye itching	A	2.68 ± 1.99	1.56 ± 1.58	1.48 ± 1.81	0.044	0.019
	B	2.04 ± 1.93	1.48 ± 1.61	1.28 ± 1.74	0.250	0.014
Tearing	A	1.24 ± 1.36	1.20 ± 1.26	0.92 ± 1.08	0.992	0.382
	B	1.12 ± 1.36	1.30 ± 1.24	0.68 ± 1.41	0.271	0.297
Eye redness	A	2.00 ± 2.02	1.12 ± 1.24	0.88 ± 1.20	0.150	0.003
	B	1.12 ± 1.42	0.80 ± 1.32	0.84 ± 1.31	0.337	0.292

2.96 ± 1.72 (group B) at V2 and a score of 3.12 ± 2.11 (group A) and 2.80 ± 2.06 (group B) at V3. There were no significant differences between both groups.

Similarly, the patients' assessment of efficacy was "good to satisfactory" both on day 7 (group A: 2.76 ± 1.89; group B: 2.96 ± 1.81) and on day 14 (group A: 2.56 ± 2.00; group B: 2.44 ± 2.16) without statistical differences between groups.

Following 1 week of treatment, the tolerability was judged as "very good" in group A (1.24 ± 1.30) and as "good" (2.40 ± 1.53) in group B. Following crossover of groups, tolerability of the treatment was judged as "satisfactory" (3.0 ± 2.16) in group A and as "very good" (0.88 ± 1.05) in group B. The changes of tolerability between both groups were highly significant ($P < 0.001$), thus indicating that tolerability was significantly better following a 7-day treatment with ectoine containing nasal spray in comparison to 7-day treatment with cromoglycic acid nasal spray.

Those differences in tolerability scoring were also clearly visible in the patients' assessment of tolerability. Whereas tolerability was judged as "very good" (1.30 ± 1.48) within the first days of treatment in group A, scoring for the second week decreased to a mean score of 2.65 ± 1.89 corresponding to "good to satisfactory." Group B showed the opposite development with a tolerability scoring of 2.35 ± 1.68 ("good") within the first 7 days which improved to a mean score of 0.97 ± 1.24 ("very good") within the second half of the treatment. Details of the patients' tolerability assessment are depicted in Figure 12. In summary, patients judged the tolerability significantly better under treatment with ectoine containing nasal spray compared to treatment with cromoglycic acid nasal spray ($P < 0.001$).

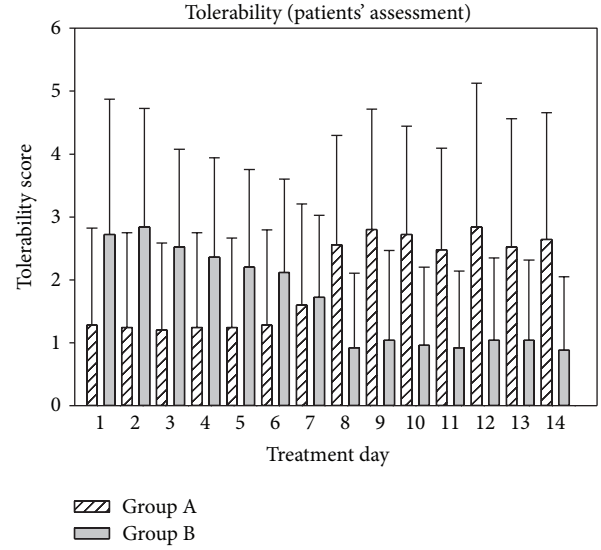

Figure 12: Patients' assessment of tolerability of treatments during study 2.

The compliance was assessed as very good by the physician, and values were not statistically different between groups (see Table 10).

Adverse Events (AEs). During the study, no serious adverse events (SAEs) occurred. No adverse events were observed during treatment with ectoine containing nasal spray. In contrast, 13 patients complained about a burning sensation during treatment with cromoglycic acid nasal spray. One

Table 8: Physician's assessment of palate itching and turbinate hyperplasia during study 2.

Symptom	Group	Mean V1	Mean V2	Mean V3	*P* value V1 versus V2	*P* value V1 versus V3
Palate itching	A	2.32 ± 2.54	1.12 ± 2.05	0.84 ± 1.52	0.054	0.003
	B	2.20 ± 2.36	1.24 ± 2.13	0.92 ± 1.80	0.048	0.002
Turbinate hyperplasia	A	4.68 ± 1.25	3.56 ± 1.08	3.76 ± 1.69	<0.001	0.007
	B	4.80 ± 1.15	3.84 ± 1.25	3.56 ± 1.19	0.005	0.001

Table 9: Patients' assessment of palate itching during study 2.

Symptom	Group	Mean d1	Mean d7	Mean d14	*P* value d1 versus d7	*P* value d1 versus d14
Palate itching	A	1.80 ± 2.35	1.56 ± 1.87	1.12 ± 1.62	0.962	0.425
	B	1.56 ± 1.89	1.24 ± 2.09	0.92 ± 1.78	0.053	0.035

Table 10: Compliance scores (assessed by the investigator) following 1 week (V2) and 2 weeks (V3) of treatment during study 2.

Group	Mean V2	Mean V3
A	1.28 ± 1.24	1.12 ± 0.97
B	0.88 ± 0.93	1.16 ± 1.03

patient complained about displeasing smell, and another patient complained about dehydration effect. The correlation between the observed AEs (burning sensation, displeasing smell, and dehydration effect) was judged as probable.

4. Conclusions

The current studies investigated the efficacy and safety of ectoine containing nasal spray and eye drops in comparison with commonly applied pharmacological treatments of allergic rhinitis. In two noninterventional trials, ectoine products were compared with either azelastine or cromoglycic acid containing products. Although this paper covers results of two separate studies, they were summarized in one document as the indication was very similar and both studies aimed to compare ectoine containing products with other topical medications. As the study with cromoglycic acid was one of the first studies with ectoine products, dosage was slightly higher than in the study comparing ectoine and azelastine products. As a placebo-controlled, randomized trial with ectoine nasal spray and eye drops which was conducted after the study 2 had confirmed that the dose of 4 uses per day was sufficient to show significant superiority over placebo treatment, this dosage was chosen in study 1 [14]. Both studies demonstrated that allergic rhinitis can be successfully and safely treated with ectoine containing products, thus offering a potential new treatment strategy for allergic rhinitis sufferers.

Both studies were intentionally designed as noninterventional studies based on German law. Although this study design forbids randomization of patients, use of placebo, and blinding of study medication, it still reflects the most realistic standard clinical practice. Thus, patients were included independently on their prior medication and no washout period had to be kept. In order to ensure homogeneity of patients, all had to show a certain degree of symptoms at inclusion reflected by a minimum of TNSS values. Additionally, symptom scores were correlated with pollen count scores in order to include objective measures into the analysis. Importantly, sites specialized in the area of ear nose throat practice were chosen to warrant a very precise assessment of symptoms by specialized physicians and to have a homogeneous patient population. Although we believe that valuable results can be drawn from noninterventional trials, one drawback of this study design is the fact that one cannot include a placebo group into the study population. On the other hand, it has been demonstrated that double-blind randomized placebo-controlled trials clearly have their limitations and disadvantages and that particularly patients' awareness of a placebo arm can lead to modifications of results due to patients' expectations and interpretations [15]. This was confirmed by a comparison of open and controlled study designs in neuroleptic studies, indicating that results of well performed open studies earn more attention [16].

In study 1, it was shown that both the ectoine and the azelastine products resulted in a clear decrease of symptoms of allergic rhinitis over the study period of 7 days. According to the physicians' evaluation, the symptoms nasal obstruction, rhinorrhea, sneezing, nose itching, conjunctivitis (azelastine group only), eye itching, and tearing were significantly reduced. The mean decrease of TNSS was −58.85% in the ectoine group and −57.11% in the azelastine group, thus demonstrating a strong clinical relevance. Similarly, mean decreases in TOSS were −45.96% in the ectoine group and −44.98% in the azelastine group and therewith reflect strong clinical relevance, too.

Study 2 also demonstrated a significant decrease of symptom scores upon treatment: within the first week of the study, TNSS values decreased by −50.96% (group A) and by −45.21% (group B), and decreases within the entire study period of 2 weeks were −62.74% (group A) and −49.14% (group B) according to the physician's assessment. Nasal obstruction is often caused by an enlargement of the nasal turbinates which are located on the lateral walls on each side of the nose.

Thus, the significant improvement of turbinate hyperplasia as assessed by the physician underlined the efficacy of both treatments in reducing nasal obstruction.

In comparison to the physicians' assessment of symptoms, generally less strong symptom decreases were observed by patients. This might be most likely due to the fact that starting symptom values as assessed by patients themselves were lower than the physicians' values. This in turn is at least partly accredited to the fact that patients' first assessments of symptoms were documented at the end of the first treatment days whereas physicians documented baseline symptoms during the first site visit prior to the start of treatment. A recent placebo-controlled study in an environmental challenge chamber showed that 3 hours after application of the ectoine nasal spray and eye drops the symptoms were decreased by ~20%. This decrease reflects roughly the difference between the first assessment by the physicians and the first patient diary entry [14]. In addition, physicians are able to carry out ranking of symptoms based on their experience with many patients; thus, their judgment might be considered more objectively. On the other hand, symptoms such as itching of eyes, nose, and palate cannot be measured with a scientifically valid method and are thus prone to personal perception and difficult to be assessed by physicians together with patients. Taken together, an overestimation by the physician or an underestimation by the patients is not likely.

In study 1, the patients' assessment showed that for the azelastine group, decreases were significant for the symptoms nasal obstruction, sneezing, and nasal itching. For the ectoine group, values decreased significantly in the symptom nasal obstruction, whereas a clear but not significant decrease in the symptoms nasal itching, sneezing, and rhinorrhea was observed. In total, decreases of TNSS were −24.68% in the ectoine group and −35.26% in the azelastine group, thus confirming a clinical relevance of the treatment. Clear decreases in the ocular symptoms tearing and eye itching were assessed by patients of both groups; however, values did not reach significance. The symptom conjunctivitis was clearly (but not significantly) decreased in the ectoine group, whereas it became slightly worse in the azelastine group. In total, TOSS as assessed by patients decreased by −19.57% in the ectoine group and by −11.81% in the azelastine group.

Although no ocular treatment was applied in study 2, ocular symptoms of allergic rhinitis clearly improved upon treatment with ectoine or cromoglycic acid nasal spray. According to the physicians' assessment, TOSS values decreased by −59.09% in group A and by −39.32% in group B after 1 week of treatment and by −73.74% in group A and by −45.47% in group B after 2 weeks of treatment. It is not surprising that local and nonpharmaceutical nasal treatment might also influence ocular symptoms as recent studies suggest a crosstalk between the nose and eyes. The mechanism of the influence of symptoms via the nasolacrimal duct is not fully understood but thought to be via a mucosal connection, possibly via a nose-eye reflex.

The patients' assessment of ocular symptom development in study 2 confirmed a significant decrease in the symptom eye itching after two weeks' treatment in both groups. A significant reduction of the symptom eye redness was observed in group A but not in group B. However, as ocular symptom scores were generally rather small in this study and treatment aimed mainly to reduce nasal symptoms, further studies will be needed to evaluate the efficacy of treatments on ocular symptoms. Interestingly, another published study with azelastine eye drops, cromoglycate eye drops, or placebo eye drops showed superiority of both active treatments versus placebo without significant differences between the two active treatments [17], and a future study comparing treatment with ectoine eye drops only with other pharmacological eye drop formulations would be desirable.

The current studies both showed that ectoine nasal spray and eye drops can safely be applied in patients with allergic rhinitis. Patients judged the tolerability of the products as similarly good as the azelastine products and significantly better than cromoglycic acid nasal spray, and the very low numbers of AEs reflected a very good safety profile of the used treatments.

The crossover design of study 2 bears difficulties as no washout period between the crossover was carried out. However, as symptoms were assessed on a daily basis, effects following one treatment only (after one week) can be analyzed separately from the results following two treatments. As clear improvements of symptoms were already observed after one week of treatment, this time span seems sufficient to evaluate effects of either treatment A or B.

As the efficacy and safety of azelastine has been studied in a huge range of clinical trials during the last decades, one can use historical data to bring the results of the current study into a broader context (for results from comparator studies see Table 11). In addition, results from placebo groups of comparator studies can be used in order to rank the current results. Thus, comparable data confirmed a superiority of azelastine versus placebo treatment, and values indicate that effects of azelastine are usually about 2-3-fold higher than placebo. However, comparing the actual values of the current study with other studies is rather difficult as design (e.g., randomized versus nonrandomized, placebo-controlled versus not placebo-controlled, differences in length of treatment, and differences in scaling of symptoms) and dose (azelastine concentration and number of daily applications) of available studies differs enormously. In addition, most studies used nasal spray only and assessed solely nasal symptoms, whereas the current study is one of few studies acknowledging also ocular symptoms of allergic rhinitis. Taken together, results of the current study 1 showed that effects of ectoine containing products are almost comparable with those of azelastine, a well-studied drug, which has a proven superiority to placebo treatment and is commonly prescribed against allergic rhinitis.

Cromoglycic acid has been a common drug in treating allergic rhinitis, and although it is thought not to be as effective as intranasal steroids or antihistamines, it has been shown to reduce both nasal and ocular symptoms and it is therefore a reasonable therapy option. In particular, its good safety profile makes it an interesting treatment option both for children and pregnant women. As no published studies were identical with the current study design (study

Table 11: Comparison of the current study 1 with other azelastine studies.

Study	Treatment	% improvement from baseline to end of treatment	
		TNSS	TOSS
Current study 1[1]	Ectoine	58.85	45.96
	Azelastine	57.11	44.98
Current study 1[2]	Ectoine	24.68	19.57
	Azelastine	35.26	11.81
Lumry et al. 2007 (study 1)[3] [18]	Azelastine	14.1	n.d.
	Placebo	4.5	n.d.
Lumry et al. 2007 (study 2)[3] [18]	Azelastine	22.1	n.d.
	Placebo	12.0	n.d.
Shah et al.[4] [19]	Azelastine 0.1%	24.4	n.d.
	Azelastine 0.15%	29.7	n.d.
	Placebo	12.0	n.d.
Howland et al.[5] [20]	Azelastine 0.15%	19.3	16.7
	Placebo	11.4	6.0
Van Bavel et al.[6] [21]	Azelastine 0.15%	18.7	n.d.
	Placebo	10.5	n.d.
Falser et al.[7] [22]	Azelastine	83.56*	n.d.
	Levocabastine	70.42*	n.d.
Charpin et al.[8] [23]	Azelastine	60.2	65.0
	Cetirizine (tablet)	63.3	60.8

[1]Physicians assessment; [2]patients' assessment; [3]one spray of 0.1% azelastine nasal spray per nostril twice daily for 14 days; [4]two sprays of 0.1% or 0.15% azelastine nasal spray per nostril twice daily for 14 days; [5]two sprays of 0.15% azelastine nasal spray per nostril once daily for 14 days; [6]two sprays of 0.15% azelastine nasal spray per nostril once daily for 2 weeks; [7]azelastine 1.12 mg/day and levocabastine 0.4 mg/day nasal spray administered twice daily for 4 weeks, *TNSS: sneezing, nasal itching, and rhinorrhoea; [8]one spray of 0.1% azelastine nasal spray per nostril twice daily for 14 days. n.d.: not determined.

2) in terms of treatment duration and analysis of end points, only general comparisons to cromoglycic acid studies can be drawn. Several studies have confirmed that cromoglycic acid is superior to placebo in patients with allergic rhinitis [24]. Thus, Schuller and colleagues [25] investigated the efficacy of Cromolyn sodium in comparison to nedocromil sodium and placebo. Over a treatment period of 8 weeks, it was demonstrated that Cromolyn resulted in a clear improvement of nasal symptoms, particularly in the symptom "stuffy nose." A further placebo-controlled study confirmed that cromoglycate acid nasal spray provided significant relief of nasal symptoms within 2 weeks of treatments which were significant for the symptoms sneezing and nasal congestion and clearly visible but not significant for the symptoms rhinorrhea and nasal pruritus [26].

Taken together, ectoine containing nasal spray and eye drops have been demonstrated to be promising alternatives to pharmacological drugs with both good efficacy and a very good safety profile. As the ectoine nasal spray and eye drops act purely physically on the nasal and ocular mucosa, it makes those products particularly interesting for patients with reservations about pharmacological therapy. An additional study in children and adolescents with seasonal allergic rhinitis (data not yet published) has confirmed the safety of ectoine containing nasal spray and eye drops in the pediatric population. Further studies should be undertaken to further investigate the onset of action and compare it to commonly applied pharmacological drugs. Quick relief of symptoms is crucial for patients and understood to be advantageous when comparing, for example, azelastine with intranasal corticosteroids [27] and should therefore be assessed for the ectoine products. A controlled, ramdomised study which was carried out using a controlled environmental exposure chamber showed a quick onset of action of ectoine nasal spray and eye drops and confirmed the efficacy in reducing both nasal and ocular symptoms [14]. Additional studies applying the ectoine eye drops only are needed to further elucidate their impact on ocular symptoms during allergic rhinitis.

Conflict of Interests

Dr. Nina Werkhäuser and Dr. Andreas Bilstein are employees of bitop AG, a company where medical devices, including the ectoine nasal spray and eye drops, are developed and registered. bitop AG sponsored the trials discussed in this paper. Dr. Uwe Sonnemann received sponsorship by bitop AG to conduct the studies.

Acknowledgment

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Review Article

Thermal Water Applications in the Treatment of Upper Respiratory Tract Diseases: A Systematic Review and Meta-Analysis

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Background. Thermal water inhalations and irrigations have a long tradition in the treatment of airway diseases. Currently there exists no systematic review or meta-analysis on the effectiveness of thermal water treatment in upper respiratory tract diseases. *Methods*. A systematic search in the databases of MEDLINE, EMBASE, CENTRAL, ISI Web of Science, and MedPilot was accomplished. *Results*. Eight evaluable outcome parameters from 13 prospective clinical studies were identified for 840 patients. Mucociliary clearance time improves significantly ($P < 0.01$) for the pooled thermal water subgroup and the sulphurous subgroup after 2 weeks (−6.69/minutes) and after 90 days (−8.33/minutes), not for isotonic sodium chloride solution (ISCS). Nasal resistance improved significantly after 2 weeks (Radon, ISCS, and placebo), after 30 days (sulphur and ISCS), and after 90 days (sulphur). Nasal flow improved significantly with the pooled thermal water, radon alone, and ISCS subgroups. For the IgE parameter only sulphurous thermal water ($P < 0.01$) and ISCS ($P > 0.01$) were analyzable. Adverse events of minor character were only reported for sulphurous treatment (19/370). *Conclusion*. Thermal water applications with radon or sulphur can be recommended as additional nonpharmacological treatment in upper airway diseases. Also in comparison to isotonic saline solution it shows significant improvements and should be investigated further.

1. Introduction

Upper airway diseases compass acute and chronic conditions. In this study, we focus on recurrent upper respiratory tract infections (RURT), allergic rhinitis (AR), nonallergic rhinitis (NAR), and acute and chronic rhinosinusitis (ARS/CRS) with and without nasal polyps. These disorders are extremely common and present in all ages, all ethnic populations, and all countries [1]. Apart from their high socioeconomic burden [2], "comorbidities are common and increase the complexity of the management and costs" [1].

Rhinitis is a symptomatic inflammation of the nasal mucosa including nasal symptoms like rhinorrhea, nasal obstruction, nasal itching, and sneezing [3]. The most common form of noninfectious rhinitis is AR with immunoglobulin E- (IgE-) mediated immune response after allergen exposure [1]. Nonallergic rhinitis shows periodic or perennial symptoms, which are not IgE-dependent such as infectious or vasomotor rhinitis [4]. Infectious rhinitis has either viral, bacterial, or other infectious agents origin [3] and affects millions of people annually [5].

Rhinitis and sinusitis mostly coexist and have been proposed as *rhinosinusitis* [6]. The European Position Paper on Rhinosinusitis and Polyps *EPOS* 2012 [7] defines rhinosinusitis as an inflammation of the nose and paranasal sinuses characterised by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge. Further, either endoscopic or CT proof is obligatory. Acute and chronic rhinosinusitis are distinguished in length of illness and grade of decay of symptoms. It is characterised by a duration of more than 12 weeks without complete resolution of symptoms and affects approximately 5–15% of the general population [7–9].

State-of-the-art documents ARIA [3] and EPOS [7] provide evidence-based treatment guidelines where "anti-inflammatory medication represents the first-line treatment"

[10]. Along with steroid/pharmacological treatment, EPOS [7] recommends nasal saline irrigation as additional first-line treatment in acute and chronic rhinosinusitis and after sinonasal surgery [7]. Some reviews also state nasal irrigation as adjunctive treatment for allergic rhinitis, acute upper respiratory tract infections, and rhinitis of pregnancy [11–13]. Data for nasal inhalation is limited; EPOS [7] mentions inhalation treatment only in acute rhinosinusitis without evidence. Other guidelines from the German Society of Otorhinolaryngology have an open suggestion for inhalation in rhinosinusitis for symptomatic relief [14].

The current treatment regimens for CRS and AR are effective in the majority of patients, but there are a number of patients still suffering from symptoms [10]. Especially under chronic conditions with long-term drug consumption like glucocorticosteroids, many patients hesitate to take medicine. A publication by Kaschke points out that 64% of AR patients have steroid phobia [15]. It is observed that more and more patients inquire about nonpharmacological therapy approaches in the treatment of rhinosinusitis [16]. Possible approaches besides the proven saline irrigation could be the use of thermal water irrigations and inhalations.

Concerning medical spending, a study by Bhattacharyya points out that the annual costs for medication to treat CRS with intranasal steroids, nonsedating antihistamines, and antibiotic therapy were $213, $227, and $335 in 2003 [2]. Averaging the annual cost of sinus medications including over-the-counter remedies, nasal steroid sprays, and antibiotics, the calculations of Gliklich and Metson result in $1220 per patient [17].

In the United States the medical spending for AR almost doubled from $6.1 billion in 2000 to $11.2 billion in 2005 [18]. Nasal saline irrigation in AR patients helps to reduce medicine consumption by an average of 2.99% [19]. Thermal water applications present an inexpensive nonpharmacological adjunction as well and could therefore further reduce the medicine consumption and costs.

Thermal water treatment belongs to Balneology. Balneology (lat. *balneum*: bath) is the science of natural curative waters, curative gases, and peloides and their use in the treatment of diseases not only as baths, inhalations, or irrigations but also as drinking cures or mud packs [20]. Thermal inhalations and irrigations are century-old practices and already the Romans appreciated the health-promoting effects of different thermal sources.

According to German regulations, natural curative waters are characterised by a minimum content of 1 g dissolved minerals per liter. The designation "thermal water" requires a temperature of a minimum of 20°C when emerging from the spring [21]. Among the many different types of thermal waters our focus is on sulphurous and radon waters. Sulphurous water and its therapeutical use belong to the oldest forms of balneology. It is said to break disulphide bonds of the mucin and activate breathing and blood circulation and helps to reduce inflammation [20, 22, 23]. Radon is a radioactive gas which emits alpha rays. Its very low content in thermal sources has biopositive effects which stimulate cellular activity [24].

Several studies show significant results in thermal water treatment with inhalation or irrigation treatment [25–29]. Up to now no systematic review or meta-analysis on thermal water application in upper airway diseases exists.

2. Materials and Methods

A comprehensive search in the databases of MEDLINE (Medical Literature Analysis and Retrieval System Online), CENTRAL (Cochrane Central Register of Controlled Trials), EMBASE (Excerpta Medica Database), Web of Science, and MedPilot was conducted. In the systematic search the terms "thermal water," "Spa therapy," "thermal water inhalations," and "Spa treatment" were combined with the terms "rhinitis," "rhinosinusitis," "allergic rhinitis," "chronic rhinitis," and "nasal irrigation" with the Boolean operator "and" in all fields. Furthermore, the terms "Radon Spa therapy," "Balneotherapy," "Sulphurous Water," "Bromide Water," "Iodic Water," "Salty Water," and "Radon Water" were linked through "and" with "rhinitis" or "rhinosinusitis." No limitation was made in language, publication date, and duration of the study or the demographic data of patients. Literature published up to and including 27 February 2014 was included.

The inclusion criteria for this meta-analysis were as follows: clinical studies conducted with thermal water for the upper airway diseases, allergic, chronic, or acute rhinosinusitis, at least three or more points on the modified JADAD scale [30], and the presence of the complete statistical data sets consisting of mean deviation, standard deviation, and sample size (if appropriate, calculation using standard error or the upper and lower quartile) at defined follow-up time points.

The following outcome parameters were examined in this study: the mucociliary clearance time (MCT), nasal respiratory flow (Flow), nasal resistance (R), immunoglobulin values A, E, G, and M, and adverse events (AE).

3. Data Collection and Analysis

The search described above initially resulted in 2113 matches. Duplicates and studies that were either nonclinical or disease-specific not relevant for this analysis were excluded so that the abstracts of 50 remaining studies were examined. Another 15 could be excluded after the perusal of the abstract. 35 studies were investigated in full-text which led to the exclusion of another seven studies due to divergent treatment modalities [22, 31, 32], mismatching disease patterns [33, 34], unavailability [35], or the conduction of the study with animals [36].

The remaining 28 articles were evaluated by two independent reviewers with the modified Jadad Scale by Oremus et al. [30]. It amplifies the original 3-item Jadad Scale [37] consisting of randomization, blinding, and study dropouts by adding inclusion/exclusion criteria, side effects, and statistical methods. Additionally, it features two bonus points for appropriate randomisation method and double-blinding. If this does not apply, these points are deducted. The minimum score is 0 points; the maximum score is 8 points.

We set a minimal score of at least three points to establish a qualitative homogeneity essential for our meta-analysis. Thus another 15 studies were excluded [38–52] and 13 studies remained for our analysis. Four of those are in Italian and nine in English.

The different thermal waters used in the studies included were pooled concerning their different substances. This resulted in two main groups: sulphurous water with nine studies [25–28, 53–57] and radon water with two studies [29, 58]. Salt-bromine-iodine water [59] and hypermineral chloride sodium water [60] were used once. We also pooled a common thermal water group to compare it to isotonic sodium chloride solution (ISCS) and placebo. Figure 1 shows a flowchart of the literature identification process. Tables 1 and 2 show the included studies in a systemic overview.

3.1. Statistical Methods. The statistical calculations were performed using the statistic software Comprehensive Meta-Analysis Version 2.2.064 (Biostat, Inc.). The study values for the identified outcome parameters were sorted according to the time of measurement (baseline, 12 days, 2 weeks, 30 days, and 90 days). Studies with the same parameter and different (follow-up) times of measurement, of 12 and 14 days, were combined to one-time measurement (2 weeks). All points of measurements of the different studies were summarised and analysed using the random-effect model. The mean and, respectively, the standard error of the mean are depicted in the figures (Figures 2–5). In the following analysis we assumed a significance if the P value was less than or equal to 5% ($P \leq 0.05$). For clarification, significant improvements/changes are marked with * in Tables 4–7. For some identified parameters data were available from one study only and therefore had to be excluded from the meta-analysis but are, for the sake of completeness, included in the figures with an interrupted line.

4. Results

In total, 13 studies published between 1998 and 2013 have been included in this analysis.

All forms of applications were pooled. The specific form of application and the number of patients is depicted in Table 3. An aerosol therapy is categorised under inhalations. Altogether, 430 patients received irrigations and 557 patients inhalations.

4.1. Study Design. All studies feature a prospective study design. Five studies are randomised, controlled, and double-blind [25–28, 54]; other three studies are randomised and controlled [56, 59, 60]; one study is controlled and double-blind [29]; one is only double-blind [55]. The remaining studies are not randomised, blinded, or controlled [53, 57, 58]. ISCS was used for the control groups, drinking water or distilled water for placebo groups. The duration of the studies varies between 12 days and 6 months.

4.2. Selection of Patients. A total number of 840 patients aged between 2 and 100 years took part in these studies, 510 of them received an application with thermal water, 285 were treated with ISCS [25–28, 56, 59], 20 inhaled drinking water [29], and 25 inhaled distilled water [54].

4.3. Mucociliary Clearance Time. MCT was examined in seven studies with 422 patients in total (Table 4). Thermal water (radon, sulphur, and salt-bromine-iodine) applications showed a significant improvement of MCT compared to baseline at both points of measurement (Figure 2). The measurement for ISCS compared to baseline showed no significance after two weeks but after 90 days in the followup. Only one study was conducted with placebo which did not show any significance neither did radon water applications ($P = 0.059$). Sulphurous water applications showed significant lower values after two weeks compared to the baseline value ($P < 0.01$) and are also significant lower after 90 days compared to the baseline values ($P < 0.01$).

In an internal comparison between the ISCS group and the sulphurous water group we had nonsignificant initial situations ($P = 0.211$), but after 2 weeks and 90 days the outcome differed significantly ($P < 0.01$). The ISCS and the radon group already differed significantly in baseline values ($P < 0.05$) and had almost parallel curves.

Figure 2 illustrates all values calculated in the meta-analysis and noted in Table 4.

4.4. Nasal Resistance. Nasal resistance was measured in six of the included trials with a total number of 347 patients (Table 5). Thermal water treatment was not significant after two weeks but after 30 and 90 days compared to baseline. ISCS treatment showed significance after two weeks and 30 days compared to baseline, but after 90 days there was no significance. This graph (Figure 3, ISCS graph) showed a very erratic curve due to the very heterogeneous study design and the different points of measurement of the three studies included. Both the treatment with placebo and the treatment with radon water showed significance after two weeks. The treatment with sulphurous water showed significance after 30 ($P < 0.01$) and 90 days ($P < 0.05$) but not after two weeks ($P = 0.118$).

4.5. Nasal Flow. The nasal flow was specified in three of the included trials with 117 patients (Table 6). All of these patients received inhalation and aerosol therapy. Figure 4 shows all included studies, using this outcome parameter, the dotted lines for placebo and sulphur indicate single studies. Compared to baseline, the combined thermal water group ($P < 0.05$), as well as the radon ($P < 0.05$) and the sulphurous water group ($P < 0.01$), showed significant improvement after two weeks, whereas drinking water application ($P = 0.425$) showed no improvement or significance.

4.6. Immunoglobulins E, A, G, and M. Immunoglobulin concentrations in the blood were examined in two of the included trials [25, 26] with a total number of 180 patients. Both studies compared sulphurous water treatment to ISCS treatment. Distribution of the number of patients (90 patients per group) was equal in both studies.

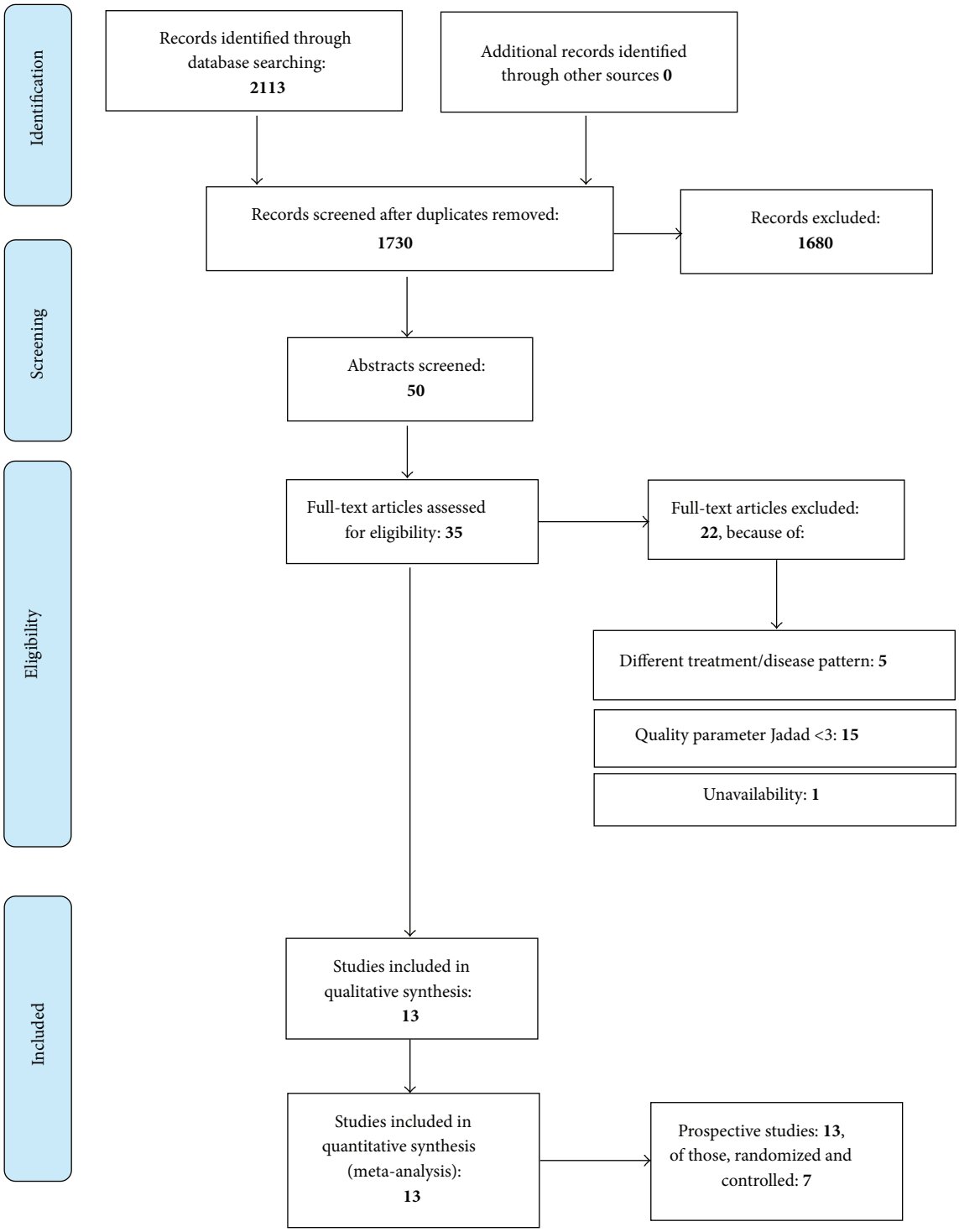

Figure 1: Flow chart. Source: PRISMA 2009 Flow chart [61], augmented with exclusions and types of included studies.

4.7. IgE. Figure 5 illustrates the meta-analysis outcome for IgE. Both groups started from a comparable initial position. Sulphurous water treatment rose significantly ($P < 0.01$) at both measurement points 12 and 90 days compared to baseline in contrast to ISCS treatment which was neither significant after 12 days ($P = 0.442$) nor after 90 days ($P = 0.567$).

4.8. IgA, G, and M. No significant differences could be revealed in the analysis of the immunoglobulins A, G,

Table 1: Included studies.

Author	Year	Study design	Thermal water/control	Application	Adverse events	MCT	Flow	Resistance	IgA	IgE	IgG	IgM
De Luca et al. [53]	2006	Prospective, nonrandomized, noncontrolled	Sulphur/—	Inhalation 1x daily 10 min for 12 days	X	X						
Marullo and Abramo [29]	2000	Prospective, nonrandomized, controlled, double-blind	Radon/Tap water	Inhalation 10 min, Aerosol 10 min, 1x daily for 12 days	X	X	X	X				
Miraglia del Giudice et al. [60]	2011	Prospective, randomized, controlled	Hypermineral chloride sodium water/ISCS	Aerosol 15 days a month for 3 months/ ISCS daily for 3 months	X							
Ottaviano et al. [27]	2012	Prospective, randomized, controlled, double-blind	Sulphur-Arsenic-Ferrous water diluted to 10% solution with distilled water/ISCS	Irrigation 1x daily 20 mL for 1 month	X			X				
Ottaviano et al. [28]	2011	Prospective, randomized, controlled, double-blind	Sulphur-Salt-Bromine-Iodine/ISCS	Irrigation 4x daily 5 mL for 1 month	X			X				
Passali et al. [58]	2013	Prospective, nonrandomized, noncontrolled	Radon/—	Inhalation, Aerosol, each 10 min 1x daily for 14 days	X	X	X	X				
Passali et al. [59]	2008	Prospective, randomized, controlled	Salt-Bromine-Iodine/ISCS	Inhalation 10 min, Aerosol 10 min, Irrigation 5 min 1x daily for 14 days	X	X						
Passariello et al. [57]	2012	Prospective, nonrandomized, noncontrolled	Sulphate-Sodium-Chloride/—	Aerosol 15 min 1x daily for 15 days	X							
Salami et al. [26]	2010	Prospective, randomized, controlled, double-blind	Sulphur/ISCS	Inhalation, 10 min Irrigation 6 min 1x daily for 12 days	X	X		X	X	X	X	X
Salami et al. [25]	2008	Prospective, randomized, controlled, double-blind	Sulphur/ISCS	Inhalation 10 min 1x daily for 12 days	X	X			X	X	X	X
Staffieri et al. [56]	2008	Prospective, randomized, controlled	Sulphur-Arsenic-Ferrous water diluted to 10% solution with distilled water/ISCS	Irrigation 4x daily 20 mL for 6 months	X							
Staffieri and Abramo [55]	2007	Prospective, nonrandomized, noncontrolled, double-blind	Sulphur-Arsenic-Ferrous water/—	Inhalation, Aerosol each 10 min, 1x daily for 12 days	X	X	X	X				
Staffieri et al. [54]	1998	Prospective, randomized, controlled, double-blind	Sulphur-Salt-Bromine-Iodine/distilled water	Inhalation, 10 min, Aerosol 10 min 1x daily for 12 days	X							

Table 2: Systematic presentation of the studies included.

Author	Year	Jadad score	Number of patients thermal water/control	Age span/average age	Duration of treatment	Measurement time points	Inclusion criteria	Exclusion criteria
De Luca et al. [53]	2006	3	40/—	74–100/86,5	12 days	Baseline Follow-Up: Day 12	(i) Presence of chronic rhinitis or chronic rhinosinusitis (ii) Allergic or vasomotor nasal hyperactivity (iii) Chronic laryngitis (iv) Chronic pharyngitis	(i) Acute pathologies in ENT region (ii) Steroids, mucolytics, antihistamine, NSAIDs, vasoconstrictive drugs or antibiotics in the last 30 days (iii) Autoimmune disease (iv) Patients with malignant neoplasms, surgical intervention, and/or radio chemotherapy
Marullo and Abramo [29]	2000	3	27/20	15–81/52.4	12 days	Baseline Follow-Up: Day 12	(i) At least 3 confirmed episodes of sinonasal infection in the previous 12 months (ii) Evidence of chronic sinonasal inflammation at objective otorhinolaryngologic evaluation	(i) Vasoconstrictive drugs, local and systemic steroids, NSAIDs, antihistamine, and mucolytics in the last 2 months (ii) Patients who for various reasons were not able to ensure the conclusion of the study (iii) Patients who present anterior rhinoscopy
Miraglia del Giudice et al. [60]	2011	5	20/20	6–14/	15 days a month for 3 months	Baseline Follow-Up: Week 12, 14	(i) Age from 6 to 14 (ii) History of seasonal moderate to severe allergic rhinitis for at least 2 years (iii) Positive skin prick test to *Parietaria* pollen (iv) History of mild intermittent asthma	(i) Antihistamines, intranasal, bronchial, or systemic corticosteroids, cromolyn sodium, and leukotriene modifiers in the previous 6 weeks (ii) sinus and/or upper or lower respiratory tract infection, persistent asthma, nasal surgery within the last year, respiratory tract abnormalities, and systemic diseases
Ottaviano et al. [27]	2012	5	35/35	18–65/ not specified	12 weeks	Baseline Follow-Up: Day 30, 90	(i) Age from 18 to 65 (ii) Nonallergic chronic rhinitis (iii) Cigarette smoking habit for at least 5 years	Autoimmune diseases, cystic fibrosis, and diabetes
Ottaviano et al. [28]	2011	8	40/40	18–65/ not specified	1 month	Baseline Follow-Up: Day 30	(i) Age from 18 to 65 (ii) Nonallergic chronic rhinosinusitis	Autoimmune diseases, cystic fibrosis, and diabetes
Passali et al. [58]	2013	3	33/—	12 years and older/ not specified	14 days	Baseline Follow-Up: Day 14	(i) Nasal obstruction evaluated by a 10-point Visual Analog Scale (1: nasal airways completely free; 10: nasal airway completely blocked) higher than 7 in the previous 2 months (ii) Chronic rhinosinusitis, persistent allergic rhinitis, vasomotor rhinitis with inferior turbinate hypertrophy	(i) Acute viral rhinitis (ii) Obstructive polyposis (iii) Nasal steroid, vasoconstrictive drug therapies or systemic NSAIDs, oral steroids and mucolytic treatment in the previous 2 months

Table 2: Continued.

Author	Year	Jadad score	Number of patients thermal water/control	Age span/average age	Duration of treatment	Measurement time points	Inclusion criteria	Exclusion criteria
Passali et al. [59]	2008	3	60/60	15–65/not specified	14 days	Baseline Follow-Up: Day 14	Chronic rhinosinusitis with/or nasal polyposis of I second degree of the Lund-Mackay-Classification [62]	Not specified
Passariello et al. [57]	2012	3	60/—	2–12/3.4 ± 1	15 days	Baseline Follow-Up: Day 15	(i) Age from 2 to12 (ii) CRS (iii) One or more of the following sinonasal symptoms: nasal discharge, congestion, obstruction, postnasal drip, daytime cough, and foul breath (iv) Failed courses of antibiotics, saline irrigation, nasal steroids, or antihistamine (v) Persistent symptoms for ≥1 month	(i) Steroids, nonsteroidal anti-inflammatory drugs, antihistamines, and vasoconstrictors in the previous 4 weeks (ii) Primary diagnosis of obstructive sleep apnea syndrome caused by tonsillar hyperplasia (iii) Chronic diseases, immunodeficiency, and neurological impairment (iv) Varicose veins of the nasal septum, and suspect ciliary abnormalities (v) Previous sinonasal surgery (vi) Malformation of the upper airway sinonasal osteogenesis, tumors, and obstructive lesions (vii) History of facial trauma that distorted the sinus anatomy
Salami et al. [26]	2010	5	40/40	26–58/46.4	12 days	Baseline Follow-Up: Day 12, 90	CRS without polyps	(i) Immunostimulant or immunosuppressive agents in the previous 6 months (ii) Genetic and congenital condition: cystic fibrosis and primary ciliary dyskinesia (iii) Nasal polys (iv) Positive allergy testing (v) Anatomic abnormalities (severe septal deviation among others) (vi) Acquired mucociliary dysfunction (vii) Neoplasms (viii) Acute contemporary bacterial and/or viral rhinosinusitis, middle ear, and respiratory tract (ix) Bronchopulmonary disease (x) Nasal trauma (xi) Smoker (xii) Previous nasal and sinus surgery

Table 2: Continued.

Author	Year	Jadad score	Number of patients thermal water/control	Age span/average age	Duration of treatment	Measurement time points	Inclusion criteria	Exclusion criteria
Salami et al. [25]	2008	7	50/50	6–14/9	12 days	Baseline Follow-Up: Day 12, 90	At least 3 acute episodes of upper respiratory tract infections in the last year	(i) Immunostimulant or immunosuppressive agents in the previous 6 months (ii) Previous adenoidectomy and/or tonsillectomy (iii) Anatomic anomalies (iv) Other acute infections (v) Allergic rhinitis (vi) Congenital immunodeficiency's (vii) Pulmonary disease
Staffieri et al. [56]	2008	5	40/40	18–65/not specified	6 months	Baseline Follow-Up: Day 30, 90, 180	(i) Age from 18 to 65 (ii) CRS not responding to medical treatment (iii) No contraindications to general anaesthesia and FESS	(i) Autoimmune disease (ii) Cystic fibrosis (iii) Diabetes (iv) Sinonasal inverted papilloma or sinonasal malignancy
Staffieri and Abramo [55]	2007	4	37/—	40/not specified	12 days	Baseline Follow-Up: Day 12	(i) At least 3 confirmed episodes of sinonasal infection in the previous 12 months (ii) Evidence of chronic sinonasal inflammation at otorhinolaryngologic endoscopic evaluation	Nasal steroid, vasoconstrictive drug therapies or systemic NSAIDs, and steroid or mucolytic treatments in the previous 2 months
Staffieri et al. [54]	1998	3	25/25	18–83/50.5	12 days	Baseline Follow-Up: Day 12	Chronic rhinopharyngotubaric inflammation	Not specified

Table 3: Type of application.

Application	Inhalation	Inhalation + aerosol	Irrigation	Inhalation + irrigation	Inhalation + aerosol + irrigation	Aerosol
Number of patients	140	117	230	80	120	100
Study	De Luca et al. [53] Salami et al. [25]	Marullo and Abramo [29] Passali et al. [58] Staffieri and Abramo [55] Staffieri et al. [54]	Ottaviano et al. [27] Ottaviano et al. [28] Staffieri et al. [56]	Salami et al. [26]	Passali et al. [59]	Miraglia del Giudice et al. [60] Passariello et al. [57]

Table 4: Results of MCT.

	Patients	MCTt	CI 95%	*P* value
Thermal water (sulphur + salt-bromine-iodine + radon)				
Baseline	265	19.67020883	[17.40; 21.94]	
2 weeks	265	12.98258754	[11.34; 14.63]	<0.01*
90 days	90	11.34482778	[10.91; 11.78]	<0.01*
ISCS				
Baseline	137	21.81138859	[18.19; 25.05]	
2 weeks	137	18.54655442	[17.09; 20.01]	0.107
90 days	90	17.60438587	[17.17; 18.04]	<0.05*
Placebo				
Baseline	20	19.8	[17.78; 21.82]	
2 weeks	20	18.5	[15.65; 21.35]	0.465
Radon				
Baseline	60	15.84974132	[11.85; 19.85]	
2 weeks	60	11.95305164	[11.33; 12.58]	0.059
Sulphur				
Baseline	205	19.43087224	[18.89; 19.97]	
2 weeks	205	12.57267757	[10.30; 14.85]	<0.01*
90 days	90	11.34482778	[10.91; 11.78]	<0.01*

*Significant in comparison to baseline ($P < 0.05$).

Table 5: Results of nasal resistance.

	Patients	NasRes	CI 95%	*P* value
Thermal (sulphur +radon)				
Baseline	212	0,442260114	[0.26; 0.62]	
2 weeks	137	0.257467503	[0.22; 0.30]	0.051
30 days	66	0.113901251	[0.05; 0.18]	<0.01*
90 days	64	0.159927083	[0.003; 0.32]	<0.05*
ISCS				
Baseline	115	0.516633	[0.13; 0.90]	
2 weeks	40	1.28	[1.16; 1.40]	<0.01
30 days	62	0.124408602	[0.10; 0.15]	<0.05
90 days	68	0.617283393	[−0.36; 1.60]	0.851
Placebo				
Baseline	20	0.23	[0.19; 0.27]	
2 weeks	20	0.19	[0.17; 0.21]	<0.05*
Radon				
Baseline	60	0.364606147	[0.33; 0.40]	
2 weeks	60	0.240700629	[0.15; 0.33]	<0.05*
Sulphur				
Baseline	152	0.495021367	[0.22; 0.77]	
2 weeks	77	0.272182942	[0.26; 0.29]	0.118
30 days	66	0.113901251	[0.05; 0.18]	<0.01*
90 days	64	0.159927083	[0.003; 0.32]	<0.05*

*Significant in comparison to baseline ($P < 0.05$).

Table 6: Results of nasal flow.

	Patients	NasFlow	CI 95%	*P* value
Thermal (sulphur + radon)				
Baseline	97	604.1	[513.68; 694.45]	
12–14 days	97	721.5	[697.18; 745.84)	<0.05*
Placebo				
Baseline	20	714.3	[664.13; 764.47]	
12–14 days	20	687.5	[644.79; 730.21]	0.425
Radon				
Baseline	60	633.4	[540.95; 725.90]	
12–14 days	60	738.8	[703.64; 773.89]	<0.05*
Sulphur				
Baseline	37	558.4	[526.53; 590.27]	
12–14 days	37	705.6	[671.86; 739.34]	<0.01*

*Significant in comparison to baseline ($P < 0.05$).

and M, neither beyond the subgroups between ISCS and sulphur nor in the individual groups between the baseline and the maximal treatment duration of 90 days.

4.9. Adverse Events. All adverse events that occurred during the studies in the entire patient population were extracted and illustrated in forest plots. In total, 19 patients out of 840 treated patients suffered from study related adverse events. All adverse events occurred under the treatment with sulphurous water: 13 patients experienced mild nasal irritation and a sensation of burning after application and five suffered from very limited epistaxis, one from an aggravation of the symptoms, and one from dermatological hypersensitivity. No adverse events are reported for the treatment with another thermal water, ISCS, or placebo.

For sulphurous water, 19 adverse events occurred in a total group of 370 patients. This led to an adverse event rate of

Table 7: Results of IgE.

	Patients	IgE	CI 95%	*P* value
Sulphur				
Baseline	90	105.11	[98.53; 111.69]	
12 days	90	75.65	[70.13; 81.18]	<0.01*
90 days	90	74.79	[69.38; 80.19]	<0.01*
ISCS				
Baseline	90	101.69	[94.03; 109.35]	
12 days	90	97.10	[88.22; 105.98]	0.442
90 days	90	98.30	[89.60; 107.00]	0.567

*Significant in comparison to baseline ($P < 0.05$).

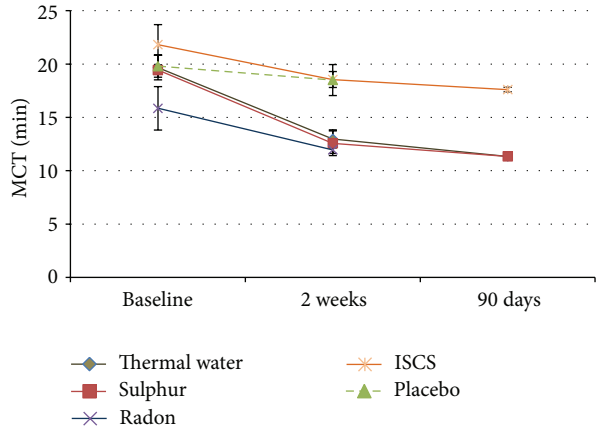

Figure 2: Mucociliary clearance time.

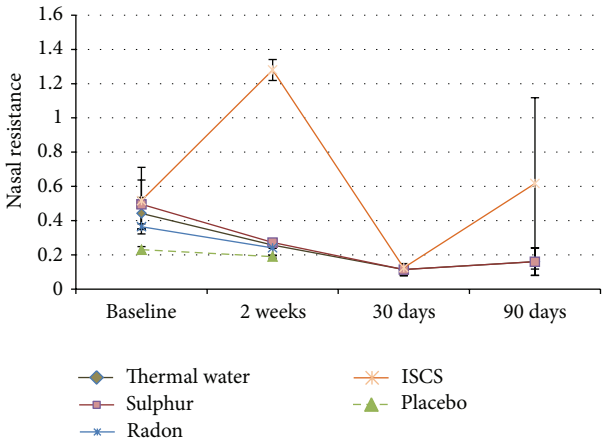

Figure 3: Nasal resistance.

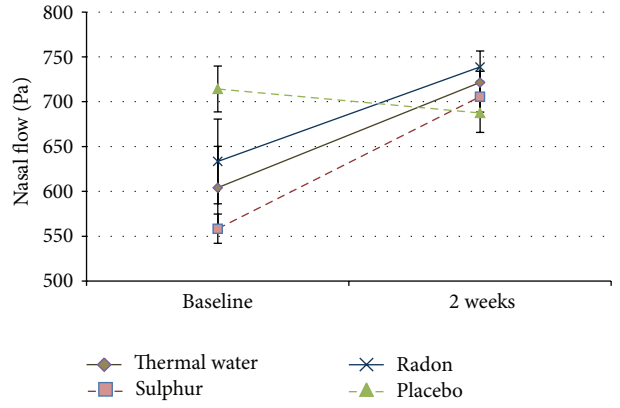

Figure 4: Nasal flow. Dotted lines present single studies.

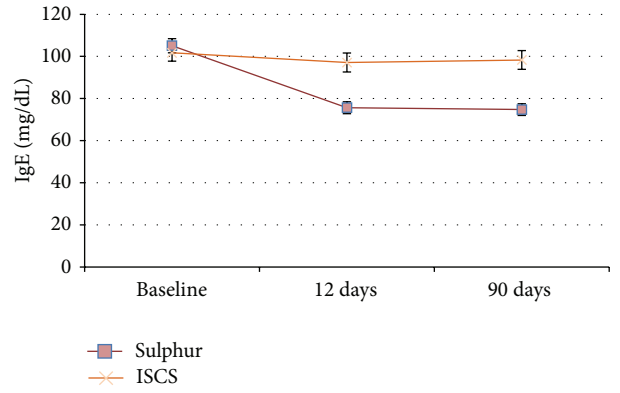

Figure 5: IgE in serum.

11.9%. The assumed rate of adverse events ranged from 7.8 to 17.6% (Figure 6).

By pooling all thermal water subgroups we received a total number of 510 treated patients with 19 adverse events. This led to an adverse event rate of 9.8% with an assumed range from 6.6 to 14.4% (Figure 7).

5. Discussion

This review and its appertaining meta-analysis is the first systematic approach to thermal water treatment in upper respiratory tract diseases. For the identified outcome parameters some significant improvements could be found in the treatment with thermal water irrigation and inhalation.

In order to ensure methodological quality of the included trials, two independent reviewers applied the modified Jadad Scale to every study with a minimal score of 3.

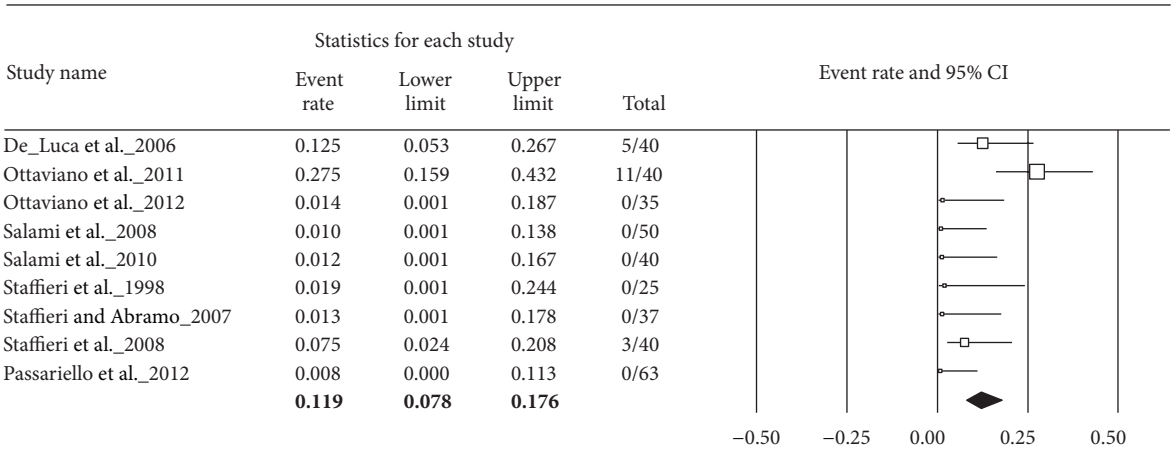

Figure 6: Adverse events for sulphurous water.

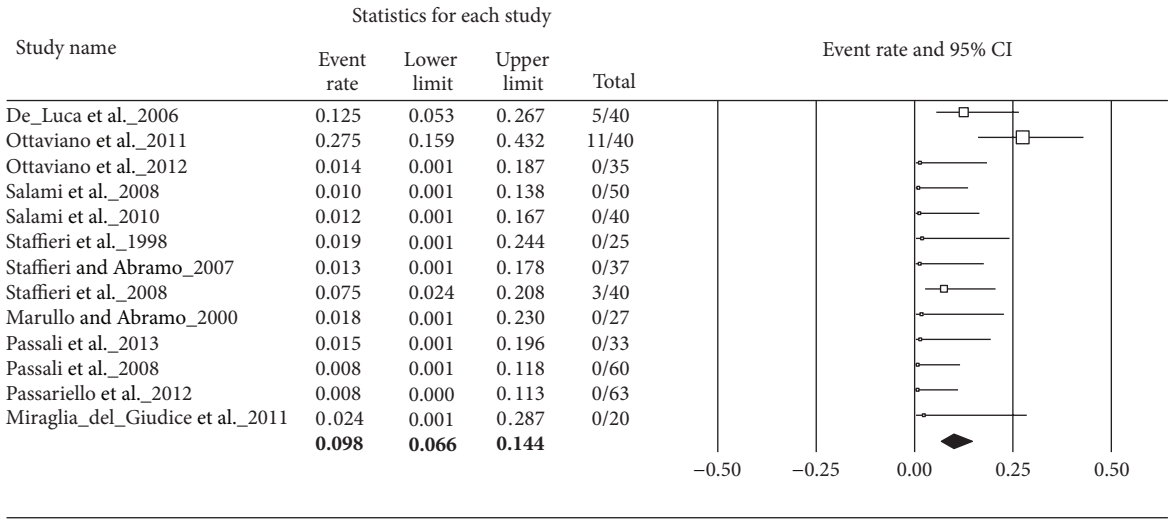

Figure 7: Adverse events for thermal water.

Further this meta-analysis is calculated by the "random effects" model, which takes possible heterogeneity more into consideration than the "fixed effect" model. The confidence intervals are broader and thus capture the true value of the meta-analysis. Where relative overestimation of smaller studies can result in greater inaccuracy, this is a more conservative and cautious estimation. It constitutes a higher risk for bias of the results [63].

In addition, we pooled the clinical pictures of allergic, acute, and chronic rhinosinusitis as well as studies with children, adults, and elderly people. In two studies patients with minimally invasive functional endoscopic sinus surgery (FESS) before treatment were included. This can be assumed as selection bias for this meta-analysis. Furthermore, only published studies were included in this meta-analysis. Thus, publication bias may occur.

Besides irrigation, also inhalation therapy was used in the different studies. Both treatments reduced inflammatory mediators in nasal secretions [64]. Nasal irrigation had a direct physical cleansing effect by flushing out thick mucus, crusts, debris, allergens, and air pollutants [65]. In a review by Hermelingmeier et al. the conclusion drawn is that there is no clear data available naming the most advantageous form of application in nasal saline irrigation [19].

The present meta-analysis shows a significant advantage of mucociliary clearance changes with thermal water in comparison to isotonic saline solution. This leads to a more detailed view of the results of thermal water applications found in this meta-analysis.

The MCT parameter comprises the best set of data in our meta-analysis with seven included studies and 422 patients. Mucociliary clearance is an important defence mechanism for both upper and lower airways and "its impairment[...] predisposes to chronic infection of the nose, paranasal sinuses and the respiratory tree" [66]. The average MCT values range below 15 minutes with a test duration of less than

1 hour [67]. In this meta-analysis a significant improvement of the mucociliary clearance could be determined in thermal water applications. Hereby, the transport time could be reduced from 19.67 minutes initially to 12.98 minutes after two weeks and to 11.34 minutes after 90 days. Especially the application of sulphurous water showed a high significance ($P < 0.001$) after the two-week treatment period. At the same time the treatment with radon thermal water ($P = 0.059$) only just lacked a significant improvement ($P = 0.059$).

The literature available for these two subgroups was 5 : 2 with only 60 patients in the radon group which might have had an influence on the significance. In contrast to the thermal water group, the ISCS group only showed significance after 90 days of treatment. In turn the literature available was quite strong with 4 studies and a total of 205 patients.

Based on Marullo's study only it was not possible to conduct a meta-analysis and draw a valid conclusion on the use of placebo [29]. The meta-analysis of the mucociliary clearance time showed a significant benefit especially of the pooled thermal water treatment and sulphurous water over ISCS.

The "nasal resistance is the resistance offered by the nasal cavity to inspired air" [68] and it is measured in Pascal (Pa). All studies used for this analysis already resulted in significant changes after two weeks of treatment with radon thermal water. ISCS differed significantly after two weeks and after 30 days of treatment. The meta-analysis revealed significant variations in the three pooled ISCS studies. Especially the study of Salami et al. displayed a high deviation from the baseline of 13.1 Pa, which was reduced to 1.28 Pa after two weeks and remained rather high after 90 days of treatment with 1.12 Pa.

Opposed to these findings were those of the two other studies of this pool [27, 28], which began with much lower baseline values of 0.14 and 0.17 Pa and had different followups. Therefore these led to an unsteady curve and limited the possibility of a serious interpretation of the healing process. Nevertheless, each of these three studies showed a reduction of the nasal resistance.

The treatment with sulphurous water showed good results throughout the whole treatment period of 90 days. The results were even better after 30 and 90 days than at the beginning, which allows for the assumption that a more permanent improvement is gained here.

Based on this meta-analysis we can assume that radon water application shows significant improvement in nasal flow. The data is quite limited with the results of only one study for placebo and one for sulphurous treatment, so that we cannot compare it to the pooled results.

The use of radon thermal water as well as the entire thermal water subgroup showed a significant improvement in the nasal flow after two weeks of treatment.

In our meta-analysis of IgE, sulphurous water treatment was highly significant after 12 and 90 days. ISCS treatment showed no significance. The present IgE results were measured in patients with chronic inflammatory conditions, where eosinophil cells in the mucus are increased [69]. Reduction of eosinophil cells after thermal water treatment was also reported in Passali et al. [58] and significantly decreased in Staffieri and Abramo [55]. Hypereosinophilia is related to high levels of serum concentrations of IgE [26, 70]. The IgE concentration which decreased significantly after the application with sulphurous water confirmed beneficial effects on chronic inflammatory disorders. Sulphurous water helps to clean the nasal mucosa from irritations and reduces immune responses at a local level [25, 71]. These results support the assumption that sulphurous water has an anti-inflammatory effect.

IgA, IgG, and IgM values in the blood did not increase significantly neither with sulphurous water nor with ISCS. Unfortunately the literature available on the secretory IgA, which is secreted across the mucosa and plays a significant role in specific immune defence by preventing or blocking the adhesion of bacteria and defending the mucous membranes from common infection [72, 73], was not sufficient for a statistical analysis. Similarly, comparable studies investigating the IgM and IgG in the mucosa were missing.

Generally speaking, thermal water application is a safe treatment. Adverse events occurred in 19 out of 510 thermal water treatments and mainly consisted of mild nasal irritation, a sensation of local burning after application, and very limited epistaxis. All of these adverse events occurred under the treatment with sulphurous water. Neither for radon water, ISCS, nor placebo treatment adverse events were reported. It should be noted that both the studies by Staffieri et al. [56] and Ottaviano et al. [28] were conducted in a postoperative setting, which makes the occurrence of such adverse events more likely. Further, the study by De Luca et al. [53] was conducted with elderly people between 72 and 100 years.

6. Conclusion

Nasal application of thermal water results in a significant improvement of MCT, nasal flow, nasal resistance, and IgE concentration. The systematic review and the meta-analysis demonstrate an advantage of thermal water treatment over isotonic saline solution and placebo. Even though this aspect needs to be investigated further with randomised controlled trials in bigger cohorts and longer follow-up periods, it was shown that the application with thermal water can serve as additional nonpharmacological alternative.

Abbreviations

AE: Adverse events
AR: Allergic rhinitis
ARIA: Allergic rhinitis and its impact on asthma
ARS: Acute rhinosinusitis
CRS: Chronic rhinosinusitis
EPOS: European Position Paper on Rhinosinusitis and Polyps
FESS: Functional endoscopic sinus surgery
Flow: Nasal respiratory flow
Ig: Immunoglobulin
ISCS: Isotonic sodium chloride solution
MCT: Mucociliary clearing time
NAR: Nonallergic rhinitis
Pa: Pascal

R: Resistance
RURT: Recurrent upper airway infections.

Conflict of Interests

Sarah Keller, Volker König, and Ralph Mösges report no conflict of interests concerning this paper.

Authors' Contribution

All authors have contributed to, seen, and approved the paper.

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Clinical Study

Noninterventional Open-Label Trial Investigating the Efficacy and Safety of Ectoine Containing Nasal Spray in Comparison with Beclomethasone Nasal Spray in Patients with Allergic Rhinitis

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Objectives. The current study aimed to compare the efficacy and safety of a classical anti-inflammatory beclomethasone nasal spray in comparison to a physic-chemical stabilizing ectoine containing nasal spray in the treatment of allergic rhinitis. *Design and Methods*. This was a noninterventional, open-label, observational trial investigating the effects of beclomethasone or ectoine nasal spray on nasal symptoms and quality of life. Over a period of 14 days, patients were asked to daily document their symptoms. Efficacy and tolerability were assessed by both physicians and patients. *Results*. Both treatments resulted in a significant decrease of TNSS values. An equivalence test could not confirm the noninferiority of ectoine treatment in comparison with beclomethasone treatment. Although clear symptom reduction was achieved with the ectoine products, the efficacy judgment showed possible advantages for the beclomethasone group. Importantly, tolerability results were comparably good in both groups, and a very low number of adverse events supported this observation. Both treatments resulted in a clear improvement in the quality of life as assessed by a questionnaire answered at the beginning and at the end of the trial. *Conclusion*. Taken together, it was shown that allergic rhinitis can be safely and successfully treated with beclomethasone and also efficacy and safety were shown for ectoine nasal spray.

1. Introduction

Allergic rhinitis is a common disease with estimated 600 million patients suffering from this disease worldwide [1]. According to a large scale investigation, about 20% of the European population suffers from allergic rhinitis [2] and numbers are increasing, particularly in industrial states. Although not being a life-threatening disease, allergic rhinitis has a considerable impact on general well-being and work/school performance, and particularly its impact on comorbidities such as, for example, asthma reflects the need for good treatment options.

A number of pharmacological treatments against allergic rhinitis exist, such as antihistamines, leukotriene receptor agonists, mast cell stabilizing agents, and glucocorticosteroids. According to the ARIA (Allergic Rhinitis and its Impact on Asthma) guidelines, intranasal glucocorticosteroids are recommended as pharmacological treatment of allergic rhinitis and should be prescribed preferable to intranasal antihistamines and oral leukotriene receptor agonists [1]. However, many patients have reservations to use corticosteroids, and phobia of their usage can result in bad compliance [3]. This together with the fact that patients

often seek alternative treatments to pharmacological options reflects the need for new treatment strategies.

The current noninterventional trial compared efficacy and safety of treatment of allergic rhinitis patients with intranasal spray containing either the glucocorticoid beclomethasone or the natural, nonpharmacological substance ectoine. Overview of the results are shown in Figure 9.

Ectoine is a compatible solute which is produced by microorganisms living under harsh environmental conditions such as extreme salinity or dryness [4–6]. In those microorganisms, ectoine acts as natural cell protectant. Halophilic microorganisms living in habitats of high ionic strength cope with hyperosmotic stress by changing the composition of membrane lipids and by regulating the intracellular concentration of low molecular weight solutes such as the compatible solute Ectoine. As a result of the latter response, the cells are able to maintain proper osmotic balance under conditions of hyperosmotic stress, which is crucial to prevent the cell from leaking water, hence avoiding irreversible plasmolysis and dehydration, and to generate turgor pressure within limits necessary for growth [7, 8]. Ectoin is industrially produced via the "bacterial milking process" using the gram negative bacterium *Halomonas elongata* [9, 10]. It is known that ectoine works via an entropy-driven mechanism called "preferential exclusion" or "preferential hydration" during which ectoine influences the characteristics of the water shell surrounding biomolecules like membranes. By excluding osmolytes from the direct hydrate shell of proteins and membranes, a preferential hydration of such proteins or membranes occurs, thereby stabilizing their native confirmation and making them less vulnerable to external stressors [11, 12]. Preclinical studies have demonstrated that the beneficial effects of ectoine can be transferred to human or animal models, and they have also shown that ectoine possesses membrane-stabilizing and inflammation-reducing properties [13–16]. Additional experiments on human nasal epithelial cell lines have confirmed the protective action of ectoine against osmotic stress (data not published). Recent developments have demonstrated that this attribute can be successfully transferred to a number of application forms such as ectoine containing creams, nasal sprays, or eye drops which can be used on humans for treatment of rhinosinusitis and atopic dermatitis [11, 12] and also congress reports point towards efficacy when applied to patients with allergic rhinitis [17–20].

This study assessed development of nasal symptoms, changes in quality of life, and judgment of efficacy and tolerability upon treatment with either the well-known steroid beclomethasone or barrier stabilizing, physically acting ectoine nasal spray in order to compare the effect levels of these different treatment concepts in patients with allergic rhinitis.

2. Materials and Methods

2.1. Treatment and Study Design. This was a controlled, open-labelled, noninterventional, multicenter study assessing the efficacy and safety of ectoine containing nasal spray in comparison to beclomethasone containing nasal spray. The patients could freely choose their treatment: they were treated with either ectoine nasal spray or beclomethasone containing nasal spray (0.05% beclomethasone).

According to §23b of the German medical device law, this study was carried out with the CE-marked medical device ectoine nasal spray without changes in its intended use; therefore, §§20–23a of the MPG had not been complied with. Open observational trials are health authority accepted in Germany for nonpharmaceutical treatment options. According to a general statement by ethical committees, this type of study does not allow for a placebo group, because this would involve a lack of benefit for patients.

Ectoine nasal spray is an isotonic solution containing 2% ectoine, natural salt, and water. According to the instructions for use, one puff of the spray had to be applied into each nostril three times daily. The beclomethasone spray was used in accordance with the instructions for use: two puffs of the spray had to be applied into each nostril twice a day. Each puff of the nasal spray contains 0.05 mg beclomethasone diproprionate. Further ingredients are benzalkonium chloride (preservative), polysorbate, glucose, cellulose, sodium carmellose, water, sodium hydroxide, and hydrochloric acid for pH adjustment.

Male and female patients aged 18–70 years with documented diagnosed seasonal allergic rhinitis were eligible for the study, based on the discretion of the investigator. In order to be sure of the allergic symptoms, the nasal symptom score (TNSS) at study start had to be ≥ 6.

Patients had to attend two site visits (V1 on day 0 and V2 on day 14 ± 2). During the entire treatment period, patients were asked to document their symptoms in patient diaries once daily. Assessments of symptoms by physicians were carried out during site visits V1 and V2.

The medication was handed over to the patients by the physician. After completion of the study, no drug accountability was performed.

For simplification reasons, patients of the ectoine group will be termed group 1 in this paper, and patients of the beclomethasone group will be termed group 2.

2.2. Endpoints. Primary endpoints were changes in the single symptoms nasal obstruction, rhinorrhea, nasal itching, and sneezing as well as changes in the sum of nasal symptoms (TNSS). Secondary endpoints were the assessment of the symptom itchy ear/palate and assessment of efficacy and tolerability as well as analysis of safety data.

2.3. Scoring of Nasal Symptoms. Single nasal symptoms as well as ear/palate itching were assessed with a score described as follows: 0 (no symptoms), 1 (slight symptoms), 2 (moderate symptoms), and 3 (severe symptoms). Patients assessed their symptoms reflectively, and scores describing the symptoms within the last 24 h were documented in the patients' diaries. Physicians scored the current symptoms during both patient visits (V1 and V2).

In order to account for the influence of pollen intensity on the nasal symptoms, the quotient TNSS/pollen count score

was calculated. Pollen count scores were derived from online available daily pollen counts for the relevant local area. The scoring of pollen counts was as follows: no pollen count was scored with 0.1, low pollen count with 1, moderate pollen count with 2, and strong pollen count with 3.

2.4. Scoring of Efficacy and Tolerability. At the end of the study, patients assessed both efficacy and tolerability with a score of 0 (no efficacy, bad to tolerate), 1 (moderate efficacy, moderate tolerability), 2 (good efficacy, good tolerability), and 3 (very good efficacy, very good tolerability).

2.5. Quality of Life Questionnaire. A modified, nonvalidated quality of life questionnaire based on the RQLQ from Juniper et al. was used in this study. During both site visits (V1 and V2), patients were asked to fill out the questionnaire. It contained 14 questions covering three topics (daily life activities, general wellbeing, and emotional status) which were to be answered on a score from 0 (none) to 6 (very/always).

2.6. Data Management and Statistics. Data of this open-label trial were collected by the physicians in an anonymized paper CRF and by the patients in diaries and questionnaires. Proper data management was monitored during the study. A SAP was set up before study closure and the data were analyzed according to the SAP. Source data from the CRFs, diaries, and questionnaires were transferred to digital data format by the physicians. The statistical analysis was carried out with SPSS Statistics 20 and SigmaPlot version 12. The primary endpoint TNSS was summarized descriptively for both V1 and V2, and differences between V1 and V2 were documented. Noninferiority of the ectoine product versus the beclomethasone containing spray was assessed with an equivalence range of 15%. Analysis of secondary parameters was done descriptively. In addition, changes during the period of the study were analyzed via Bowker's test of symmetry. Group comparisons were analyzed via Chi-square test or Fisher's exact test. All significance levels were set to 5%. Unavailable data were treated as "missing values."

3. Results

The current study was conducted in accordance with the Declaration of Helsinki. All investigations were carried out with the understanding and consent of all participants. The study took part at two German ear nose throat (ENT) practices starting in May 2011 and being completed at the end of June 2011. In total, 50 patients (34 women, 16 men) diagnosed with seasonal allergic rhinitis were included in the study. Mean age of the patients was 33.3 years. Of the 50 patients, 25 received ectoine and 25 patients received beclomethasone nasal spray. All patients completed the study. Distribution of patients is shown in Figure 1.

3.1. Total Nasal Symptom Score (TNSS). The development of the total nasal symptom score (sum of nasal obstruction, rhinorrhea, sneezing, and nasal itching) was judged by both patients and the investigators.

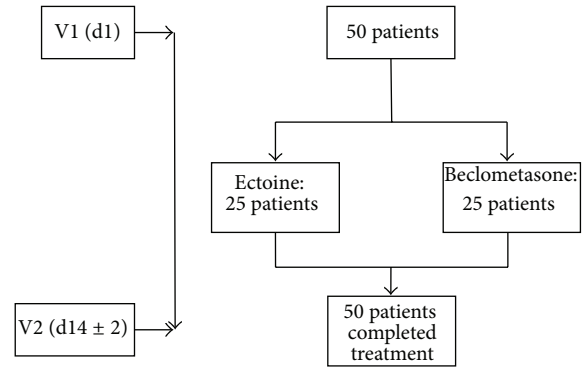

Figure 1: Patient flow during the study. On day 1 (V1), patients were asked to participate in the study. 25 patients received ectoine nasal spray, and 25 patients received beclomethasone nasal spray for a treatment period of 14 ± 2 days. All 50 patients finished the study with the final study visit V2.

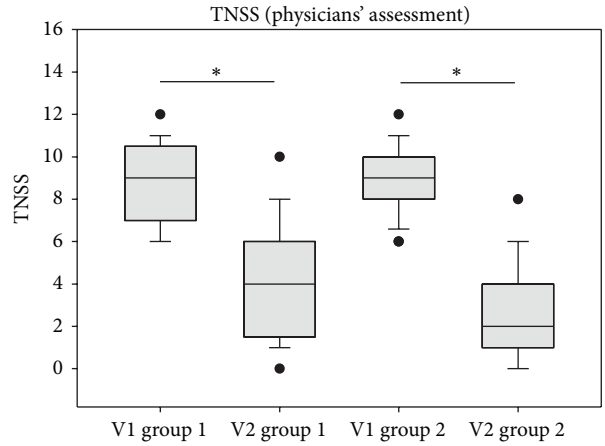

Figure 2: TNSS development during the study based on the physicians' assessment of symptoms. $^{*}P < 0.001$. Lines within the box mark the median; the upper and lower ends of the box indicate the 75th and 25th percentiles, respectively. Whiskers above and below the box indicate the 90th and 10th percentiles. Dots (•) represent outlying points.

Results of the investigators' assessment are shown in Figure 2. TNSS values decreased significantly in both groups ($P < 0.001$). In the ectoine group, values decreased from 8.76 ± 1.79 (V1) to 4.04 ± 2.75 (V2) corresponding to a total decrease of −4.72 (−51.20%), whereas values in the beclomethasone group decreased from 9.04 ± 1.53 (V1) to 2.52 ± 2.22 (V2) corresponding to a total decrease of −6.52 (−71.49%).

According to the patients' assessment (see Figure 3(a)), TNSS values decreased clearly in the ectoine group ($P = 0.072$, decrease by −12.86%) and a significant decrease was observed in the beclomethasone group ($P < 0.001$, decrease by 39.69%).

In order to consider the influence of pollen on the strength of nasal symptoms, quotients of TNSS values and pollen count scores were calculated. Those confirmed the statistically significant decrease of TNSS values from V1

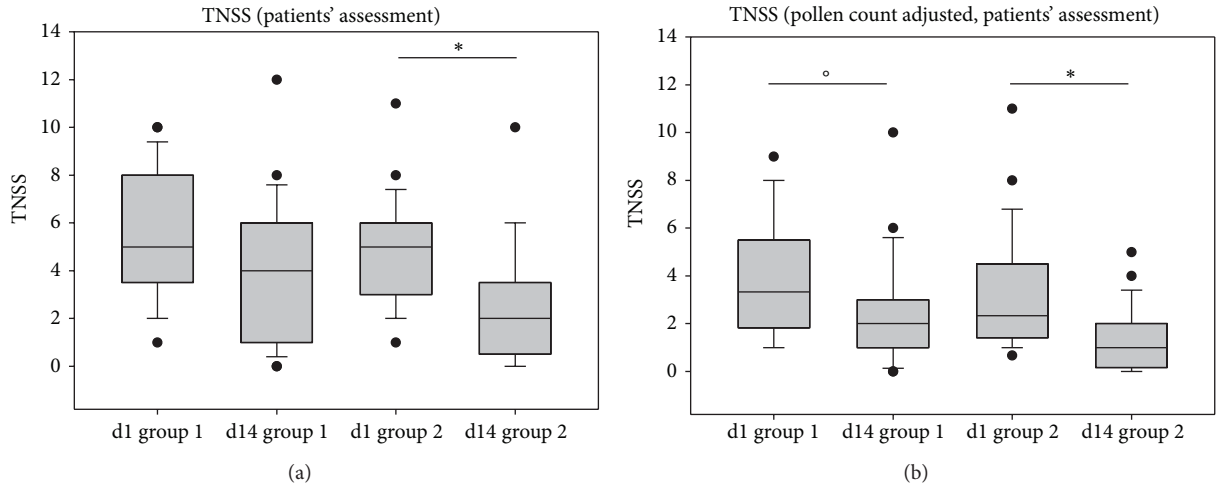

Figure 3: TNSS development during the study based on the patients' assessment of symptoms. (a) TNSS values on day 1 (d1) and day 14 (d14); (b) TNSS values adjusted for pollen counts, $^{*}P < 0.001$, $^{\circ}P = 0.043$. Lines within the box mark the median; the upper and lower ends of the box indicate the 75th and 25th percentiles, respectively. Whiskers above and below the box indicate the 90th and 10th percentiles. Dots (•) represent outlying points.

to V2 as assessed by investigators ($P < 0.001$ for both groups, details not shown). When patients' TNSS values were normalized to the pollen count scores, TNSS decreased in both groups and reached statistical significance ($P = 0.043$ for group 1, $P < 0.001$ for group 2; see Figure 3(b)).

3.2. Equivalence Test. An equivalence test was carried out to investigate the hypothesis that ectoine nasal spray is not inferior to beclomethasone containing nasal spray. As shown in Table 1, no significant differences could be shown. Thus, noninferiority of the ectoine nasal spray could not be confirmed.

3.3. Comparison of TNSS Values from V1 until the End of the First Treatment Day. In order to study the time of onset of both treatments, TNSS value development within the first 12 hours of treatment was analyzed. As shown in Figure 4, both groups showed a significant decrease of TNSS values from the first site visit until the first patient assessment at the end of the first day of treatment ($P < 0.001$ for both groups). This indicates that a comparably quick reduction of allergic symptoms was achieved within the first day of treatment in both groups.

3.4. Development of Single Symptom Scores. In order to correlate group affiliation and development of single symptoms, data were further analyzed with Fisher's exact test. Table 2 lists the number of patients with reduced, unchanged, or deteriorated symptoms as assessed by patients themselves or by the physicians. The analysis of data demonstrated that only the patients' assessment of the symptom sneezing revealed a statistically significant correlation ($P = 0.039$), indicating that this symptom improved significantly better in the patient group treated with beclomethasone nasal spray.

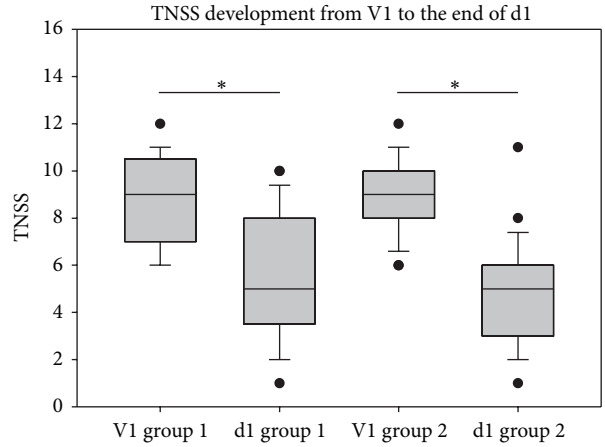

Figure 4: TNSS development from site visit 1 (V1) until the end of the first treatment day (d1). $^{*}P < 0.001$.

3.5. Ear/Palate Itching. In addition to the assessment of nasal symptoms, development of ear/palatal itching was assessed both by investigators and by patients. Results are depicted in Table 3 showing that there was no significant correlation between symptom development and group affiliation detectable neither in accordance with the investigators' nor in accordance with the patients' assessment.

3.6. Results of the Quality of Life Questionnaire. At the beginning and at the end of study participation, patients were asked to fill out a quality of life questionnaire which consisted of 14 questions. In order to investigate a correlation between changes in life quality and group affiliation, all single parameters of the questionnaire were analyzed via Fisher's exact test. A comparison of the patients' evaluation of quality of life both at d1 and d14 did not show statistical differences

Table 1: TNSS differences from treatment day 1/site visit 1 (d1/V1) to treatment d14/V2, respectively. Values are given as absolute value differences (abs) or as percentage differences (%).

TNSS difference variable	Equivalence range	TNSS difference of mean values			P value
		Value	SD	95% CI	
d14 (abs)	−2.32 ± 0.35	−1.10	0.92	[−2.64; 0.44]	$P = 0.939$
d14 [%]	−39.69 ± 5.95	−26.84	20.86	[−61.96; 8.29]	$P = 0.938$
V2 (abs)	−6.52 ± 0.98	−1.80	0.87	[−3.26; −0.34]	$P = 0.999$
V2 [%]	−71.49 ± 10.72	−20.29	8.91	[−35.25; −5.32]	$P = 0.999$

SD: standard deviation, CI: confidence interval.

Table 2: Development of single nasal symptoms during the study. Improvement, deterioration, or unchanged status of single symptoms was assessed by patients and investigators.

	Patients' assessment		Total	Physicians' assessment		Total
	Group 1	Group 2		Group 1	Group 2	
Rhinorrhoea						
Reduced	11 (47.8%)	12 (48.0%)	23 (47.9%)	17 (68.0%)	22 (88.0%)	39 (78.0%)
Unchanged	9 (39.1%)	11 (44.0%)	20 (41.7%)	6 (24.0%)	3 (12.0%)	9 (18.0%)
Deteriorated	3 (13.0%)	2 (8.0%)	5 (10.4%)	2 (8.0%)	0 (0.0%)	2 (4.0%)
Total	**23 (100.0%)**	**25 (100.0%)**	**48 (100.0%)**	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
Fisher's exact test		$P =$ **0.919**			$P =$ **0.221**	
Nasal itching						
Reduced	7 (30.4%)	12 (48.0%)	19 (39.6%)	20 (80.0%)	22 (88.0%)	42 (84.0%)
Unchanged	13 (56.5%)	10 (40.0%)	23 (47.9%)	4 (16.0%)	2 (8.0%)	6 (12.0%)
Deteriorated	3 (13.0%)	3 (13.0%)	6 (12.5%)	1 (4.0%)	1 (4.0%)	2 (4.0%)
Total	**23 (100.0%)**	**25 (100.0%)**	**48 (100.0%)**	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
Fisher's exact test		$P =$ **0.440**			$P =$ **0.830**	
Nasal obstruction						
Reduced	11 (47.8%)	10 (40.0%)	21 (43.8%)	18 (72.0%)	22 (88.0%)	40 (80.0%)
Unchanged	7 (30.4%)	13 (52.0%)	23 (41.7%)	6 (24.0%)	2 (8.0%)	8 (16.0%)
Deteriorated	5 (21.7%)	2 (8.0%)	7 (14.6%)	1 (4.0%)	1 (4.0%)	2 (4.0%)
Total	**23 (100.0%)**	**25 (100.0%)**	**48 (100.0%)**	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
Fisher's exact test		$P =$ **0.258**			$P =$ **0.347**	
Sneezing						
Reduced	5 (21.7%)	12 (48.0%)	17 (35.4%)	18 (72.0%)	23 (92.0%)	41 (82.0%)
Unchanged	10 (43.5%)	11 (44.0%)	21 (43.8%)	3 (12.0%)	2 (8.0%)	5 (10.0%)
Deteriorated	8 (34.8%)	2 (8.0%)	10 (20.8%)	4 (16.0%)	0 (0.0%)	4 (8.0%)
Total	**23 (100.0%)**	**25 (100.0%)**	**48 (100.0%)**	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
Fisher's exact test		$P =$ **0.039**			$P =$ **0.115**	

Table 3: Development of ear/palate itching during the study (d1 to d14 or V1 to V2) as assessed by patients and investigators. The total number of patients (% given in brackets) where symptoms were reduced, unchanged, or deteriorated is shown.

Ear/palate itching	Patients' assessment		Total	Physicians' assessment		Total
	Group 1	Group 2		Group 1	Group 2	
Reduced	4 (17.4%)	12 (48.0%)	16 (33.3%)	12 (48.0%)	14 (56.0%)	26 (52.0%)
Unchanged	14 (60.9%)	9 (36.0%)	23 (47.9%)	12 (48.0%)	8 (32.0%)	20 (40.0%)
Deteriorated	5 (21.7%)	4 (16.0%)	9 (18.8%)	1 (4.0%)	3 (12.0%)	4 (8.0%)
Total	**23 (100.0%)**	**25 (100.0%)**	**48 (100.0%)**	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
Fisher's exact test		$P =$ **0.088**			$P =$ **0.357**	

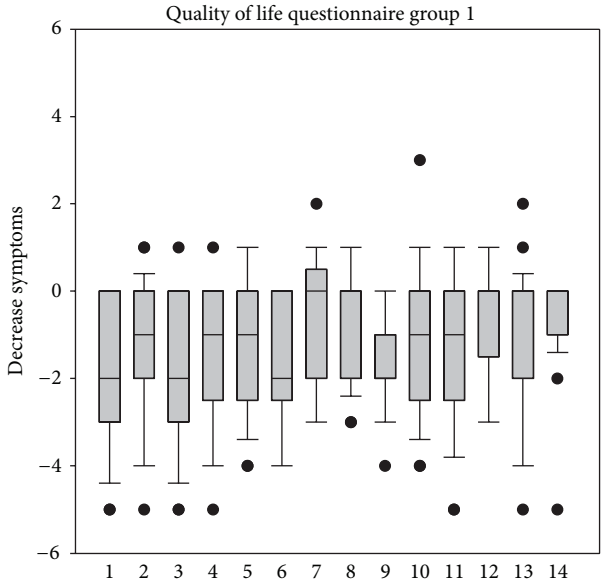

Figure 5: Reduction of scores of the quality of life questionnaire from V1 to V2 in group 1. 1 = frequency of tissue use, 2 = rubbing eyes and nose, 3 = frequency of brushing of nose, 4 = bad sleep, 5 = bad work performance, 6 = fatigue, 7 = thirst, 8 = lack of concentration, 9 = general well-being, 10 = headache, 11 = bad temper, 12 = general disconcertment, 13 = frustration, and 14 = reactions of others to the allergy.

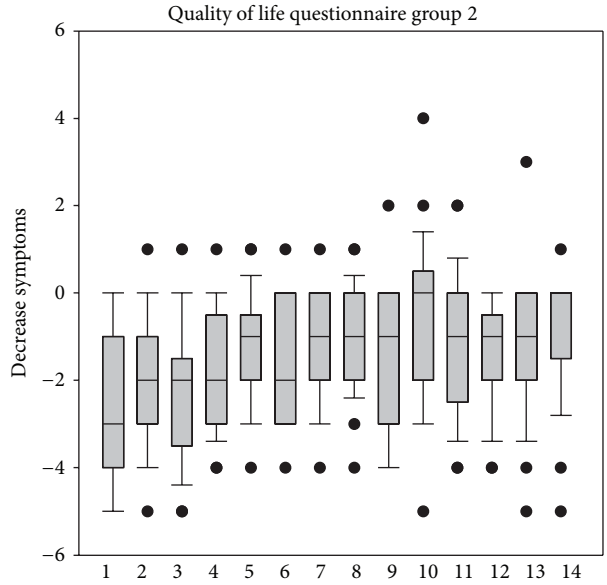

Figure 6: Reduction of scores of the quality of life questionnaire from V1 to V2 in group 1. 1 = frequency of tissue use, 2 = rubbing eyes and nose, 3 = frequency of brushing of nose, 4 = bad sleep, 5 = bad work performance, 6 = fatigue, 7 = thirst, 8 = lack of concentration, 9 = general well-being, 10 = headache, 11 = bad temper, 12 = general disconcertment, 13 = frustration, and 14 = reactions of others to the allergy.

between groups 1 and 2 in any of the questions asked (details not shown). Analysis of the results of the quality of life questionnaire as assessed by the investigators only showed a statistical significance ($P = 0.008$) for the parameter "frequency of brushing the nose" indicating that this parameter improved significantly better in the beclomethasone group (for details, see Table 4).

In addition to the analysis described above, total decreases of scores of the quality of life questionnaires were analyzed. As depicted in Figures 5 and 6, treatment resulted in decreases of all questioned parameters, thus indicating that all bothersome points which were covered in the questionnaire of life had improved during treatment.

3.7. Efficacy and Tolerability. Patients and investigators evaluated both the efficacy and tolerability of treatments during the study. Judgment of patients was given on a daily basis, whereas the investigators assessed those parameters at the end of the study (V2). As shown in Figure 7, patients judged the tolerability of both products similarly, and mean values of 2.42 ± 0.72 (group 1) and 2.53 ± 0.55 (group 2) corresponded to good to very good tolerability. No significant differences were detectable between groups. Similarly, assessment by the investigators during V2 confirmed a good tolerability of the treatments which was comparable between groups (see Figure 8).

The results of the assessment of efficacy of both treatments are depicted in Figures 10 and 11. As shown in Figure 10, efficacy assessment was similar during the first two days of treatment but increased over the treatment period of 14 days in the beclomethasone group compared to the ectoine group.

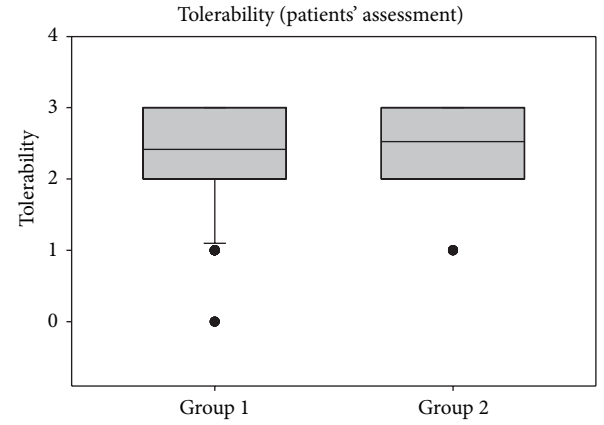

Figure 7: Tolerability assessments of patients during the entire study period of 14 days. Lines within the box mark the median; the upper and lower ends of the box indicate the 75th and 25th percentiles, respectively. Whiskers below the box indicate the 10th percentiles. Dots (•) represent outlying points.

In group 1, mean values of 1.09 ± 0.78 (mean values of entire study period) reflected moderate efficacy assessed by patients and a value of 1.44 ± 1.00 showed similar judgment by the physicians. In group 2, the efficacy was judged as good by patients (1.73 ± 0.94) and as very good by investigators (2.60 ± 0.58).

3.8. Adverse Events (AEs). In total, 3 adverse events were reported. Details are given in Table 5. No serious adverse

Table 4: Results (changes from V1 to V2) of the quality of life questionnaire documented by physicians.

		Group 1	Group 2	Total
	Frequency of tissue use			
	Reduced	12 (48.0%)	15 (60.0%)	27 (54.0%)
$P = \mathbf{0.568}$	Unchanged	11 (44.0%)	7 (28.0%)	18 (36.0%)
	Increased	2 (8.0%)	3 (12.0%)	5 (10.0%)
	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
	Rubbing eyes and nose			
	Reduced	14 (56.0%)	14 (56.0%)	28 (56.0%)
$P = \mathbf{0.999}$	Unchanged	8 (32.0%)	7 (28.0%)	15 (30.0%)
	Increased	3 (12.0%)	4 (16.0%)	7 (14.0%)
	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
	Frequency of brushing of nose			
	Reduced	12 (48.0%)	21 (84.0%)	33 (66.0%)
$P = \mathbf{0.008}$	Unchanged	8 (32.0%)	4 (16.0%)	12 (24.0%)
	Increased	5 (20.0%)	0 (0.0%)	5 (10.0%)
	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
	Bad sleep			
	Reduced	15 (60.0%)	17 (68.0%)	32 (64.0%)
$P = \mathbf{0.878}$	Unchanged	9 (36.0%)	7 (28.0%)	16 (32.0%)
	Increased	1 (4.0%)	1 (4.0%)	2 (4.0%)
Gesamt	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
	Bad work performance			
	Reduced	15 (60.0%)	20 (80.0%)	35 (70.0%)
$P = \mathbf{0.328}$	Unchanged	7 (28.0%)	4 (16.0%)	11 (22.0%)
	Increased	3 (12.0%)	1 (4.0%)	4 (8.0%)
	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
	Fatigue			
	Reduced	16 (64.0%)	15 (60.0%)	31 (62.0%)
$P = \mathbf{0.690}$	Unchanged	8 (32.0%)	7 (28.0%)	15 (30.0%)
	Increased	1 (4.0%)	3 (12.0%)	4 (8.0%)
	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
	Thirst			
	Reduced	10 (40.0%)	14 (56.0%)	24 (48.0%)
$P = \mathbf{0.178}$	Unchanged	8 (32.0%)	9 (36.0%)	17 (34.0%)
	Increased	7 (28.0%)	2 (8.0%)	9 (18.0%)
	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
	Lack of concentration			
	Reduced	10 (40.0%)	15 (60.0%)	25 (50.0%)
$P = \mathbf{0.389}$	Unchanged	13 (52.0%)	8 (32.0%)	21 (42.0%)
	Increased	2 (8.0%)	2 (8.0%)	4 (8.0%)
	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
	General well-being			
	Reduced	20 (80.0%)	16 (64.0%)	36 (72.0%)
$P = \mathbf{0.462}$	Unchanged	4 (16.0%)	6 (24.0%)	10 (20.0%)
	Increased	1 (4.0%)	3 (12.0%)	4 (8.0%)
	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
	Headache			
	Reduced	16 (64.0%)	10 (40.0%)	26 (52.0%)
$P = \mathbf{0.081}$	Unchanged	8 (32.0%)	9 (36.0%)	17 (34.0%)
	Increased	1 (4.0%)	6 (24.0%)	7 (14.0%)
	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**

Table 4: Continued.

		Group 1	Group 2	Total
	Bad temper			
	Reduced	13 (52.0%)	17 (68.0%)	30 (60.0%)
$P = \mathbf{0.549}$	Unchanged	8 (32.0%)	6 (24.0%)	14 (28.0%)
	Increased	4 (16.0%)	2 (8.0%)	6 (12.0%)
	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
	General disconcertment			
	Reduced	12 (48.0%)	19 (76.0%)	31 (62.0%)
$P = \mathbf{0.099}$	Unchanged	11 (44.0%)	6 (24.0%)	17 (34.0%)
	Increased	2 (8.0%)	0 (0.0%)	2 (4.0%)
	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
	Frustration			
	Reduced	10 (40.0%)	16 (64.0%)	26 (52.0%)
$P = \mathbf{0.195}$	Unchanged	14 (56.0%)	8 (32.0%)	22 (44.0%)
	Increased	1 (4.0%)	1 (4.0%)	2 (4.0%)
	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**
	Reactions of others to the allergy			
	Reduced	7 (28.0%)	10 (40.0%)	17 (34.0%)
$P = \mathbf{0.377}$	Unchanged	18 (72.0%)	14 (56.0%)	32 (64.0%)
	Increased	0 (0.0%)	1 (4.0%)	1 (2.0%)
	Total	**25 (100.0%)**	**25 (100.0%)**	**50 (100.0%)**

In addition to the analysis described above, total decreases of scores of the quality of life questionnaires were analyzed. As depicted in Figure 5 and Figure 6, treatment resulted in decreases of all questioned parameters, thus indicating that all bothersome points which were covered in the questionnaire of life had improved during treatment.

Table 5: Adverse events during the study.

Description AE	Treatment group	Relationship
Headache	Ectoine	Highly unlikely
Headache	Ectoine	Highly unlikely
Burning of nose	Beclomethasone	Probably

events (SAEs) occurred during the study. Both AEs occurring in the ectoine group were assessed as highly unlikely by the investigators, whereas the correlation of the AE "burning of nose" with the study medication was judged as probable in the beclomethasone group.

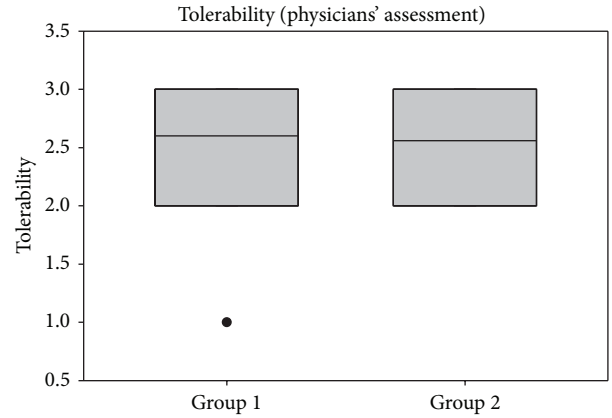

Figure 8: Assessment of tolerability at V2 assessed by the physicians. Lines within the box mark the median; the upper and lower ends of the box indicate the 75th and 25th percentiles, respectively. Dots (•) represent outlying points.

4. Conclusions

The current noninterventional, open-label study investigated treatment of allergic rhinitis comparing the intranasal glucocorticoid beclomethasone with that of ectoine containing nasal spray. Within the study, different mode of action, on the one hand the glucocorticoid, was compared to a physical, membrane stabilizing molecule. Importantly, it was shown that nasal symptom scores of both treatment groups improved significantly over the study period of 14 days. Although advantages of the beclomethasone spray in comparison with the ectoine spray were shown, results of the ectoine group showed its potential clinical efficacy. Glucocorticoids bind to specific glucocorticoid receptors which are present on almost all cells of the body. Following binding, transcription of a number of inflammatory cytokines and chemokines can be modulated, which in turn results in decreased recruitment and activation of inflammatory cells [21]. In allergic rhinitis, this results in a quick improvement of inflammatory symptoms which was confirmed in the results of the beclomethasone group. Oppositely, ectoine acts physically via a mechanism called "preferential exclusion." In the presence of ectoine, membranes and lipids are protected indirectly. As ectoine is expelled from the surface of proteins

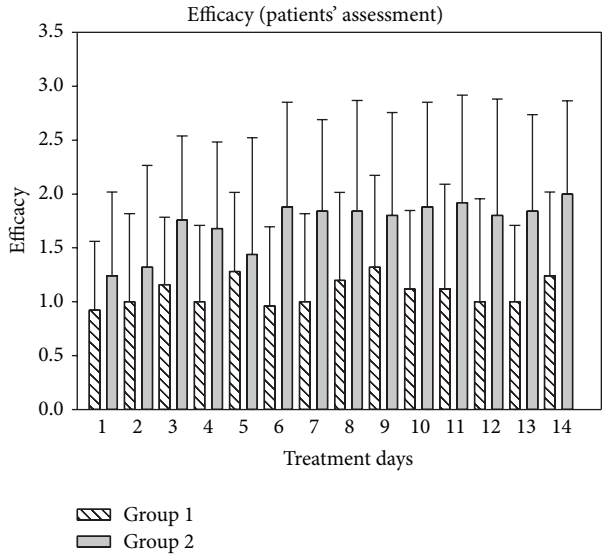

Figure 9: Efficacy assessment of treatments assessed by patients over a study period of 14 days. Mean values ± SD are depicted.

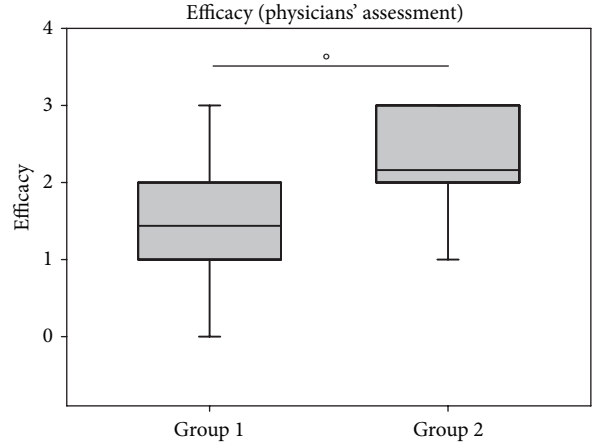

Figure 11: Assessment of efficacy of both treatments at site visit 2 (V2) by investigators. $^{\circ}P = 0.009$. Lines within the box mark the median; the upper and lower ends of the box indicate the 75th and 25th percentiles, respectively. Whiskers above and below the box indicate the 90th and 10th percentiles. Dots (•) represent outlying points.

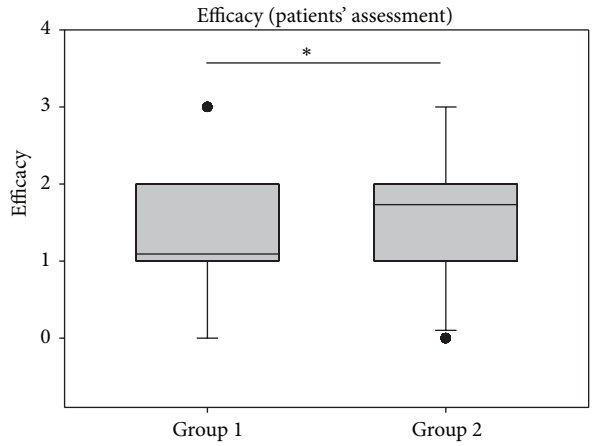

Figure 10: Assessment of efficacy of treatments in groups 1 and 2 over a period of 14 days (mean). $^{*}P < 0.001$. Lines within the box mark the median; the upper and lower ends of the box indicate the 75th and 25th percentiles, respectively. Whiskers above and below the box indicate the 90th and 10th percentiles. Dots (•) represent outlying points.

and lipids, those are protected by a water shell, thereby increasing the fluidity of membranes and resulting in the preferential formation of the native conformation of proteins [8, 16, 22–25]. This might stabilize mucous membranes such as lining epithelia of the nose, thereby protecting those from invading allergens and reducing allergen-induced inflammations as shown in different model systems [13, 26, 27] and as reported in congress report [28, 29]. It is understood that many allergens which cause allergic rhinitis symptoms have protease activities which act by impairing epithelial barrier function. This in turn results in increased penetration of allergens into nasal mucosa [30]. The barrier stabilizing properties of ectoine may counteract this scenario by improving the epithelial barrier and stabilizing membranes. In allergic rhinitis, this might protect the nasal mucosae from invading allergens, resulting in improvement of symptoms.

The study was intentionally performed as noninterventional study, reflecting the most realistic standard clinical practice and German law. However, this study design forbids randomization of patients, use of placebo, and blinding of study medication. Thus, patients were included independently of their prior medication and no wash-out period had to be kept. All patients had to show a certain degree of symptoms, measured by a minimum TNSS, at study start. Although we believe that valuable results can be drawn from noninterventional trials, one drawback of this study design is the fact that one cannot include a placebo group into the study population. On the other hand, it has been demonstrated that double-blind randomized placebo-controlled trials clearly have their limitations and disadvantages; for example, a comparison of open and controlled study designs in neuroleptic studies indicated that results of well performed open studies can earn more attention. The study design, however, reduces the grade of evidence delivered by the study data from Ib to IIa. On the other hand, it presents a realistic view of the most common clinical practice. Importantly, patient parameters in the current trial seemed to be well balanced between the two groups, with no major differences existing in terms of baseline values at the beginning, demography, history, and symptoms/health status before treatment.

As confirmed in the current study, beclomethasone acts rather quickly, and reduction of nasal symptoms was already observed within the first 12 hours of treatment. Surprisingly, the ectoine nasal spray seems to work comparably quick and results in a clear improvement of symptoms within the same time period of 12 h. Although the percentage of symptom decline was slightly larger in the beclomethasone group (decrease by −47.35%) in comparison to the ectoine group (decrease by −37.44%), decreases were both statistically

significant ($P < 0.001$). Within the following treatment days, nasal symptoms decreased further, and at the final visit, TNSS values had decreased by −51.20% in the ectoine group and by 71.49% in the beclomethasone group. The degree of improvement following treatment with nasal corticosteroids corresponds to comparable data from the literature, describing decreases of total nasal symptoms of about 40–85% [31–33]. All those studies reported that corticosteroid treatment of allergic rhinitis worked significantly better than placebo treatment, and although the current study does not include a placebo group, it allows bringing the current results into a broader context.

The decreases of TNSS as assessed by the physicians were confirmed by the patients, with stronger decreases documented in the beclomethasone group in comparison with the ectoine group. In total, patients' baseline TNSS values were lower than the physicians' scores, whereas TNSS values at the end of the study were comparable between physicians and patients assessments. This difference can be explained with the fact that physicians' assessment of baseline values took part prior to treatment, whereas the first patients' documentations of TNSS values were performed at the end of the first treatment day and confirmed the quick onset of action of both treatments.

The aim of the current study was to investigate if ectoine nasal spray is as equally effective as treatment with a glucocorticoid nasal spray. As evaluated with an equivalence test of TNSS values assessed both by physicians and by patients, noninferiority of ectoine versus beclomethasone could not be confirmed. It is noteworthy that the safety profile of both treatments was assessed as good to very good both by investigators and by patients which was underlined by the very low number of adverse events. This goes in line with reports confirming that intranasal glucocorticosteroids can be applied safely [34], even in children and for chronic rhinitis [35]. Positive treatment effects of the current study were also reflected by the results of the quality of life questionnaire which demonstrated improvements in all questioned areas covering daily life activities, general well-being, and the emotional status of patients.

Additional support to the potential efficacy of the ectoine nasal spray comes from similar studies. In a single center, double-blind, placebo-controlled cross-over study consisting of 5 visits involving patients suffering from allergic rhinoconjunctivitis, it could be demonstrated that ectoin nasal spray and eye drops relieved all of the hallmark symptoms of allergic rhinoconjunctivitis with minimal side-effects thereby showing a statistically significant effect over the placebo group. Corresponding data has been presented on a scientific congress [19]. Furthermore, additional noninterventional studies and another placebo-controlled clinical trial involving ectoine containing products in the treatment of allergic rhinitis were analyzed together. Both nasal and ocular symptoms decreased significantly upon treatment with ectoine products. The strength of effects of ectoine products was assessed by comparison of symptom scores on day 7 and baseline values on day 1 with reference products (azelastine, beclomethasone, or cromoglycic acid) or placebo treatment and showed comparable (nasal obstruction and rhinorrhea) or better (nasal itching and sneezing) efficacy of the ectoine products in comparison to control substances ([36], congress report, paper accepted).

Taken together this study supports that allergic rhinitis can be successfully treated with beclomethasone and also it was shown that ectoine nasal spray may be a future treatment option. Whereas efficacy of the pharmaceutically active steroid beclomethasone seems to be superior to that of the natural, barrier stabilizing substance ectoine, with its nonpharmaceutical mode of action, the safety profiles of both treatment groups were comparable. Thus, after proving the hints towards efficacy with further studies, ectoine containing products might become interesting alternative treatment strategies for symptom reduction in allergic rhinitis, particularly for patients seeking nonpharmaceutical treatments, as they contain a natural substance and are free of preservatives. Those alternative treatments are highly demanded but not yet generally recommended [37] and, thus, should be evaluated in more detail in further studies.

Disclosure

Bilstein is an employee of Bitop AG, a company where medical devices, including the ectoine nasal spray, were developed and registered. Bitop AG sponsored the trial discussed in this paper. Sonnemann and Möeller received sponsorship by Bitop AG to conduct the study.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

Liposomal Nasal Spray versus Guideline-Recommended Steroid Nasal Spray in Patients with Chronic Rhinosinusitis: A Comparison of Tolerability and Quality of Life

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Objective. To investigate the tolerability and impact on quality of life of liposomal nasal spray compared to guideline-recommended steroid-based therapy in patients with chronic rhinosinusitis. Symptom reduction and use of antisymptomatic medication were also examined. *Methods.* In this monocenter, prospective, controlled, open, and noninterventional study, 60 patients with chronic rhinosinusitis were treated with liposomal nasal spray and 30 patients received steroid-based therapy. The study comprised five visits occurring at intervals of two to four weeks. Efficacy was determined according to the sinusitis symptom score documented daily. The polyp score was recorded at the initial and final visits. Tolerability was determined through the Nasal Spray Evaluation Questionnaire, and quality of life was ascertained with the SNOT-20 Score. *Results*. Both treatments achieved a significant reduction of sinusitis symptoms ($P < 0.05$) and also rhinoscopic improvement ($P < 0.05$). The majority of patients assessed the treatments as "good" or "very good," and the quality of life improved significantly ($P < 0.05$). There was no significant difference in symptom reduction, QoL, and endoscopic exams between both treatments. *Conclusion*. The treatment of chronic rhinosinusitis with liposomal nasal spray results in a similar, significant reduction of symptoms and significant improvement in quality of life as guideline-recommended treatment and is therefore a comparable alternative.

1. Introduction

With a lifetime prevalence of about 5%, chronic rhinosinusitis (CRS) is one of the most frequently occurring chronic disorders worldwide [1, 2]. The German, European, and US-AWMF guidelines recommend as treatment the topical application of glucocorticoids since they represent an important treatment principle in addition to antibiotic treatment in conservative therapy [2, 3]. Nasal irrigation or sprays with hypertonic buffered solutions can also provide symptom relief in CRS disorders and are therefore recommended by guidelines. These sprays improve mucociliary clearance by liquefying nasal secretion and have been observed to have vasoconstrictive and decongestant effects [4].

Treatment alternatives should be pointed out to patients who have a critical view of guideline-recommended steroid-based therapy. One such alternative therapy concept is the nasal application of (phospholipid) liposomes. Several studies have already demonstrated the efficacy of this nonpharmacological mechanism of action in allergic rhinitis [5, 6]. Three precursor studies which investigated the application of a liposomal nasal spray in patients with seasonal allergic rhinitis showed significant symptom improvement and good tolerability of the liposomal nasal spray, also in comparison to guideline combination therapy with glucocorticoids and antihistamines [7–9].

Because the incidence of allergic rhinitis (AR) in adults with CRS is 40%–80%, liposome therapy therefore represents

an interesting, steroid-free treatment alternative [10]. Since two-thirds of patients with AR alone have steroid phobia, the probability is high that the fear of medication containing cortisone also exists among patients with CRS [11]. The liposomes, produced from phosphatidylcholine, stabilize the surfactant film and prevent the moisture film lining the airways from tearing. A liposomal nasal spray (LN) therefore represents an entirely drug-free treatment concept [12]. The present study investigates symptom reduction after the application of a LN in patients with CRS. Tolerability and the impact on quality of life were also determined.

The study was carried out in compliance with the requirements for noninterventional studies [13]. Since both products can be purchased without a prescription, it was not necessary to seek approval from an ethics committee. Nevertheless, a consultation with the competent ethics committee with respect to professional regulations took place before the study commenced.

2. Methods

2.1. Study Design. This investigation was a monocenter, prospective, controlled, open, noninterventional study (NIS). Sixty patients with CRS symptoms were treated with LN, and 30 patients received guideline-recommended therapy with a steroid nasal spray. Patients were first offered the guideline-recommended therapy. Those patients having reservations towards pharmacological therapy were alternatively offered treatment with a liposomal nasal spray. No prior wash-out period was required. Patients 18 years or older were included who due to their disorder had already been undergoing treatment at the study center.

The NIS took place from 15 March 2011 to 31 January 2013 and consisted of five visits at intervals of two to four weeks within a total treatment period of three months. Efficacy was determined on the basis of the sinusitis symptom score (SSS), which was documented daily by the patients themselves in a patient diary; furthermore, the investigator recorded the SSS at every visit. For monitoring purposes, the investigator also determined the polyp score (PS) based on the size of polyps at the first and last visits [14]. The nasal spray sensory scale was used at the first and last visits to assess tolerability [15]. Quality of life (QoL) was determined at every visit by means of the SNOT-20 score [16].

2.2. Medication. The liposomal nasal spray *LipoNasal Pflege* (LN), manufactured by Optima Pharmazeutische GmbH, Moosburg/Wang, Germany, was applied in this study. The liposomes contained in this product consist of highly purified soy lecithin, which is composed of 94% phosphatidylcholine and a small proportion of other phospholipids. Other components of the nasal spray are sodium chloride, ethanol, dexpanthenol, vitamin A palmitate, tocopherol, and water for injection. Treatment was carried out according to leaflet instructions, with the spray being applied an average of 2-3 sprays per nostril daily.

The comparative treatment used in this study was *Livocab direkt mit Beclometason 0.05%* (LB), a corticoid having an anti-inflammatory effect for nasal application, manufactured by Orion Corporation Orion Pharma, Finland. This preparation is primarily applied short-term for seasonal allergic rhinitis. Its active ingredient is beclomethasone, which in this product is available as beclomethasone dipropionate. Beclomethasone is a synthetic glucocorticoid with vasoconstrictive, immunosuppressive, antiallergic, and anti-inflammatory properties that is used to treat asthma, allergic rhinitis, and sinusitis. Beclomethasone dipropionate is used as a prodrug and is subject to a first-pass effect in the liver, thereby limiting toxicity and systemic bioavailability.

Other components of the product are benzalkonium chloride, polysorbate 80, D-glucose, microcrystalline cellulose, carmellose sodium, purified water, sodium hydroxide, and hydrochloric acid for pH regulation.

One milliliter of nasal spray contains 0.555 mg (approx. 0.05%) beclomethasone dipropionate as the active ingredient.

One spray application (approx. 0.09 mL) contains 0.05 mg beclomethasone dipropionate.

The recommended dose for patients aged 12 years or older is 2 sprays per nostril and application. The maximum daily dosage is 4 sprays per nostril.

2.3. Study Protocol. On Day 1 of treatment (Visit 1), the investigator documented the detailed medical history and the SSS as well as the PS and conducted a regular rhinoscopy. Videoendoscopy and/or a smell test were optional.

Patients documented the number of sprays applied per nostril daily. They also specified when an onset of action occurred after the first-time application of the nasal spray (<5 min, 5–10 min, 10–30 min, 30–60 min, 1-2 h, 2–4 h, 4–8 h, >8 h, no onset of action).

Efficacy was recorded by means of the SSS, which was chosen based on the EPOS Paper [17]. The present study, however, implemented a slightly adapted version of the score to enable a direct comparison with another study that investigated steroid treatment of CRS [18]. The score was recorded at every visit by the physician as well as daily by the patient in a patient diary. The five main symptoms of rhinosinusitis (rhinorrhea, nasal obstruction, headache, facial pain, and postnasal drip) were evaluated on the basis of an ordinal scale from 0 to 3 (0 = no, 1 = mild, 2 = moderate, and 3 = severe), and the individual values were added together to obtain a sum score. Furthermore, a rhinoscopic examination was conducted at every visit to ensure an additional objective assessment of efficacy. In the process, the symptoms "edema," "secretion," and "redness" were evaluated on a 3-point scale (0 = no, 1 = mild, and 2 = severe), and the rhinoscopy score (RS) was calculated thereafter from the data obtained.

In addition, polyps were measured via endoscopy at the first and last visits to monitor polyp size. The PS was calculated from these data based on a 4-point scale (0 = no polyps, 1 = small polyps, 2 = medium-sized polyps, and 3 = large polyps) [14].

The tolerability of the nasal spray was determined by means of the Nasal Spray Sensory Scale [15]. Patients answered 14 questions pertaining to sensory parameters on a visual analog scale (0 = poor evaluation and 100 = good

Table 1: Demographic data.

	Female		Male		Valid		Missing	Total
	n	%	*n*	%	*n*	%	*n*	*n*
Liposomal nasal spray	*35*	58.3	*25*	41.7	*60*	100	*0*	*60*
Beclomethasone	*16*	53.3	*14*	46.7	*30*	100	*0*	*30*

Table 2: Distribution of allergies.

	Trees		Weeds		Grasses		Mites		Mold	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Liposomal nasal spray										
Patients with allergy	*14*	24.1	*2*	3.4	*16*	27.6	*8*	13.8	*3*	5.2
Patients without allergy	*44*	75.9	*56*	96.6	*42*	72.4	*50*	86.2	*55*	94.8
Valid	*58*	100	*58*	100	*58*	100	*58*	100	*58*	100
Beclomethasone										
Patients with allergy	*8*	26.7	*1*	3.3	*6*	20	*8*	26.7	*3*	10
Patients without allergy	*22*	73.3	*29*	96.7	*24*	80	*22*	73.3	*27*	90
Valid	*30*	100	*30*	100	*30*	100	*30*	100	*30*	100

evaluation) immediately after applying the nasal spray as well as two minutes thereafter.

Quality of life was recorded using a validated questionnaire, the "Sino-Nasal Outcome Test German Adapted Version" (SNOT-20 GAV), which patients completed at every visit [19, 20]. This form consists of 20 individual questions relating to symptoms as well as social and emotional consequences, which the patient was able to assess on a 6-point scale (0 = no problem, 1 = very minor problem, 2 = small problem, 3 = moderate problem, 4 = severe problem, and 5 = it cannot get any worse). From these 20 individual questions, the patients were also able to choose the five items most important to them. In addition, the patients assessed their subjective condition daily on a visual analog scale (0 = very poor and 100 = very good).

At Visit 5, a final evaluation was made during which the investigator assessed the medication applied in terms of effect and tolerability. Patients were also able to evaluate tolerability and efficacy of the nasal spray at the end of the treatment period with a final diary entry.

2.4. Statistics. The program SPSS 21 for Windows was used to conduct the statistical analysis. To reduce any input errors, double data entry was carried out. Unreported values were treated as "missing values."

First, all data were analyzed descriptively and tested for normal distribution using the Kolmogorov-Smirnov test. In addition, the mean values of the variables from Visits 1 and 5 were compared with the aid of the t-test for paired samples. The level of significance was set at $\alpha = 0.05$.

3. Results

3.1. Homogeneity of Treatment Groups. Overall, 35 women and 25 men aged 18 to 77 years (mean age = 42 years) were included in the LN group, and 16 women and 14 men aged 22 to 74 years (mean age = 46 years) were in the comparison group. The statistical analysis and the comparison of the demographic data showed no relevant differences between the groups at the beginning of treatment (Table 1). The analysis of the symptom scores for the previous year revealed that most patients suffered from rhinoconjunctivitis complaints (LN = 55.2% and comparison group = 51.7%) and also from asthma, rhinoconjunctivitis, and conjunctivitis. Allergies were also frequent (LN = 54.2% and comparison group = 51.7%) (Table 2). Overall, 22% in the LN group and 30% in the comparison group suffered from polyps.

3.2. Onset of Action. In the LN group, the onset of action on Day 1 occurred within 30 minutes in 47.8% of the patients, with 39.1% not noticing any onset of action at all.

The onset of action on Day 1 in the beclomethasone group took place within 30 minutes in 20% of the patients, with 48% noticing no onset of action whatsoever.

3.3. Efficacy. The liposomal nasal spray and the steroid alternative were both able to improve sinusitis symptoms significantly, with rhinoscopy findings also demonstrating distinct improvement. The sinusitis symptom score in the LN group, for instance, declined from a baseline score from 6.61 (±2.668) to 3.88 (±3.674) and in the comparison group from 6.57 (±3.012) to 4.83 (±3.601) (see Table 3 and Figure 1). The sum score of the rhinoscopic evaluation also decreased in the LN group from 3.78 (±1.368) to 1.85 (±1.477) and in the steroid group from 4.26 (±1.096) to 2.30 (±1.222) (see Table 3). The analysis of the polyp scores showed no relevant change with respect to polyp size.

No relevant differences with regard to symptom reduction could be determined in the statistical analysis of the patient diaries.

Overall, the morning SSS, which consisted of the diary items "runny nose," "itching," "sneezing," "postnasal drip," "facial pain," "headache," and "nasal obstruction," was 4.06 in the LN group and 4.01 in the steroid group out of 15

Table 3: Sinusitis sum score (SSS), rhinoscopy sum score (RS), and SNOT-20 total score.

	V1	V2	V3	V4	V5	Improvement V1–V5
Liposomal nasal spray						
SSS						
MV	**6.61**	**5.00**	**4.63**	**4.45**	**3.88**	**2.73**
SD	*2,668*	*2.327*	*2.797*	*2.615*	*2.674*	*2.849*
RS						
MV	**3.78**	**2.50**	**2.28**	**1.60**	**1.85**	**1.93**
SD	*1.368*	*1.177*	*1.724*	*1.676*	*1.477*	1.639
SNOT						
MV	**32.57**	**23.64**	**21.51**	**19.57**	**18.43**	**14,14**
SD	*10.786*	*12.694*	*13.204*	*13.590*	*13.372*	*12,731*
Beclomethasone						
SSS						
MV	**6.57**	**5.26**	**4.91**	**4.91**	**4.83**	**1.74**
SD	*3.012*	*3.441*	*4.231*	*4.100*	*3.601*	*3.151*
RS						
MV	**4.26**	**3.04**	**2.65**	**2.87**	**2.30**	**1.96**
SD	*1.096*	*1.261*	*1.112*	*1.604*	*1.222*	*1.147*
SNOT						
MV	**39.91**	**30.04**	**28.04**	**25.83**	**26.00**	**13.91**
SD	*19.776*	*18.165*	*19.427*	*21.777*	*22.076*	*19.246*

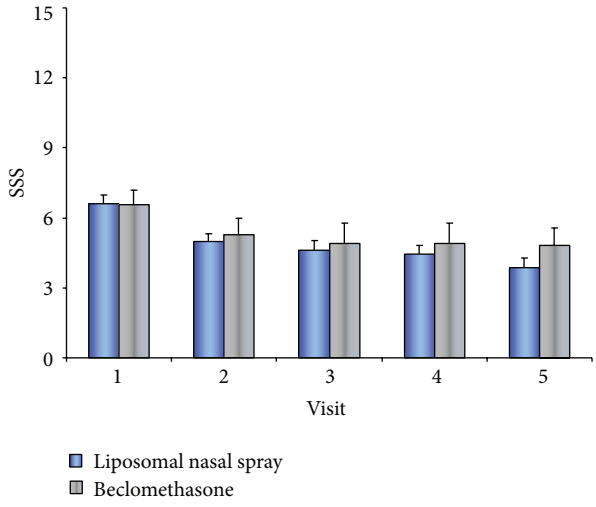

Figure 1: Sinusitis symptom score over the course of five visits in both groups.

possible points. Over the three-month observation period, the patients' daily documented sinusitis sum scores in both groups decreased from approximately 4 at baseline to 2.16 on Day 86. The difference between both groups was not significant.

At the final evaluation, the majority of the patients in both groups rated efficacy "good" or even "very good" (Table 7).

3.4. Tolerability and Safety. In the immediate evaluation of the Nasal Spray Sensory Scale, the steroid nasal spray group achieved somewhat better results than the LN group (P = 0.178), but ultimately there was no significant difference between both groups at V5 (P = 0.564) (Table 4).

In the evaluation after two minutes, both groups showed higher values, which means that the application was perceived as more pleasant. The value for the LN group was 80.4 at Visit 1 and 78.8 at Visit 5. In the group receiving beclomethasone, the value was 85.1 at Visit 1 and 78.3 at Visit 5. No significant difference existed between both nasal sprays comparing its tolerability immediately (P = 0.594) or after 2 minutes (P = 0.815), neither at V1 nor at V5 (Table 4).

In the final assessment of tolerability, the majority of evaluable patients in both groups rated both treatments "good" or even "very good" (Table 7).

Overall, both treatment modalities were tolerated well; no significant difference between both groups was observed (P = 0.306).

3.5. Dropouts and Adverse Events. Altogether, 20 patients from the LN group and seven patients from the cortisone group dropped out of the study. In most cases, the reasons for discontinuation remained unknown; only one patient from the LN group and two patients from the cortisone group dropped out of the study because of an adverse event (AE). AEs occurred in a total of 23 patients, 10 events of which were reported in the LN group and 13 in the group receiving the steroid nasal spray.

Table 4: Sensory evaluation immediately and two minutes after application.

	Immediately after application		Two minutes after application	
	V1	V5	V1	V5
Liposomal nasal spray				
MV	**75.25**	**72.42**	**80.39**	**78.81**
SD	*13.933*	*14.826*	*16.146*	*15.686*
Beclomethasone				
MV	**80.34**	**73.82**	**85.13**	**78.35**
SD	*13.549*	*16.714*	*11.2686*	*17.975*

One patient from the LN group and five patients from the beclomethasone group also experienced a second AE (Table 5).

None of these incidents were documented by the study investigators as serious adverse events in the serious adverse event (SAE) form. An association with the study drug could not be ruled out for five AEs in the beclomethasone and eight AEs in the LN group.

3.6. Quality of Life. The application of both preparations resulted in a significant improvement in quality of life as early as V2 ($P \leq 0.05$). The treatments themselves did not differ from each other significantly ($P \geq 0.05$).

In the LN group, therapy caused a drop in the total sum score of the SNOT-20 Quality of Life Scale from 32.57 ± 10.786 to 18.43 ± 13.372; the score decreased in the comparison group from 39.91 ± 19.776 to 26 ± 22.076 (see Table 3 and Figure 2). When dividing the SNOT-20 Quality of Life Scale into "primary nasal symptoms," "secondary rhinogenic symptoms," and "general quality of life," significant improvements could also be observed within these subareas ($P \leq 0.05$) (Table 6). No significant difference existed between the groups.

Besides the evaluation of the SNOT-20, patients recorded their subjectively perceived condition daily in a diary as a further parameter for determining quality of life.

Figure 3 shows the three-month course of the patients' mean subjective condition over the treatment period.

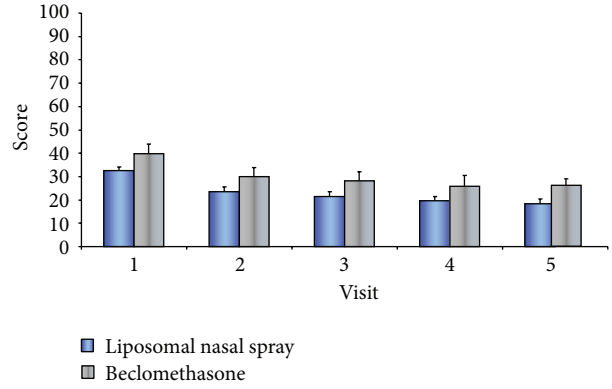

Figure 2: Course of the SNOT total score V1 to V5.

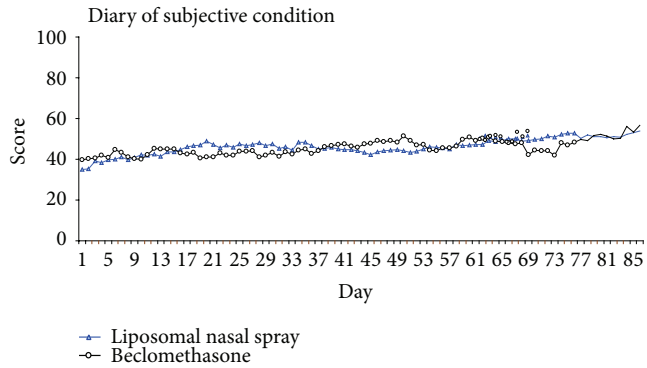

Figure 3: Diary assessment of the subjective condition of patients.

4. Discussion

Besides antibiotics, topical treatment with corticosteroids is the guideline-recommended treatment of choice for symptomatic CRS [2], although so-called "cortisone phobia" represents an increasing problem. This circumstance often results in lacking patient compliance and the change to an alternative steroid-free medication [21]. As demonstrated in previous studies on allergic rhinitis and rhinoconjunctivitis, therapy with a liposomal nasal spray is an equally effective and tolerable treatment alternative [7–9]. In terms of efficacy, it is assumed that the phospholipids supplemented via nasal spray stabilize or restore the impaired "nasal surfactant," thereby maintaining the natural moisture film protecting and moisturizing the nasal mucosa and as a basis of mucociliary clearance [12]. The present study was able to illustrate that the application of liposomes also represents a promising treatment option for CRS. Since CRS is often accompanied by allergic rhinitis, a possible explanatory approach may also lie in the wound-healing and anti-inflammatory properties of liposomes [22]. The barrier function of the nasal mucus layer is impaired due to the inflamed mucosa. Liposomes primarily consist of phosphatidylcholine, which in terms of quantity make up the largest proportion of nasal surfactant and are thus able to stabilize the liquid film that moisturizes and protects the mucus membrane [12].

Since symptom scores can always be subjectively influenced [23] and to obtain the most objective evaluation of efficacy as possible, we chose a combination of investigator evaluation and patient evaluation. The symptoms for the SSS, which patients entered in their diaries and the physician filled out at the visits, were selected based on the EPOS Paper 2007 [17]; for better comparability with a steroid, however, the study implemented a slightly adapted score [18]. Ultimately, the items "nasal secretion," "postnasal drip," "facial pain," "headache," and "nasal obstruction" were used. Application of the liposomal nasal spray led to a significant improvement in the SSS of 2.7 from V1 to V5, corresponding to improvement by 41.4%; improvement in the comparison group was 26.5%. The sum score of the rhinoscopic evaluation also decreased significantly in the LN group from 3.78 to 1.85 (51% improvement) and in the steroid group from 4.26

Table 5: Adverse events.

	(1) Adverse event	(2) Adverse event
Beclomethasone	Acute sinusitis	Acute rhinitis
	Minor hemorrhoid bleeding	Acute viral rhinopharyngitis
	Cephalgia	Acute sinobronchitis
	Acute bacterial sinusitis	Acute bacterial sinusitis
	Rhinitis, cough	
	Acute viral rhinopharyngitis	
	Viral upper respiratory tract infection	
	Acute viral infection	
	Acute exacerbation of CRS	
	Gastroenteritis	Tonsillitis
	Acute viral rhinopharyngitis with rhinosinusitis	
	Bronchitis	
	Dysesthesia of nasal mucosa and facial pain	
Liposomal nasal spray	Recurrence of chronic lymphatic leukemia	
	Acute exacerbation of chronic rhinosinusitis	
	Acute bronchitis	
	Cephalgia	Fatigue
	Rhinitis	
	Infection of paranasal sinuses	
	Capsulitis DII right hand	
	Arthrosis of both hip joints	
	Acute exacerbation of chronic pansinusitis	
	Acute sinusitis	

Table 6: SNOT-20 subscales:primary nasal symptoms, secondary rhinogenic symptoms, and general quality of life.

	V1	V2	V3	V4	V5	Improvement V1–V5
Liposomal nasal spray						
Primary nasal symptoms						
MV	**40.24**	**31.02**	**28.57**	**26.20**	**24.90**	**15.34**
SD	*14.976*	*13.204*	*17.531*	*18.295*	*18.042*	*−3.066*
Secondary rhinogenic symptoms						
MV	**32.52**	**20.68**	**21.97**	**19.52**	**18.44**	**14.08**
SD	*17.220*	*15.811*	*14.528*	*14.337*	*14.563*	*2.657*
General quality of life						
MV	**29.22**	**21.42**	**18.39**	**16.78**	**15.70**	**13.52**
SD	*16.726*	*17.451*	*16.341*	*16.217*	*15.634*	*1.092*
Beclomethasone						
Primary nasal symptoms						
MV	**45.00**	**33.33**	**31.33**	**28.33**	**28.50**	**16.5**
SD	*3.012*	*3.441*	*4.231*	*4.100*	*3.601*	*−0.589*
Secondary rhinogenic symptoms						
MV	**35.00**	**28.89**	**27.64**	**25.14**	**24.86**	**10.14**
SD	*19.009*	*17.629*	*20.489*	*20.990*	*21.714*	*−2.731*
General quality of life						
MV	**40.10**	**29.18**	**26.57**	**25.51**	**25.99**	**14.11**
SD	*24.255*	*21.496*	*22.267*	*25.662*	*25.806*	*−1.551*

Table 7: Patients' final evaluation of efficacy and tolerability.

	Efficacy	Tolerability
Liposomal nasal spray		
Very good	17.8%	39.4%
Good	53.8%	50%
Satisfactory	17.9%	7.9%
Poor	10.3%	2.6%
Beclomethasone		
Very good	17.4%	26.1%
Good	47.8%	60.9%
Satisfactory	13%	4.3%
Poor	21.7%	8.7%

to 2.3 (46% improvement). In a precursor study on allergic rhinoconjunctivitis, the application of the liposomal nasal spray resulted in nasal symptom relief of 33.2% and global improvement of 41.4%, which support the results of the present study [9].

Overall, tolerability of the liposomal nasal spray was assessed positively; 50% of the valid percentages rated tolerability "good," 39.4% "very good," 7.9% "satisfactory," and only 2.6% evaluated tolerability "poor." Some patients commented on the smell of the liposomal spray. Since it was decided to deliberately forgo the addition of artificial aromas in the product to avoid possible allergic reactions or intolerances, the natural scent of lecithin (phospholipids) is perceptible.

4.1. Quality of Life. A study by Rudmik and Smith showed that CRS leads to a significant loss of quality of life, among other things due to symptoms such as sleeplessness, headache, and facial pain, and also emotional consequences such as sadness and a sense of shame [24]. In this study, a significant decrease resulted in the total sum score of the SNOT-20 Quality of Life Scale and in the subareas "primary nasal symptoms" and "secondary rhinogenic symptoms" as well as in the area "general quality of life." Thus, significant improvement in quality of life could be verified through the use of a nonpharmacological product in CRS. Both preparations, however, do not differ significantly.

5. Conclusion

All in all, both of the applied treatments led to significant improvement in the patients' condition, with no significant differences resulting between both study medications for the most part.

The values calculated in this study show that liposomal nasal spray is an effective treatment alternative for patients with CRS. Its application resulted in significant symptom reduction and improved quality of life. Furthermore, the majority of patients assessed its tolerability very positively. Liposomal nasal spray is therefore a suitable steroid-free method for treating CRS, particularly for patients who take a somewhat critical view of guideline-recommended therapy with cortisone.

Abbreviations

AE: Adverse event
AR: Allergic rhinitis
CRS: Chronic rhinosinusitis
LN: Liposomal nasal spray
LB: Livocab beclomethasone
NIS: Non-interventional study
PS: Polyp score
QoL: Quality of life
RS: Rhinoscopy score
SAE: Serious adverse event
SSS: Sinusitis symptom score.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Meta-Analysis of the Efficacy of Ectoine Nasal Spray in Patients with Allergic Rhinoconjunctivitis

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Objectives. The meta-analysis aims to investigate the efficacy of ectoine nasal spray and eye drops in the treatment of allergic rhinitis and rhinoconjunctivitis symptoms. *Design and Methods.* This meta-analysis is based on yet unpublished data of four studies. Both nasal and eye symptoms were documented in patient diary cards. All scales were transformed into a 4-point scale: 0 = no, 1 = mild, 2 = moderate, and 3 = severe symptoms. Each symptom was analysed individually in a meta-analysis of the area under the curve values as well as in a meta-analysis of pre- and posttreatment comparison. *Results.* After seven days of treatment with ectoine nasal spray both nasal and ocular symptoms decreased significantly. A strong reduction of symptom severity was shown for the parameters rhinorrhoea (31.76% reduction) and nasal obstruction (29.94% reduction). Furthermore, the meta-analyses of individual symptoms to investigate the strength of effect after seven days of medication intake showed significant improvement for nasal obstruction, rhinorrhoea, nasal itching, sneezing, itching of eyes, and redness of eyes. The improvement of the symptom nasal obstruction was associated with a strong effect 0.53 (±0.26). *Conclusions.* The ectoine nasal spray and eye drops seem to be equally effective as guideline-recommended medication in the treatment of rhinoconjunctivitis symptoms.

1. Introduction

Allergic rhinitis is clinically defined as an inflammation of the nose with characteristic symptoms such as rhinorrhoea, nasal obstruction, sneezing, and/or itching of the nose. The symptomatic disorder of the nasal mucosa and tissue is associated with an IgE-mediated immune response to allergens and is characterised by two phases: an immediate response after allergen exposure (early phase) and a late phase occurring up to 12 hours later, which predominantly causes nasal congestion [1]. If a concurrent respiratory infection is present, a patient's probability of developing bronchial asthma as comorbidity increases. Likewise, the risk of developing further allergies with more severe symptoms rises over the time of the disease [2].

A variety of causes for rhinitis exist in both children and adults, but 50% of all cases can be ascribed to allergy [3]. Due to its prevalence, impact on quality of life, impairment of work or school performance, reducing effect on productivity, economic burden, and risk of comorbidities, allergic rhinitis is regarded worldwide as a major chronic respiratory disease. Moreover, it can be associated with significant fatigue, mood changes, cognitive impairments, depression, and anxiety [4–8].

The optimal treatment of allergic rhinitis depends on several individual factors. A stepwise therapeutic approach, however, is generally recommended. Current guidelines favour second-generation oral or topical H1 antihistamines for treating allergic rhinitis [1, 9, 10]. Moreover, intranasal glucocorticosteroids and intranasal decongestants are highly recommended as effective treatments for nasal blockage [11].

Ectoine (2-methyl-1,4,5,6-tetrahydropyrimidine-4-carbox-yclic acid) is a compatible solute which is naturally produced by bacteria, conferring resistance to external stress factors such as extreme temperatures, high salt concentrations, and ultraviolet radiation. It acts via a mechanism called "preferential exclusion" and "preferential hydration" [12]. Ectoine is expelled from proteins or lipid membranes,

resulting in the modulation of the solvent characteristic of surrounding water. Thus, ectoine is able to form a protective and stabilising hydrate capsule around the protein and therefore helps to protect biomolecules and proteins from irreversible structural modifications by inhibiting dehydration. This indirect effect leads to a more compact and more stable folding of proteins and increases the stability of lipid membranes by increasing their fluidity [13]. The effect derives from the mechanism of halophilic bacteria which stabilises the osmotic balance in the microorganic cell, where extremolytes such as ectoine are accumulated in the cytosol to equal out the varying salt concentration in the outer area [14, 15]. Stabilisation of membranes such as those lining the airways or eyes might reduce the potential water loss of such membranes and protect them against invading allergens, thereby limiting the inflammatory cascade induced by stress mediators at the membrane level, as has been shown for lung epithelia and skin cells [16]. *In vitro* experiments have further shown that ectoine inhibits apoptosis, triggered by nanoparticles [17], and likewise blocks the activity of ceramides, which are regarded as central molecules in the sphingolipid metabolism as well as in the induction of apoptosis [18]. Currently, ectoine is used in dermatological products for successfully treating skin diseases such as atopic dermatitis [19]. Still widely unknown is the use of ectoine in nasal sprays or eye drops. In such medical devices, ectoine may strengthen the hydroprotection of the nasal membrane and may alleviate the infection of the inflamed tissue [20].

Toxicological studies and results of human studies reflect the excellent safety profile of products containing ectoine, therewith making them promising candidates for the treatment of allergic rhinoconjunctivitis [14, 20].

With this meta-analysis we aimed to investigate the efficacy of ectoine nasal spray in the treatment of allergic rhinitis and rhinoconjunctivitis symptoms.

2. Material and Methods

2.1. Literature Search. In order to investigate the efficacy of treatment with ectoine, data from published as well as unpublished clinical studies were reviewed.

Bitop AG, a German medical device company, kindly supplied us with detailed results from several clinical and noninterventional studies launched between 2008 and 2011 investigating its allergy nasal spray based on ectoine. Additionally, we conducted a systematic and comprehensive search of scientific and medical databases for further studies and reports published until January 2013. For this purpose, a catalogue of search criteria was generated in due consideration of the question posed by this meta-analysis. Using PubMed's MeSH database, the literature search was based on the following search criteria: "Ectoin," "ectoine", "(*S*)-2-Methyl-1,4,5,6-tetrahydropyrimidin-4-carbonsäure," "$C_6H_{10}N_2O_2$", "1,4,5,6-tetrahydro-2-methyl-4-pyrimidine-carboxylic acid," "cryoprotective cyclic amino acid," and "rhinitis." Although several electronic databases were searched including *PubMed, Medline, Medpilot, Web of Science, CENTRAL*, *EMBASE,* and *Google Scholar,* no further studies on this topic were found. Given the lack of appropriate hits, no additional limits regarding language, participants, publishing date, or study phase were set.

Therefore, this meta-analysis is based on unpublished data provided by Bitop AG. The study data have not been published to date since the number of participants in each trial was too small. Nowadays, large randomised controlled trials with more than 250 patients per treatment group are usually required to be considered for publication [21, 22]. In total four studies were assessed which fulfilled the inclusion criteria described below. The paediatric, randomised controlled study had been formally approved by the respective ethical review committee, whereas no ethical approval was necessary for observational trials in Germany. In all studies, patients had to sign the informed consent form to be eligible for participation.

2.2. Patients and Outcome Parameters. The study population comprised both adults and children with a history of allergic rhinitis or rhinoconjunctivitis, who recorded their daily allergy symptoms for at least 7 days in a patient diary. Each symptom had to be scored numerically on a 4-point scale (0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, and 3 = severe symptoms). In case of different scaling schemes applied in a study, scores were adapted to this 4-point scale for comparability reasons.

The primary efficacy parameter was the improvement of each individual symptom (nasal congestion, rhinorrhoea, itching of the nose, sneezing, red/watery eyes, and itching of the eyes) after 7 days of treatment. Generally, patient reported that rhinitis-related symptoms occurred in the nose, eyes, and ears/palate, whereas nasal congestion and rhinorrhoea were frequently reported as most predominant.

2.3. Statistical Methods. For continuous data, we calculated individual and pooled statistics as mean differences with 95% confidence intervals. The efficacy parameters for each study included in the analysis were analysed using the ANOVA model [23]. Scores for each individual symptom after 7 days of medication intake were evaluated in comparison to the baseline values at Day 1. All deviating scaling systems for rating the intensity of rhinitis symptoms were adapted to a 4-point scale. If symptoms were originally rated from 0 to 8 (0 being no symptoms and 8 being very severe symptoms) the scores were transformed according to the following scheme: 0, 1 = no symptoms; 2, 3 = mild symptoms; 4, 5 = moderate symptoms; 6, 7, 8 = severe symptoms. Likewise, 12-point scales were translated into 0, 1, 2 = no symptoms; 3, 4, 5 = mild symptoms; 6, 7, 8 = moderate symptoms; 9, 10, 11, 12 = severe symptoms. In case of missing data the last-value-carried forward method was applied. If data of Day one were not available, we used the score of the following day as baseline value. Additionally, the area under the curve (AUC) from Day 1 to Day 7 was assessed for each symptom. The AUC expresses the cumulative effect of the investigational products over the course of seven days by adding up the baseline adjusted symptom scores of each day. A noninferiority margin δ to ensure a clinically relevant effect was not determined, since

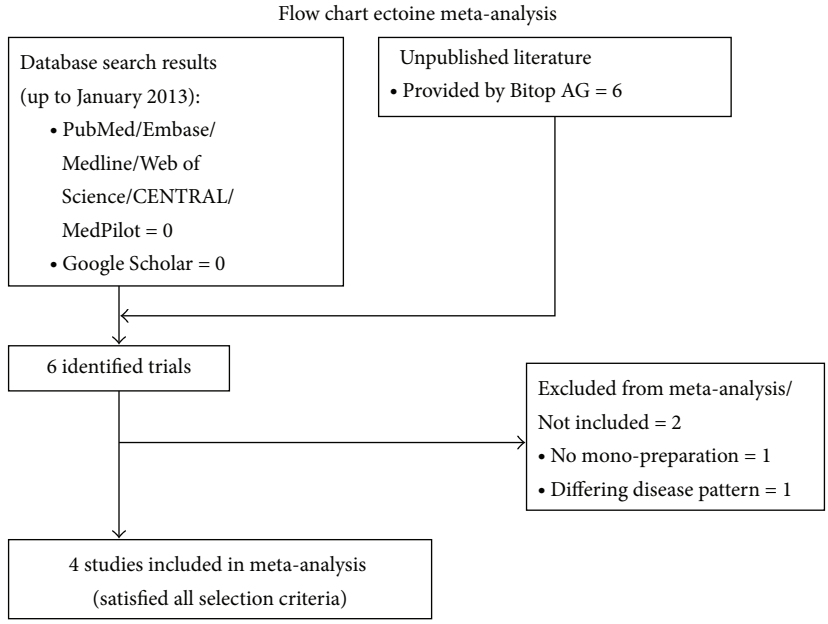

Figure 1: Flow chart.

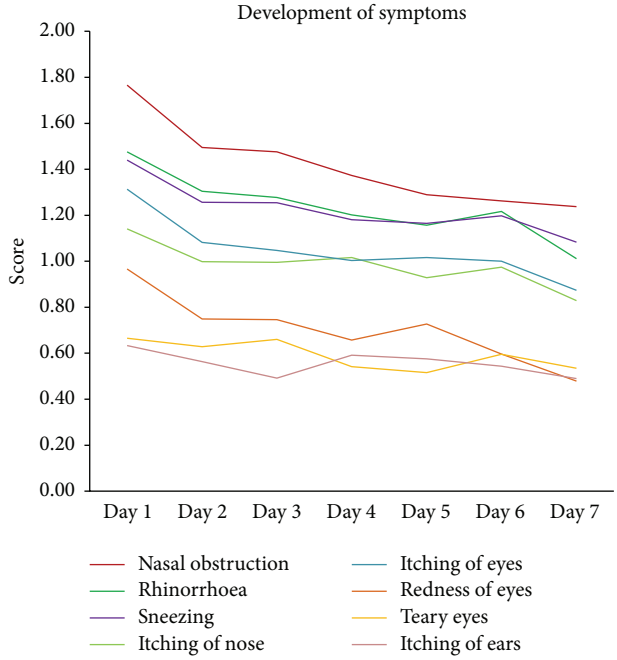

Figure 2: Development of symptoms.

no solid historical data were available. Thus, noninferiority was assumed, when the 95% confidence interval of the overall effect size included the neutral number "0."

Results were displayed graphically as forest plots with associated 95% confidence intervals according to Clopper and Pearson [24]. The area of each square (point estimator for odds ratio) is proportional to the weight of the corresponding study and therefore proportional to the number of patients included as well as to the precision of the effect. Heterogeneity was assessed using I^2 statistics and the random-effect model was applied for data synthesis [25].

SPSS version 19 and Review Manager 5 (RevMan 5) were used for statistical analyses and quantitative data synthesis.

3. Results

3.1. Literature Search and Study Population. We identified six studies with unpublished data, provided and conducted by Bitop AG, which matched our inclusion criteria (see Figure 1). One study investigating ectoine in combination with dexpanthenol had to be excluded, since the additional active agent dexpanthenol instead of a mono-preparation would have introduced a severe bias to this meta-analysis. Another study, which investigated patients suffering from Rhinitis Sicca, was rejected because of the differing disease pattern. Thus, the meta-analysis was based on data from four unpublished studies. Of these, three studies included only adults, while one study investigated the efficacy of ectoine in children. Details on the integrated studies are shown in Table 1.

All studies were performed in ENT medical practices in Germany.

In total, 112 patients were included in the analyses comparing the symptom scores on Day 7 and at baseline (Day 1), while the meta-analysis based on the AUC comprised 213 participants. This difference was due to unbalanced numbers of patients in each group of comparison. We performed the meta-analysis in line with a statement proposed by the international MOOSE group [26] about the conduct of meta-analyses of observational studies. Their recommendations concern the entire process of performing a meta-analysis—from describing background, search strategy,

Table 1: Description of included studies.

Study ID	Indication of study	Comparator	Study design	*N* (Ectoine and control)	Inclusion criteria/exclusion criteria	Duration and dosage	Outcome Parameter	Rating scales
Ectoine versus azelastine, 2010	Allergic rhinitis and conjunctivitis	Azelastine nasal spray and azelastine eye drops	Observational	Ectoine: 21 Control: 21	AR proven by a diagnostic tool or by an allergist Moderate-to-strong nasal symptoms and at least mild eye symptoms at inclusion Signed informed consent Exclusion: allergy to ectoine or azelastine, pregnancy, operation of nose	1 week, ectoine: 4 times daily Azelastine: 2 times daily	Primary: Nasal congestion, rhinorrhoea, sneezing, nasal itching, itching of eyes, itching of palate, teary eyes, conjunctivitis. Secondary: Efficacy, safety.	8-point scale
Ectoine versus cromoglicic acid 2009	Allergic rhinitis	Cromoglicic nasal spray	Observational, cross-over trial	Ectoine: 25 Control: 25	Inclusion: AR proven by skin prick test or by an allergist Moderate-to-strong nasal symptoms at inclusion Signed informed consent Exclusion: allergy to ectoine or cromoglicic acid, pregnancy, operation of nose	1 week, ectoine: 5 times daily Cromoglicic acid: 4 times daily	Primary: Nasal congestion, rhinorrhoea, sneezing. Secondary: Itching of eyes, itching of palate, teary eyes, conjunctivitis, nasal concha hyperplasia, efficacy, safety.	8-point scale
Ectoine versus Livocab, 2011	Seasonal allergic rhinitis	Levocabastine with beclomethasone 0.05% (nasal spray)	Observational	Ectoine: 25 Control: 25	Inclusion: 18–70 years Signed informed consent	2 weeks, intake of medication according to prescribing information	Primary: Total Nasal Symptom Score as sum of nasal congestion, rhinorrhoea, sneezing and nasal itching. Secondary: Itching of palate and ears, efficacy, safety.	4-point scale
Paediatric trial Ectoine versus placebo, 2011	Seasonal allergic rhinitis	Placebo nasal spray and eye drops	RCT	Ectoine: 41 Control: 30	Inclusion: 5–17 years Seasonal AR, general good health status, free of any concomitant conditions that could interfere with conduct of study, sum of TNSS >5, sum of TOSS >3 Signed informed consent (also by parents)	2 weeks Nasal spray: 1 puff per nostril 3 times daily Eye drops: 3 times daily	Primary: Safety Secondary: Efficacy assessment, Total Nasal Symptom Score as sum of runny nose, itchy nose, nasal congestion, sneezing; Total Ocular Symptom Score as sum of itchy eyes, red eyes, watery eyes.	4-point scale

Study or subgroup	Baseline Mean	SD	Total	Day 7 Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Ectoine versus Azelastine	1.9	0.94	21	1.48	1.08	21	18.3%	0.42 [−0.19, 1.03]
Ectoine versus Cromoglycin	1.92	0.91	25	1.2	0.87	25	28.1%	0.72 [0.23, 1.21]
Ectoine versus Livocab	1.68	0.99	25	1.08	1	25	22.5%	0.60 [0.05, 1.15]
Pediatric trial	1.56	1.18	41	1.2	0.98	41	31.1%	0.36 [−0.11, 0.83]
Total (95% CI)			**112**			**112**	**100.0%**	**0.53 [0.26, 0.79]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 1.26$, df = 3 ($P = 0.74$); $I^2 = 0\%$
Test for overall effect: $Z = 3.94$ ($P < 0.0001$)

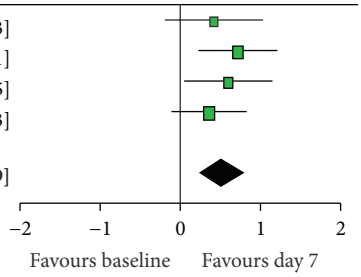

Figure 3: Nasal obstruction.

Study or subgroup	Baseline Mean	SD	Total	Day 7 Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Ectoine versus Azelastine	1.71	0.9	21	1.05	0.97	21	17.4%	0.66 [0.09, 1.23]
Ectoine versus Cromoglycin	1.4	0.91	25	0.96	0.84	25	23.7%	0.44 [−0.05, 0.93]
Ectoine versus Livocab	1.52	1.01	25	1.28	0.94	25	19.1%	0.24 [−0.30, 0.78]
Pediatric trial	1.27	0.95	41	0.76	0.77	41	39.8%	0.51 [0.14, 0.88]
Total (95% CI)			**112**			**112**	**100.0%**	**0.47 [0.23, 0.70]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 1.19$, df = 3 ($P = 0.76$); $I^2 = 0\%$
Test for overall effect: $Z = 3.88$ ($P = 0.0001$)

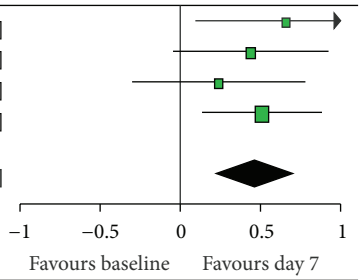

Figure 4: Rhinorrhoea.

Study or subgroup	Baseline Mean	SD	Total	Day 7 Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Ectoine versus Azelastine	1.29	0.9	21	0.81	0.75	21	28.6%	0.48 [−0.02, 0.98]
Ectoine versus Livocab	1.04	0.94	25	0.92	0.91	25	27.3%	0.12 [−0.39, 0.63]
Pediatric trial	1.1	1	41	0.76	0.86	41	44.1%	0.34 [−0.06, 0.74]
Total (95% CI)			**87**			**87**	**100.0%**	**0.32 [0.05, 0.59]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.99$, df = 2 ($P = 0.61$); $I^2 = 0\%$
Test for overall effect: $Z = 2.34$ ($P = 0.02$)

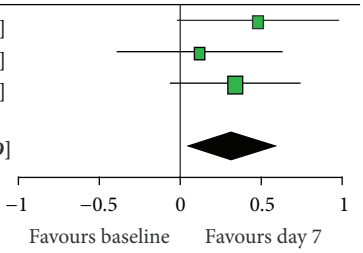

Figure 5: Nasal itching.

Study or subgroup	Baseline Mean	SD	Total	Day 7 Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Ectoine versus Azelastine	1.81	0.87	21	1.24	0.89	21	21.0%	0.57 [0.04, 1.10]
Ectoine versus Cromoglycin	1.32	1.03	25	1	0.91	25	20.6%	0.32 [−0.22, 0.86]
Ectoine versus Livocab	1.24	0.93	25	1.24	0.83	25	24.4%	0.00 [−0.49, 0.49]
Pediatric trial	1.39	1	41	0.85	0.85	41	34.0%	0.54 [0.14, 0.94]
Total (95% CI)			**112**			**112**	**100.0%**	**0.37 [0.11, 0.63]**

Heterogeneity: $\tau^2 = 0.01$; $\chi^2 = 3.47$, df = 3 ($P = 0.33$); $I^2 = 13\%$
Test for overall effect: $Z = 2.79$ ($P = 0.005$)

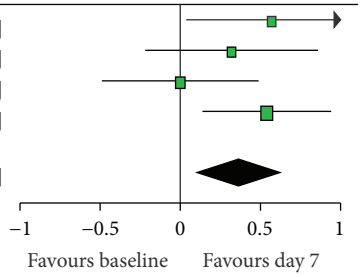

Figure 6: Sneezing.

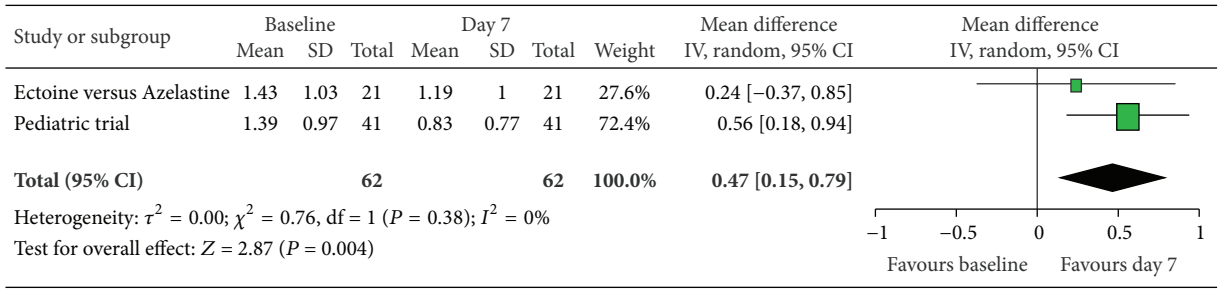

Figure 7: Itching of eyes.

and methodology applied to presentation of results and discussion.

All data presented are based on the ITT analysis set of each study.

3.2. Bias. As with any systematic review or meta-analysis, biases may be present and limit the validity of the work. The main concern of this meta-analysis may be the quality of the included studies. In contrast to large systematic reviews, this work was mainly based on observational studies with no blinding of the patients or investigators. Since only one randomised, placebo controlled trial on children has been performed and published on this specific topic, the methodological concepts of the included trials do reach the evidence level IIb but not Ib. We addressed this "garbage in/garbage out" problem [27, 28] in performing a subgroup analysis on observational studies with adults. Apart from that no major conceptional differences between the studies were apparent, which minimises the risk concerning problems with uniformity ("apple-oranges problem") [29]. Endpoints, nasal symptoms, measurements, and the study population were comparable. However, scaling systems in rating the symptom severity differed slightly and had to be transformed into a homogeneous scaling scheme. It is questionable whether this adaption leads to a loss of information or a shift of results. However, the tendency of whether symptoms were released or not is not biased by this approach. Furthermore, the variation of control groups may limit the validity of the meta-analysis. Ectoine was compared to four different control medications, since the major interest was about the efficacy of the active agent ectoine in comparison to general drugs prescribed. As already mentioned before, this meta-analysis was based on only small, unpublished clinical trials. Thus, one can speak of a very untypical publication bias with solely data from yet unpublished studies. Given the small number of included studies, we refrained from performing a funnel plot.

3.3. Development of Symptoms. Figure 2 illustrates the cumulative efficacy of ectoine-based products on both nasal and eye symptoms based on results from the included studies. The descending curve progression affirmed the positive effect of ectoine on rhinitis-related symptoms. At baseline, nasal obstruction presents the most predominant symptom of the allergic disease. After seven days of treatment, each symptom had improved to a mild level of discomfort (see Figure 2). The strongest decrease in nasal symptom severity was shown for rhinorrhoea and nasal obstruction, both being reduced by approximately 30%. For nasal obstruction, a symptom score of 1.77 at Day 1 decreased to a mean score of 1.24 and the symptom severity of rhinorrhoea eased from 1.48 to 1.01 after seven days of treatment with ectoine nasal spray.

According to the patients' diary entries, however, none of the symptoms was assessed as moderate or severe at baseline, but mild to moderate at the most. The rather mild assessment of symptoms at baseline limited the prospects of significant improvement. However, the apparent decrease in symptom severity suggests the efficacy of ectoine-based treatment.

3.4. Meta-Analyses as Comparison of Baseline (Day 1) to Day 7. The meta-analyses of individual symptoms that were conducted to determine the strength of effect after seven days of medication intake compared to baseline indicated the efficacy of ectoine. All nasal symptoms had significantly improved by Day 7 compared to Day 1.

According to Ferguson the effect size of improvement can be classified in three categories: 0–0.2 reflecting a small effect, 0.2–0.5 indicating a moderate effect, and 0.5–0.8 representing a strong effect [30]. Therefore, the improvement of the main nasal symptom "nasal obstruction" (Figure 3) with a size effect of 0.53 (±0.26) was evaluated as strong. Further nasal symptoms still showed significant moderate effects. The effect size for "rhinorrhoea" (Figure 4) was nearly as high with 0.47 (±0.24), "nasal itching (Figure 5) was calculated as 0.32 (±0.27), and for "sneezing" (Figure 6) the effect size was 0.37 (±0.26). P values of the overall effect (shown underneath each figure) demonstrate significance for all nasal symptoms: both "nasal obstruction" and "rhinorrhoea" were associated with $P < 0.0001$; the symptom "nasal itching" corresponds to $P = 0.02$; the P value for "sneezing" was calculated as $P = 0.005$.

Furthermore, we pooled data from two studies that additionally used ectoine-based eye drops to investigate the effect of ectoine on eye symptoms. After seven days of treatment, "itching of eyes" (Figure 7) and "redness of eyes" (Figure 8) showed significant improvements compared to baseline. Both parameters improved by a moderate-to-strong effect size with 0.47 (±0.32) and 0.54 (±0.30), respectively. Only the reduction of symptom severity in "teary eyes" (Figure 9) was not statistically significant.

Study or subgroup	Baseline Mean	Baseline SD	Baseline Total	Day 7 Mean	Day 7 SD	Day 7 Total	Weight	Mean difference IV, random, 95% CI
Ectoine versus Azelastine	0.95	0.92	21	0.48	0.68	21	37.6%	0.47 [−0.02, 0.96]
Pediatric trial	1.15	0.94	41	0.56	0.81	41	62.4%	0.59 [0.21, 0.97]
Total (95% CI)			**62**			**62**	**100.0%**	**0.54 [0.24, 0.84]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.14$, df = 1 ($P = 0.70$); $I^2 = 0\%$
Test for overall effect: $Z = 3.56$ ($P = 0.0004$)

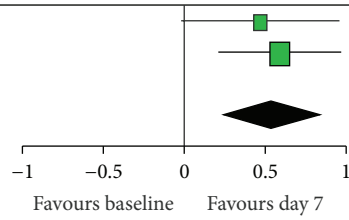

Figure 8: Redness of eyes.

Study or subgroup	Baseline Mean	Baseline SD	Baseline Total	Day 7 Mean	Day 7 SD	Day 7 Total	Weight	Mean difference IV, random, 95% CI
Ectoine versus Azelastine	0.62	0.67	21	0.62	0.74	21	48.7%	0.00 [−0.43, 0.43]
Pediatric trial	0.98	1.06	41	0.59	0.81	41	51.3%	0.39 [−0.02, 0.80]
Total (95% CI)			**62**			**62**	**100.0%**	**0.20 [−0.18, 0.58]**

Heterogeneity: $\tau^2 = 0.03$; $\chi^2 = 1.67$, df = 1 ($P = 0.20$); $I^2 = 40\%$
Test for overall effect: $Z = 1.03$ ($P = 0.30$)

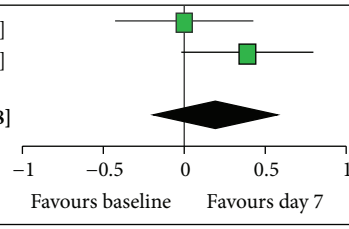

Figure 9: Teary eyes.

Throughout the analyses, the level of heterogeneity was low. As suggested by Higgins et al. [31], values for $I^2 < 25\%$ may express a low level of heterogeneity, although its categorisation and quantification are not that simple in general. Since I^2 was calculated to be in a range of 0% to 13% for most symptoms (apart from the symptom "teary eyes"), the heterogeneity across studies appears to be small.

3.5. Meta-Analyses of the Area under the Curve (AUC) Comparing Ectoine with Control Medication. The meta-analyses of AUC, comprising Day 1 to Day 7, evaluated the efficacy of ectoine treatment in comparison to placebo or to a standard medication (control) for allergic rhinitis. One study used azelastine nasal spray as comparator, in the second study a nasal spray based on cromoglicic acid served as control medication, the third study investigated ectoine nasal spray versus levocabastine (Livocab) with beclomethasone nasal spray, and the paediatric study was set up as a placebo-controlled trial. Although it is principally not recommended to pool data from studies with different control groups, the approach seemed appropriate since we were able to extract original data for each symptom individually. For all symptoms ectoine-containing nasal spray demonstrated similar or better efficacy when compared to controls. Effects were greatest for the symptoms "nasal itching" (−1.97 ± 1.54) (Figure 12) and "sneezing" (−1.69 ± 1.31) (Figure 13) which were associated with significant differences in favour of ectoine. For the remaining nasal symptoms "nasal obstruction" (Figure 10) and "rhinorrhoea" (Figure 11), the meta-analysis revealed that ectoine is similarly effective compared to the control drugs.

Since only two studies investigated the effect of the medication on eye symptoms, we pooled data from these two studies (ectoine versus azelastine and paediatric trial) to evaluate the effect of ectoine on the eyes. The analysis reveals that the symptom "teary eyes" (Figure 16) was significantly improved ($P = 0.02$) by the ectoine-containing nasal spray and eye drops with an effect size of −1.99 (±1.69). The symptoms "itching of eyes" (Figure 14) and "redness of eyes" (Figure 15) both tended slightly towards the ectoine products with effect sizes of −0.54 (±2.75) and −0.40 (±2.24), respectively. However, no statistical significance was reached here.

4. Subgroup Analyses

Subgroup analyses were performed for the two main allergic rhinitis symptoms of nasal obstruction and rhinorrhoea in order to evaluate the effect of ectoine in the specific group of adults with allergic rhinitis. Three studies with a total of 71 patients were included, whereby the level of heterogeneity decreased to 0%. Again, the three control groups of the integrated studies (azelastine, levocabastine/beclomethasone, and cromoglicic acid) were pooled into one control group versus ectoine nasal spray.

The subgroup analyses clearly emphasised the positive effect of ectoine nasal spray after seven days of treatment. Since each individual study consistently expressed the efficacy of ectoine, the overall pooled result for both nasal obstruction and rhinorrhoea was significant in favour of a seven-day medication intake. The corresponding P values were $P = 0.0002$ for the effect on nasal obstruction and $P = 0.005$ for rhinorrhoea. With a total effect of 0.6 (±0.31) for nasal obstruction (Figure 17), the efficacy of the ectoine-based nasal spray after seven days was associated with a strong

Study or subgroup	Ectoine Mean	SD	Total	Control Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Ectoine versus Azelastine	1.38	4.38	21	2.1	3.13	21	27.0%	−0.72 [−3.02, 1.58]
Ectoine versus Cromoglycin	3.52	3.82	25	0.8	4.52	25	26.9%	2.72 [0.40, 5.04]
Ectoine versus Livocab	2.32	4.16	25	2.12	5.26	25	24.7%	0.20 [−2.43, 2.83]
Pediatric trial	−2.63	7.415	41	0.1	6.07	30	21.4%	−2.73 [−5.87, 0.41]
Total (95% CI)			**112**			**101**	**100.0%**	**0.00 [−2.16, 2.17]**

Heterogeneity: $\tau^2 = 3.12$; $\chi^2 = 8.49$, df = 3 ($P = 0.04$); $I^2 = 65\%$
Test for overall effect: $Z = 0.00$ ($P = 1.00$)

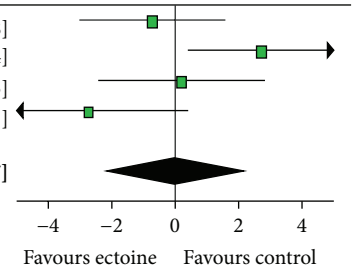

Figure 10: AUC nasal obstruction.

Study or subgroup	Ectoine Mean	SD	Total	Control Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Ectoine versus Azelastine	2.29	5.69	21	1.43	3.94	21	18.4%	0.86 [−2.10, 3.82]
Ectoine versus Cromoglycin	1.48	4.2	25	2.16	4.21	25	29.7%	−0.68 [−3.01, 1.65]
Ectoine versus Livocab	1	3.95	25	1.72	5.48	25	23.0%	−0.72 [−3.37, 1.93]
Pediatric Trial	−1.98	5.28	41	−1.03	4.8	30	29.0%	−0.95 [−3.31, 1.41]
Total (95% CI)			**112**			**101**	**100.0%**	**−0.48 [−1.75, 0.79]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 1.00$, df = 3 ($P = 0.80$); $I^2 = 0\%$
Test for overall effect: $Z = 0.75$ ($P = 0.45$)

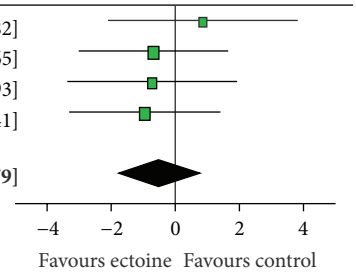

Figure 11: AUC rhinorrhoea.

Study or subgroup	Ectoine Mean	SD	Total	Control Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Ectoine versus Azelastine	1.1	5.03	21	4	5.2	21	23.1%	−2.90 [−5.99, 0.19]
Ectoine versus Livocab	0	4.3	25	0.36	5	25	32.2%	−0.36 [−2.95, 2.23]
Pediatric trial	−2.22	5.24	41	0.43	3.98	30	44.7%	−2.65 [−4.79, −0.51]
Total (95% CI)			**87**			**76**	**100.0%**	**−1.97 [−3.52, −0.43]**

Heterogeneity: $\tau^2 = 0.19$; $\chi^2 = 2.22$, df = 2 ($P = 0.33$); $I^2 = 10\%$
Test for overall effect: $Z = 2.50$ ($P = 0.01$)

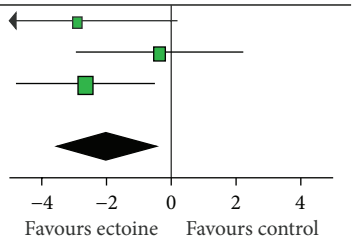

Figure 12: AUC nasal itching.

Study or subgroup	Ectoine Mean	SD	Total	Control Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Ectoine versus Azelastine	2.38	5.72	21	2.43	4.51	21	17.6%	−0.05 [−3.17, 3.07]
Ectoine versus Cromoglycin	1	3.52	25	3.08	4.97	25	30.0%	−2.08 [−4.47, 0.31]
Ectoine versus Livocab	−0.4	3.97	25	0.92	5.17	25	26.2%	−1.32 [−3.88, 1.24]
Pediatric trial	−3.02	4.93	41	−0.3	5.77	30	26.2%	−2.72 [−5.28, −0.16]
Total (95% CI)			**112**			**101**	**100.0%**	**−1.69 [−3.00, −0.38]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 1.87$, df = 3 ($P = 0.60$); $I^2 = 0\%$
Test for overall effect: $Z = 2.53$ ($P = 0.01$)

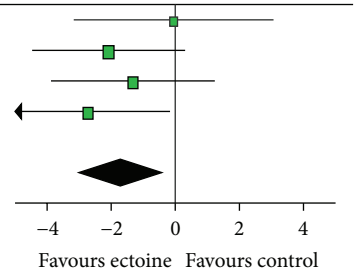

Figure 13: AUC sneezing.

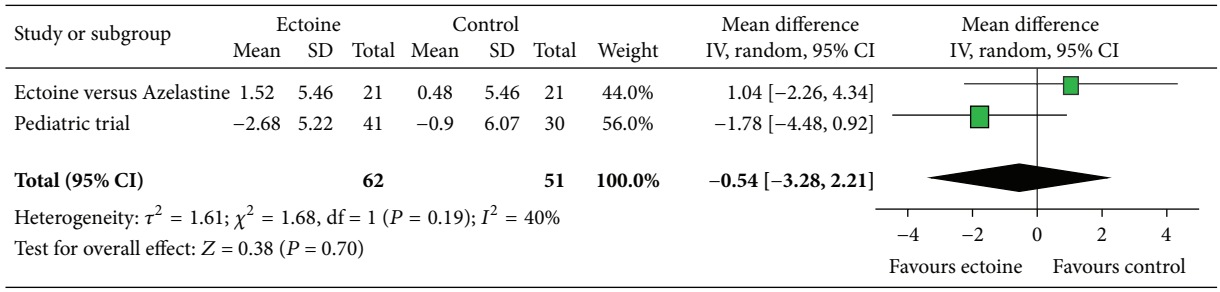

Figure 14: AUC itching of eyes.

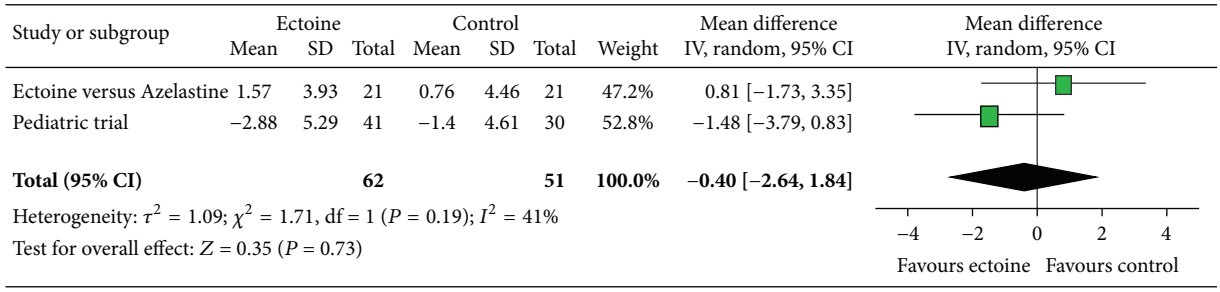

Figure 15: AUC redness of eyes.

effect size. Similarly, the total effect size of 0.44 (±0.31) for rhinorrhoea (Figure 18) signified a moderate effect of ectoine nasal spray. Therefore, the subgroup analyses confirmed the positive effect of ectoine nasal spray in alleviating the predominant symptoms of nasal obstruction and rhinorrhoea in adult patients with allergic rhinitis.

5. Discussion

This meta-analysis served to compare the treatment of allergic rhinitis with ectoine-containing nasal spray and eye drops to traditional treatment agents (antihistamine, glucocorticoid, and cromoglicic acid) or placebo treatment.

The meta-analysis involving ectoine nasal spray in the categories of baseline comparison and AUC determined a reduction in symptom severity for all relevant rhinitis symptoms. An especially strong effect was shown for the symptom of nasal congestion, which dropped significantly by 29.94% after seven days of treatment. According to the classification scheme developed by Ferguson [30], the improvement of nasal obstruction was categorised as strong, while further nasal symptoms such as rhinorrhoea, nasal itching, and sneezing were still associated with a significant improvement of moderate effect size. Likewise, significant improvements with a strong and moderate effect size were also demonstrated for nasal obstruction and rhinorrhoea in the subgroup analysis of adult SAR patients.

While ectoine-based products were shown to act significantly more effective than the control medications in easing the symptom severity of nasal itching, sneezing, and teary eyes, results for the remaining symptoms still confirmed a similar potency of ectoine nasal spray compared to standard medication.

Two studies during which ectoine-containing eye drops were used additionally to the application of ectoine nasal spray demonstrated improvement of ocular symptoms. Here, a strong size effect was shown in reducing red eyes and moderate size effect in reduction of itching eyes. Likewise, the analysis of accumulated effects revealed a significant improvement for the symptom "teary eyes" in the ectoine group. These results indicate a positive influence of ectoine eye drops on ocular symptoms in seasonal allergic rhinitis. However, further studies are needed to confirm these findings as the possibility that the effect may be explained by the inhibition of the naso-ocular reflex, as it has been suggested in studies with intranasal steroids [32], cannot be excluded based on the current results.

In this meta-analysis, we compared the efficacy of ectoine to three effective, currently guideline-recommended medications, such as the second-generation antihistamine azelastine, the glucocorticoid combination levocabastine/beclomethasone, and the classical cromoglicic acid. The comparison attested the equivalence of ectoine nasal spray to these products. Thus, the ectoine-based products can be regarded as noninferior to topical antihis-tamines, the intranasal glucocorticosteroid combination levocabastine/beclomethasone, or nasal mast cell stabilisers for the treatment of rhinitis symptoms.

The results of this meta-analysis are promising and further supported by the safety profile of products containing ectoine [20, 33]. Clinical studies have shown that treatment with ectoine results in very few adverse events (frequency comparable to placebo) and virtually no safety

Study or subgroup	Ectoine Mean	SD	Total	Control Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Ectoine versus Azelastine	−0.52	3.89	21	0.43	4.98	21	39.1%	−0.95 [−3.65, 1.75]
Pediatric trial	−2.59	5.97	41	0.07	3.24	30	60.9%	−2.66 [−4.82, −0.50]
Total (95% CI)			**62**			**51**	**100.0%**	**−1.99 [−3.68, −0.30]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.94$, df = 1 ($P = 0.33$); $I^2 = 0\%$
Test for overall effect: $Z = 2.31$ ($P = 0.02$)

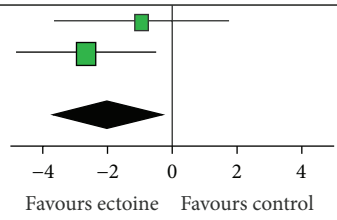

Figure 16: AUC teary eyes.

Study or subgroup	Baseline Mean	SD	Total	Day 7 Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Ectoine versus Azelastine	1.9	0.94	21	1.48	1.08	21	26.5%	0.42 [−0.19, 1.03]
Ectoine versus Cromoglycin	1.92	0.91	25	1.2	0.87	25	40.8%	0.72 [0.23, 1.21]
Ectoine versus Livocab	1.68	0.99	25	1.08	1	25	32.7%	0.60 [0.05, 1.15]
Total (95% CI)			**71**			**71**	**100.0%**	**0.60 [0.29, 0.92]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.56$, df = 2 ($P = 0.76$); $I^2 = 0\%$
Test for overall effect: $Z = 3.74$ ($P = 0.0002$)

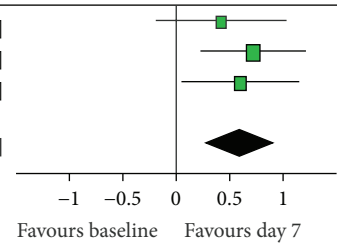

Figure 17: Subgroup nasal obstruction.

concerns [20, 33, 34]. In contrast, the traditional drugs are still associated with side effects, warranting the search for alternative treatments. Thus, particularly antihistamines, such as azelastine, even in nasal spray form, continue to cause sedation/somnolence and nasal burning occasionally (Astelin patient information). Moreover, nasal steroids can also have various adverse effects. For example, the patient information for glucocorticoid nasal spray, for example, fluticasone furoate (Veramyst), warns about possible ocular side effects including glaucoma, cataracts, and increased intraocular pressure. While—in isolated cases—growth retardation has been associated with beclomethasone treatment [35], nasal spray, and eye drops containing ectoine offer adequate relief from allergy symptoms without these added risks. However, those infrequent side effects should not be overestimated, and newer drug formulations show fewer adverse reactions than the earlier agents. Still, the absence of safety concerns makes ectoine-based products particularly interesting candidates for the treatment of allergic rhinitis in children. Since the application of corticosteroids in children has raised some concerns regarding impaired growth and abnormal development, ectoine may provide a safe and convenient alternative for physicians and parents worried about treating their allergic children with pharmaceutical products that have potential harmful side effects. However, the safety of ectoine nasal spray needs to be further investigated in future studies to confirm the safety profile of the product.

The mode of action of ectoine-based products in preventing and relieving allergic symptoms is based on the physical interaction of ectoine with water and the resulting effects on the membrane of the tissue treated. Stabilisation of cell membranes with consequent enhancement of the tissues' barrier function may reduce the allergen-membrane interactions and inflammation which usually cause ocular, nasal, and nonnasal symptoms in patients with allergic rhinitis.

There is one constraint to this meta-analysis: upon inclusion, patients had mostly mild symptoms. Hence, no large improvements could be expected from a one- to two-week course of treatment. Considering these baseline values, the verified improvement can indeed be interpreted as convincing. Future studies including patients with more severe baseline symptoms would be needed to further investigate the effectiveness of ectoine treatment in rhinitis patients. A further limitation concerns the methodology, since only data from unpublished studies are included in this meta-analysis. The included study data have not been published to date, since the number of participants in each trial was too small to show interesting results. Nowadays, large randomised controlled trials with more than 250 patients per treatment group are commonly required to be considered for publication [21, 22]. Likewise, published noninterventional studies are usually performed with numbers larger than 1000 patients to be powered adequately [36, 37]. To date, no publications investigating ectoine as a nasal spray ingredient exist.

6. Conclusion

Taken together, this meta-analysis demonstrated that the application of ectoine-based nasal spray and eye drops improves symptoms of allergic rhinitis and rhinoconjunctivitis. This easy-to-apply, well-tolerated, naturally-based nasal and ocular treatment, which has no unpleasant taste and

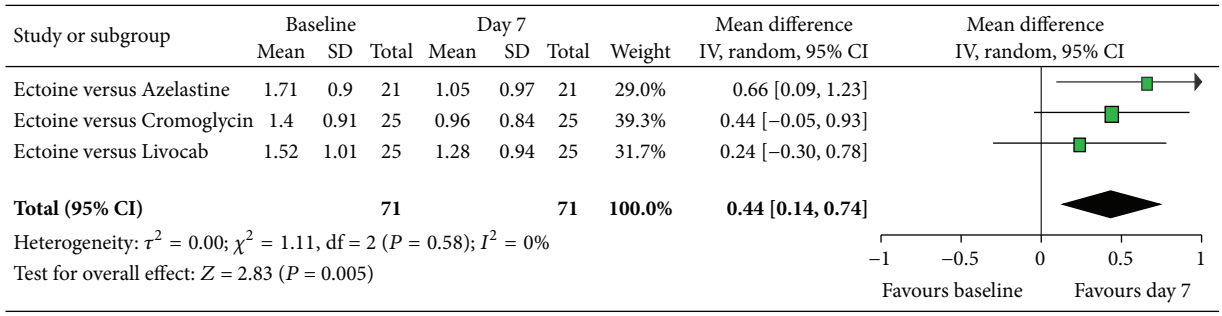

Figure 18: Subgroup rhinorrhoea.

virtually no side effects, effectively reduces allergic rhinitis symptoms and represents an exciting alternative for rhinoconjunctivitis sufferers.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Probiotics in the Treatment of Chronic Rhinoconjunctivitis and Chronic Rhinosinusitis

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Chronic rhinitis and rhinosinusitis (CRS) are relevant health conditions affecting significant percentages of the western population. They are frequently coexisting and aggravating diseases. Both are chronic, noninfectious, and inflammatory conditions sharing to a certain extent important pathophysiologic similarities. Beneficial effects of probiotics are long known to mankind. Research is beginning to unravel the true nature of the human microbiome and its interaction with the immune system. The growing prevalence of atopic diseases in the developed world led to the proposition of the "hygiene hypothesis." Dysbiosis is linked to atopic diseases; probiotic supplementation is able to alter the microbiome and certain probiotic strains have immunomodulatory effects in favour of a suppression of Th-2 and stimulation of a Th1 profile. This review focuses on randomized, double-blind, placebo-controlled trials investigating clinical parameters in the treatment of chronic rhinitis and CRS. An emerging number of publications demonstrate beneficial effects using probiotics in clinical double-blind placebo-controlled (dbpc) trials in allergic rhinitis (AR). Using probiotics as complementary treatment options in AR seems to be a promising concept although the evidence is of a preliminary nature to date and more convincing trials are needed. There are no current data to support the use of probiotics in non-AR or CRS.

1. Chronic Rhinoconjunctivitis and Chronic Rhinosinusitis

ARIA guideline defines rhinitis as a chronic inflammatory disease of the nose resulting in nasal symptoms including nasal obstruction, sneezing, and anterior or posterior rhinorrhea (occurring during two or more consecutive days for more than one hour) [1].

Allergic rhinitis (AR) is the most common form of noninfectious, chronic rhinitis affecting more than 25% percent of the European population [1, 2]. It is characterized as an eosinophilic, IgE-mediated, Th-2 dominated immune disorder. "Local allergic rhinitis" describes a condition of local allergen-specific IgE production in the nose. Prevalence data are estimated to lay between 47% and 62.5% of patients with perennial and seasonal symptoms. Interestingly, this condition is described to precede a "classic" AR [3].

Prevalence data about nonallergic forms of chronic, noninfectious rhinitis are rare. They are estimated to be almost as high as AR [1]. Non-AR includes a long list of potential causes. However, the idiopathic form remains the most frequent [4]. Although non-AR is per exclusion not a type-I allergy it resembles often the same cellular key players such as mast cells and eosinophils [5].

EPOS guidelines define chronic rhinosinusitis (CRS) by the presence of at least two of the following symptoms for at least 12 weeks per year: nasal blockage, nasal discharge, facial pain or pressure, or reduction of smell with at least one of the symptoms being nasal blockage or nasal discharge.

Chronic rhinosinusitis can occur with or without nasal polyps (CRSwNP or CRSsNP) [6].

The pathophysiology of CRS is only partially understood. It is characterized as a chronic inflammation resembling components of Th-2 (eosinophils, mast cells) and Th-1 immune responses [6–8].

Chronic rhinitis and CRS are frequently coexisting and aggravating conditions. Both are chronic, noninfectious, and inflammatory conditions sharing important pathophysiologic similarities such as a Th-2 type immune pattern.

2. Probiotics

Beneficial effects of probiotics are long known to and practiced by mankind. The Russian immunologist Metschnikow (Nobel prize laureate 1908) published the results of extensive studies about probiotics in his book "The Prolongation of Life" in 1907. Present day, the human microbiome concept and probiotics are experiencing an impressive renaissance.

A probiotic widely consists of a food product or supplement containing sufficient numbers of viable microorganisms aimed at altering the microflora of the host and, in turn, conferring beneficial health effects. The World health Organisation describes *Probiotics* as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host." Probiotic microorganisms are typically consumed in fermented foods (i.e., cheeses, yoghurts) and most commonly used *genera* include *Lactobacilli* and/or *Bifidobacterium*. They are typically anaerobic organisms and in the intestines they ferment ingested food products to produce lactic acid. Their inherent biological features enable them to predominate and prevail over potential pathogenic microorganisms in the human digestive tract.

Prebiotics are nondigestible food ingredients selectively stimulating the favorable growth and/or activity of probiotics. Prebiotics are usually oligosaccharides such as fructooligosaccharides (FOSs), inulin, or galactooligosaccharides (GOSs).

Synbiotics are the combination of probiotics and prebiotics [9].

3. The Microbiome and Dysbiosis

Our understanding of the human microbiome and its interaction with the human immune system is increasing rapidly. A PubMed search of the term probiotics presents thousands of citations during the past 10 years, compared to less than 100 for the previous 25 years. The intestine—the largest lymphoid organ in the body—and particularly the large intestine is heavily colonized and commensal bacteria outnumber human cells by a factor of 10 to 1. The intestinal microbiome plays a key role in the maintenance of mucosal health [10] and research continues to present an intensive crosstalk at play between this interface. In addition to this, the skin (the largest human organ) consists of a densely populated and diverse habitat of microbiota. Research is only just beginning to unravel the unprecedented influence this equally complex and dynamic ecosystem plays in the onset and progression of a number of chronic inflammatory diseases [11].

A disrupted microbiome (= dysbiosis) has been associated with a lengthening list of health conditions such as obesity and malnutrition, diabetes, numerous diseases of the intestines, autism, and chronic inflammatory conditions such as atopy or rhinitis [12, 13].

Subsequent to the sterile uterine environment, colonization begins at birth. By one (three) year(s) of age the microbiome has a stable adult-like signature [12, 14, 15]. Thus, postnatal microbiome development is thought to play a pivotal role in infant health. The type of delivery (vaginal or cesarean section) undoubtedly contributes to the ratio of colonized *genera* as a result of different exposures during delivery, their effects of which may persist for a period of time after birth [16]. For example, infants born from a cesarean section have been linked to higher risk categories for some immune-mediated diseases [17, 18]. Life events such as travelling, antibiotics, short/long term dietary changes, and illnesses will alter the composition of the microbiome [19, 20].

There is little doubt that the influence of probiotic bacteria has the ability to exert indirect or direct immunomodulatory effects, however their detailed mechanisms remain to be determined. Other mechanisms of action continue to be observed which include modulation of cellular metabolism and epithelial barrier functions. Interestingly, many specific effects and efficacy have been shown to be species or strain specific [21–26].

The interactions of probiotics with the host immune system are only partially understood and include, for instance, the following.

(i) *Humoral immunity*: stimulation of Th 1, suppression of Th 2, stimulation of Tregs, transforming growth factor β and an increase in local IgA production which influences mucosal defences [21, 22, 27].

(ii) *Innate immunity/adjuvant effects:* toll-like receptor signalling (TLR-2), nucleotide-binding oligomerization domain receptors (NODs)- or lectins signaling, and interaction with dendritic cells (modulation of DC maturation and their cytokine patterns) [28, 29]. Additional interactions of the microbiome and the human body are executed via the "gut-brain-axis" [12, 30]. Furthermore, probiotics can serve as mucosal delivery vehicles, exhibit a "colonization resistance" by their commercial properties, and enhance the epithelial gut barrier [28]. However, mechanistic studies are mostly based on *in vitro* cell models and make it difficult to translate or accurately portray native *in vivo* mechanisms.

Supplementation of pre- or probiotics is unlikely to resolve conditions in predisposed individuals predominated by complex genetic factors and/or sever dysregulation of their immune system. However, the association of certain mild-severe diseases linked to microbiome dysbiosis may offer realistic prophylactic or therapeutic treatment options. The beneficial effects of probiotic consumption in a variety of inflammatory diseases (e.g., irritable bowel disease, chronic respiratory diseases) have been reported [31, 32]. Due to aforementioned characteristics, it is obvious that probiotics can be studied for beneficial effects in the prevention and treatment of chronic rhinitis and CRS.

4. Dysbiosis and AR

The growing prevalence of atopic disease in the developed world led to the proposition of the "hygiene hypothesis" by Strachan in 1989 [33]. In the progression of that early hypothesis the crucial role of microbial environmental stimuli for atopy was emphasized advancing it into the "microbial

hypothesis" [34]. Considering the collective genomes of microbes that live inside and on us, in addition to our own, one has engineered the term: the human supraorganism to describe our true form [14]. Human evolution has brought the industrialization of the modern world and, with it, advances in technology which have transformed people's lives over the past century. More importantly, such environmental changes play a fundamental role in altering our biosphere, thus our health and onset and progression of diseases. The rise of atopic eczema in industrialized countries has now reached epidemic levels within the last five decades [35].

Dysbiosis could conclusively be linked to atopy—in animal studies [36] and man [37–39].

5. Probiotics and Prevention of Atopy

Probiotics that are tailored and marketed towards treating individuals suffering from a range of atopic diseases are starting to emerge and grow on the market.

Using probiotics for prevention of atopic diseases was initiated by Scandinavian trials published in high-impact journals demonstrating significant effects in the prevention of atopic dermatitis [40–42]. Here, *Lactobacillus (L.) rhamnosus* appears to be a primary candidate strain in the incidence of atopic dermatitis. However, overall data is conflicting and evidence is limited [43]. Human studies can be very difficult to compare since they can vary considerably in their design (i.e., screening, duration, clinical end-point definitions, etc.). Recent reviews see moderate effects in the prevention of atopic dermatitis (in subgroups) but not in AR, asthma, or allergic sensitizations [44, 45]. Interestingly, a recent investigation examined associations between consumption of probiotic milk products in pregnancy and infancy with questionnaire-reported atopic eczema, rhinoconjunctivitis, and asthma in 40,614 children. In this population-based cohort the consumption of probiotic milk products was related to a reduced incidence of atopic eczema and rhinoconjunctivitis, but no association was seen for incidence of asthma by 36 months of age [46]. In addition to this, a study performed by Kuitunen and colleagues [17] provided a strong hypothesis in that babies, delivered via caesarean section, who received synbiotics had fewer IgE-associated diseases (24.3%) compared to a placebo group (40.5%) at the age of 5 years. Much needed data is necessary to confirm or refute this hypothesis, since this study also concluded contradictory results in which the incidence of atopic disease in 925 neonatal infants, who each received synbiotics, was comparable to a placebo group after 2 and 5 years.

The complex crosstalk and array of effects by which prebiotics and probiotics elicit are not fully understood and this may explain, in part, why results of human studies, which use synbiotics to induce immune-health benefits, have been contradictory [47]. However, study designs are under increasing scrutiny and the need to better define validated biomarkers, valuable enough to substantiate a health claim, has yet been achieved.

6. Treatment of Chronic Rhinitis and CRS by Probiotics

6.1. Allergic Rhinitis. As explained above, dysbiosis is linked to atopic diseases, probiotic supplementation is able to alter the microbiome, and certain probiotic strains have immunomodulatory effects in favour of a suppression of Th-2 immune response and stimulation of Th-1 and Tregs. Hence, there is an objective rationale for studying probiotics in the treatment of AR. Over the last years an emerging number of randomized, dbpc trials focusing on clinical data in humans were published for the treatment of AR.

6.1.1. Seasonal AR. Wassenberg et al. studied *L. paracasei* in a dbpc cross-over trial ($n = 31$) versus placebo in grass pollen allergic patients in 2011 by means of nasal provocation (NPT) over 4 weeks of treatment. Nasal congestion in NPT was significantly improved by active treatment [48].

Ouwehand et al. analyzed the combination of *L. acidophilus* and *Bifidobacterium (B.) lactis* in 47 children suffering from birch pollen AR in a dbpc trial versus placebo over 4 months. The combination of the selected probiotics was shown to prevent the pollen-induced infiltration of eosinophils into the nasal mucosa and indicated a trend for reduced nasal symptoms [49].

B. longum significantly improved eye symptoms in 40 patients with allergic rhinoconjunctivitis due to Japanese cedar in a dbpc setting versus placebo over 14 weeks. Nasal symptoms improved as well, although not statistically significant [50, 51].

B. lactis was studied in 20 patients suffering from grass AR in a dbpc trial against placebo over 8 weeks during the grass pollen season. Total nasal symptom score improved significantly. IL-5, IL-13, and TNF-alpha were significantly decreased, likewise was the CD63 expression on activated basophils [52].

Lastly, Perrin et al. studied *L. paracasei* versus a combination of *L. acidophilus* and *B. lactis* in 31 grass pollen allergic patients in a dbpc cross-over design over 4 weeks. *L. paracasei* significantly reduced nasal pruritus while not affecting nasal congestion in that setting [32].

6.1.2. Perennial AR. Wang et al. analyzed *L. paracasei* in 80 patients suffering from house dust mite (HDM)-allergic rhinitis in a dbpc trial versus placebo over 30 days. Scores for the overall quality of life significantly decreased in the *L. paracasei* group as compared against placebo, in both frequency and level of bother [53].

L. acidophilus was analyzed in 49 HDM-allergic patients against placebo in a dbpc trial for 8 weeks. Administration of *L. acidophilus* resulted in a statistically significant improvement of nasal symptom-medication scores [54].

12-week treatment of *L. salivarius* reduced rhinitis symptoms and drug usage in 240 children suffering from HDM-AR against placebo in a dbpc trial [55].

Lin et al. conducted a 12-week dbpc trial using an interesting design: 60 children with perennial AR were randomized into two groups with 28 participants receiving levocetirizine

plus placebo and 32 participants receiving regular levocetirizine plus *L. paracasei* for the first 8 weeks, with a shift to levocetirizine as rescue treatment during the following 4 weeks. The *L. paracasei* group had significantly lower Pediatric Rhinoconjunctivitis Quality of Life Questionnaires (PRQLQ) scores even after discontinuing regular levocetirizine from week 9 to week 12. There was more improvement in individual parameters in the PRQLQ including: sneezing, itchy nose, and swollen puffy eyes, in the active group. The authors summarized that dietary supplementation with *L. paracasei* provided no additional benefit when used with regular levocetirizine in treating AR in the initial 8 weeks of our study, but there was a continuing decrease in PRQLQ, as well as a significant improvement in individual symptoms of sneezing, itchy nose, and swollen eyes, after discontinuing regular levocetirizine treatment [56].

The above listed publications demonstrate beneficial effects using probiotics in clinical dbpc trials in AR. Many questions remain open: duration of treatment, strain selection, optimal dosage of strains, potential additional positive effects using prebiotics, and so forth. Due to the limited number of published trials and factors such as "publication bias" these data are of preliminary nature to date. However, effects have been shown to be reproducible and more clinical trials will be conducted. Using pre-, pro-, or synbiotics as complementary treatment options in AR seems to be a promising concept.

Interestingly, there is an increasing body of evidence in animal models revealing future options: probiotics can provide beneficial effects for immunotherapy [57] or recombinant probiotics, producing IL-10 or allergens such as Bet v1 or HDM-allergens, could have the potential for novel treatment options for AR [58–61]. The real power of probiotics may lie in the use of genetically modified lactic acid bacteria. For example, evidence from these studies indicates that deletions to certain cell surface components can ultimately downregulate inflammatory responses [62]. However, such alterations to cell surface components of lactic acid bacteria inevitably call into question their GRAS ("generally regarded as safe") status [47].

6.2. Nonallergic Rhinitis. To the author's best knowledge there exists no trial focusing specifically on non-AR.

6.3. CRS. Mukerji et al. analysed the oral use of *L. rhamnosus* on sinonasal quality-of-life scores in CRS. 77 patients were studied in a dbpc trial against placebo over a 4-week treatment, revealing no significant results [63].

However, *Staphylococcus (Staph.) aureus* is a key pathogenic component of the CRS microbiome and is associated with increased disease severity and poor postoperative outcomes. Cleland et al. investigated the probiotic properties of *Staph. epidermidis* against *Staph. aureus* in a mouse model of sinusitis. They confirmed the probiotic potential of *Staph. epidermidis* in that model [64].

Biofilms form on moist biotic and abiotic surfaces, making them common for infections of the ears, nose, and throat and especially in CRS. Eradicating ENT biofilms is difficult. Probiotics such as *L. casei* were shown to have beneficial effects in ENT biofilms [65].

Furthermore, upper respiratory tract infections (URTI) are often preceding CRS. The use of probiotics in URTI was summarized in a Cochrane review in 2011, stating that "probiotics were better than placebo in reducing the number of participants experiencing episodes of acute URTIs, the rate ratio of episodes of acute URTI and reducing antibiotic use" [66].

Recently the same could be demonstrated for healthy physically-active adults. West et al. found a significantly reduced risk of URTI using *B. lactis* in a dbpc trial including 464 subjects over 150 days of treatment [67].

Hence, published evidence does not support the use of probiotics in CRS to date. However, more data are required to finally address the question whether probiotics are beneficial in CRS.

7. Conclusion

The paradigm of the human microbiome and the relationship of dysbiosis and distinct diseases is a fascinating concept attracting increasing attention. However, there is a requirement for more consistent data from human studies and a better understanding in their mode of action through *in vitro/in vivo* models to answer many remaining open questions. It is widely demonstrated that baseline variation exists amongst the population; non-responders are frequently reported and this may be dictated by specific and ill-defined phenotypic factors. However, through understanding the role and importance of host-dependent (e.g., genetic background, diet and lifestyle, innate microflora compositions etc.) factors, may provide the opportunity to design more personalised treatment programmes designed to confer improved clinical efficacy for specific individuals or sub-populations [47]. In addition, generating sufficient scientific evidence to support a health claim may well be achieved through a better understanding of immune phenotypes of individuals and how this dictates the immunomodulatory effects elicited through the supplementation of synbiotics.

Preliminary data exist providing beneficial results in using probiotics in the treatment of allergic rhinitis and probiotics could emerge as a novel, complementary treatment option for AR. However, there are no current data to support the use of probiotics in non-AR or CRS.

Conflict of Interests

Professor Dr. Matthias F. Kramer, M.D. is the International Medical Director of Allergy Therapeutics plc. Matthew D. Heath, Ph.D., is a Development Scientist Allergy Therapeutics plc. Allergy Therapeutics market synbiotics.

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Research Article

The Compatible Solute Ectoine Reduces the Exacerbating Effect of Environmental Model Particles on the Immune Response of the Airways

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Exposure of humans to particulate air pollution has been correlated with the incidence and aggravation of allergic airway diseases. In predisposed individuals, inhalation of environmental particles can lead to an exacerbation of immune responses. Previous studies demonstrated a beneficial effect of the compatible solute ectoine on lung inflammation in rats exposed to carbon nanoparticles (CNP) as a model of environmental particle exposure. In the current study we investigated the effect of such a treatment on airway inflammation in a mouse allergy model. Ectoine in nonsensitized animals significantly reduced the neutrophilic lung inflammation after CNP exposure. This effect was accompanied by a reduction of inflammatory factors in the bronchoalveolar lavage. Reduced IL-6 levels in the serum also indicate the effects of ectoine on systemic inflammation. In sensitized animals, an aggravation of the immune response was observed when animals were exposed to CNP prior to antigen provocation. The coadministration of ectoine together with the particles significantly reduced this exacerbation. The data indicate the role of neutrophilic lung inflammation in the exacerbation of allergic airway responses. Moreover, the data suggest to use ectoine as a preventive treatment to avoid the exacerbation of allergic airway responses induced by environmental air pollution.

1. Introduction

The exposure of humans to particulate air pollution has been correlated with the incidence of atopic allergies [1]. In particular, traffic-related air pollution is strongly linked to allergic diseases including asthmatic bronchitis [2]. It is hypothesized that an adjuvant effect of inhaled particles may influence either the process of sensitization or the immune response, at the level of the disease outcome [3]. In asthma patients, such adverse effects of particulate air pollution can be observed as an acute exacerbation of allergic lung inflammation [4–6].

Current research is focusing on the molecular mechanisms by which such a toxic potential of environmental particles is mediated. As one common denominator of particle-induced adverse health effects, oxidative stress in the airways has been identified [7]. Reactive oxygen species may be triggered by the intrinsic oxidative potential of inhaled particulate matter which depends on chemical properties like elemental composition and surface charges. But also via indirect cell mediated pathways oxidative stress is generated in the airways. Upon cell contact, in particular ultrafine or nano-sized particles may interact with cellular components and organelles which can contribute to the production of reactive oxygen species [8]. Additionally, the induction of an inflammatory response, which is a typical reaction to the inhalation of poorly soluble material, can lead to an oxidative burst from inflammatory cells like macrophages and neutrophilic granulocytes [9].

So far, it is not clear whether all these potential mechanisms contribute to the exacerbation of immune reactions of the airways or whether one of these pathways dominates the adverse effects and might therefore be a useful target for preventive and therapeutic approaches. In the system

of ovalbumin (OVA)-sensitized mice this problem was addressed by inhalation studies with pure carbon nanoparticles (CNP). Such particles are considered model particles for combustion-derived recent particulate air pollution. Inhalation of these particles prior to OVA challenge led to an aggravation of the allergic airway responses including infiltration of inflammatory cells and excretion of cytokines [10]. An intervention study employing antioxidants in this scenario demonstrated that a reduction of the oxidative stress prevents the exacerbation of the airway response [11]. Using this experimental system, it should be possible to test the preventive value of compounds for individuals who suffer from atopic asthma, which might be exacerbated after inhalation of particulate matter.

In our earlier studies, we were able to demonstrate that the compatible solute ectoine is able to reduce the neutrophilic inflammatory response of the airways after exposure to pure (CNP). In the system of particle-exposed rats, the neutrophilic lung inflammation was significantly reduced when ectoine was present during exposure [12]. At a mechanistic level, we were able to demonstrate that ectoine prevents the activation of proinflammatory reactions in lung epithelial cells by stabilizing membrane signalling platforms which are addressed by oxidative cell stress coming by the particles [13]. In this context, it has been shown that ectoine does not interact with the particles themselves. The stabilizing mechanism of ectoine was investigated by a number of "proof of principle" experiments, which demonstrated that the mechanism is not based on antioxidant properties of the substance. Additionally, investigating the effect of ectoine during an ongoing neutrophilic inflammation, we observed the prevention of antiapoptotic and therefore proinflammatory reactions of neutrophils in the inflammatory environment by ectoine [14]. This effect was observed not only in the animal system, where it led to an accelerated resolution of the inflammation, but also in the human system employing peripheral neutrophils from patients suffering from chronic obstructive pulmonary disease (COPD).

The possibility to reduce neutrophilic lung inflammation without directly interfering with the oxidative potential of the nanoparticles offers the possibility to investigate the relevance of the neutrophilic inflammation for the exacerbation of allergic lung inflammation. Ectoine is a highly compatible substance which is tolerated by higher organisms without any known side effects. Therefore, the system might give indications for possible therapeutic value for asthma patients. For that purpose, the experimental system of CNP-induced neutrophilic lung inflammation was adopted to C57/Bl6 mice and the effect of ectoine application was evaluated in these organisms. The influence of ectoine of local and systemic cytokines with immune relevance was tested. In a second step, the system was applied to OVA-sensitized mice which were challenged in the presence of CNP and ectoine.

2. Materials and Methods

2.1. Particle Suspensions. Carbon nanoparticles (CNP, Printex 90) were obtained from Degussa (Essen, Germany). Stock suspensions (1 mg/mL) of particles were prepared in phosphate buffered saline (PBS) by sonication for 60 min. Particles and particle suspensions were characterized by (i) scanning electron microscopy (JSM 7000F, JEOL Ltd., Japan), (ii) BET using FlowSorb II 2300 analyzer (Micromeritics, Norcross, USA), and (iii) light scattering using ZetratracTM NPA152 (Microtrac, Montgomeryville, PA, USA). Particle suspension characteristics were as described previously [13].

2.2. Animal Experiments. Female C57BL/6JRj mice (8 weeks old, Janvier, France) were treated with particle suspensions or control solutions via pharyngeal aspiration with a volume of 50 μL, under inhalation anaesthesia (isoflurane, 5% in synthetic air, 1-2 min). Animals were sensitized by repetitive intraperitoneal injection of 1 μg OVA/alum. At the indicated time point, mice were challenged by aerosol inhalation (1% OVA in PBS) for 30 min using a Pari-Boy Nebuliser (Pari, Starnberg, Germany). Animals were sacrificed by exsanguination under anaesthesia after the indicated exposure times. Serum was prepared from blood samples taken prior to exsanguination. Bronchoalveolar lavage was taken using 4 × 1 mL PBS. All animal experiments were performed after relevant permission according to German animal protection laws.

2.3. Lavage Parameters. Differential cell counts were performed from Giemsa/May-Grünwald stainings of lavage cells. Cell free lavage fluids were subjected either to solid-phase ELISA in order to determine KC and IL-6 (R&D systems, Minneapolis, MN) or to Mouse Cytokine Antibody Arrays (RayBiotech, Inc., Norcross, CA) according to the respective manufacturer's instructions. Signal strengths of the arrays were determined densitometrically from autoradiographs using Quantity One software (version 4.1, Biorad, Hercules, CA, USA). IL-6 serum content was determined using the above-mentioned ELISA system.

2.4. Statistical Analyses. Statistical calculations were performed using IBM SPSS statistics 22. Significant values were calculated either by ANOVA analyses with Tukey's HSD post hoc testing or by comparison of individual groups by Mann-Whitney U-test. Dose response relationships were analysed by Pearson correlations. Except for the boxplot in Figure 1, mean values with standard errors are given. Power calculations for the design of animal experiments were performed using G*Power version 3.1.5 (University of Kiel, Germany).

3. Results and Discussion

With the current studies we aimed at investigating the effect of ectoine in an in vivo experimental system suitable as a model for allergic airway diseases. In a first approach, lung inflammation in C57/Bl6 mice was investigated with respect to dose response after 24 h (Figure 1). Analyses of bronchoalveolar lavage (BAL) demonstrate a dose dependent increase of inflammatory cells. The neutrophil influx is reflected by the increase of the neutrophil recruiting cytokine KC. The effect of ectoine was then tested in a time course experiment in which animals were exposed for 12 h, 24 h,

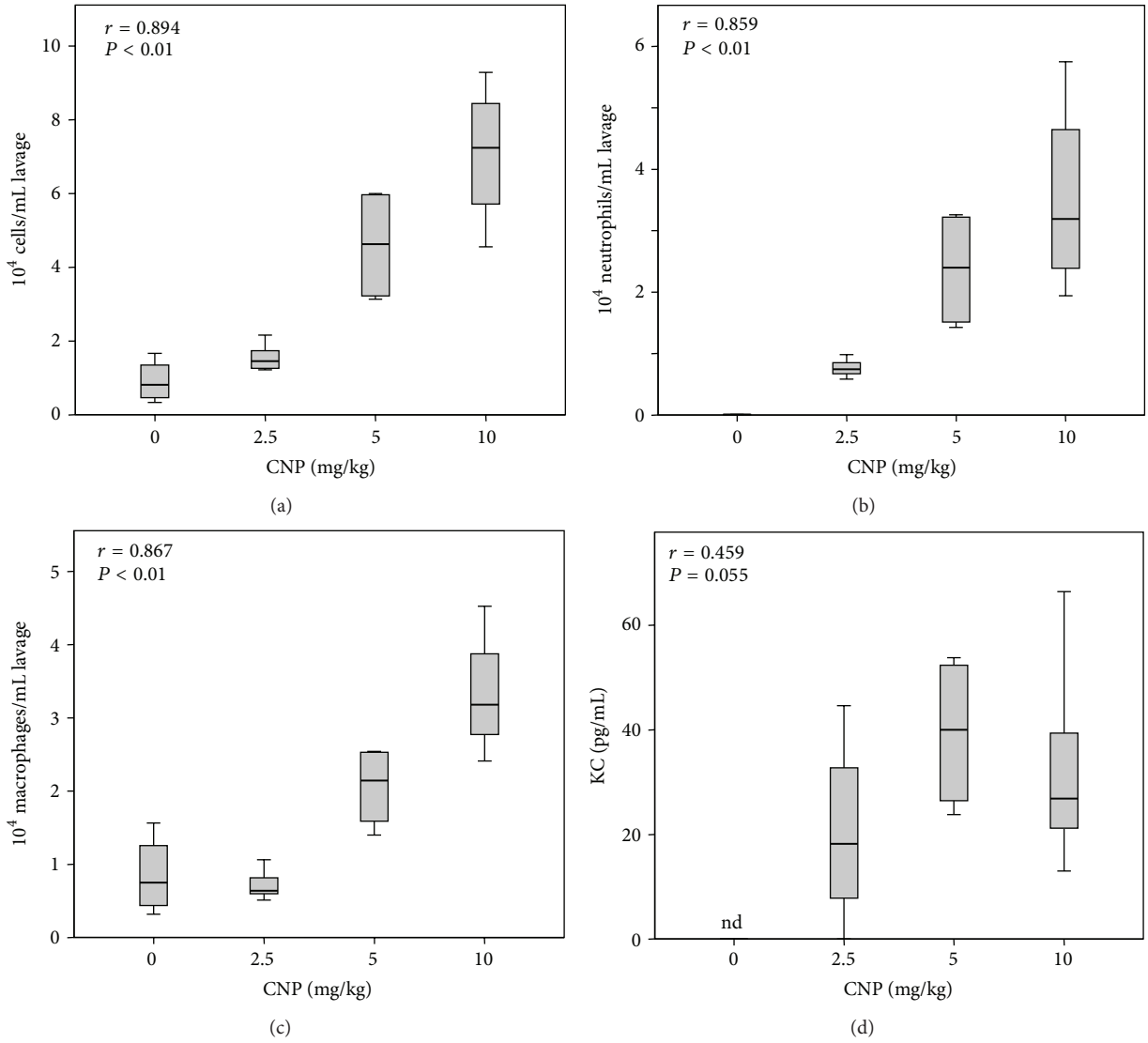

Figure 1: Lung inflammation induced by increasing doses of CNP. Female C57/Bl6 mice (8 weeks old) were exposed once to the indicated dose of CNP suspended in PBS. Animal numbers were 0 mg/kg $n = 4$, 2.5 mg/kg $n = 5$, 5 mg/kg $n = 4$, and 10 mg/kg $n = 5$. Inflammation parameters were determined 24 h after exposure. (a) Total number of cells per mL BAL; (b) total number of neutrophilic granulocytes per mL BAL; (c) total number of macrophages and monocytes per mL BAL; (d) pg/mL of KC in BAL. r, correlation coefficient of Pearson correlation; P, two sided significance; nd, not detectable.

and 48 h with 5 mg/kg CNP in the presence or absence of ectoine (Figure 2). Control animals were exposed with saline (PBS) or ectoine solution for 24 h. After a maximum of total cell counts as well as neutrophil numbers in BAL after 12 h, the inflammatory parameters attenuated during the observation period. The reduction of neutrophils was counteracted by the increase of macrophages, which help to clear the lung from apoptotic neutrophils. In the presence of 1 mM ectoine, the kinetics of the neutrophilic inflammation were mirrored at a lower level, indicating the preventive potential of the substance in the mouse system with the most striking statistical significance after 12 h.

The consequences of this reaction were furthermore tested at the level of cytokines and chemokines using membrane arrays for two BAL samples from each 12 h exposure group (Figure 3(a)). Although the differences in cytokine patterns are based on a low number of individuals, the general reduction of the selected inflammatory markers by the ectoine is obvious. In addition to these analyses, the potential of ectoine to reduce systemic inflammation was investigated by measuring IL-6 in BAL and in serum of exposed animals (Figures 3(b) and 3(c)). IL-6 levels in control animals were not detectable. The application of ectoine together with CNP led to a significant reduction of cytokine levels both in BAL and in serum of the animals. In particular, the reduction of IL-6 in the serum of the animals treated with ectoine in addition to CNP highlights the potential of this kind of treatment to reduce systemic effects of the lung inflammation. IL-6 has been discussed as a serum marker for asthma which also might be involved in the pathogenesis of asthma [15]. This

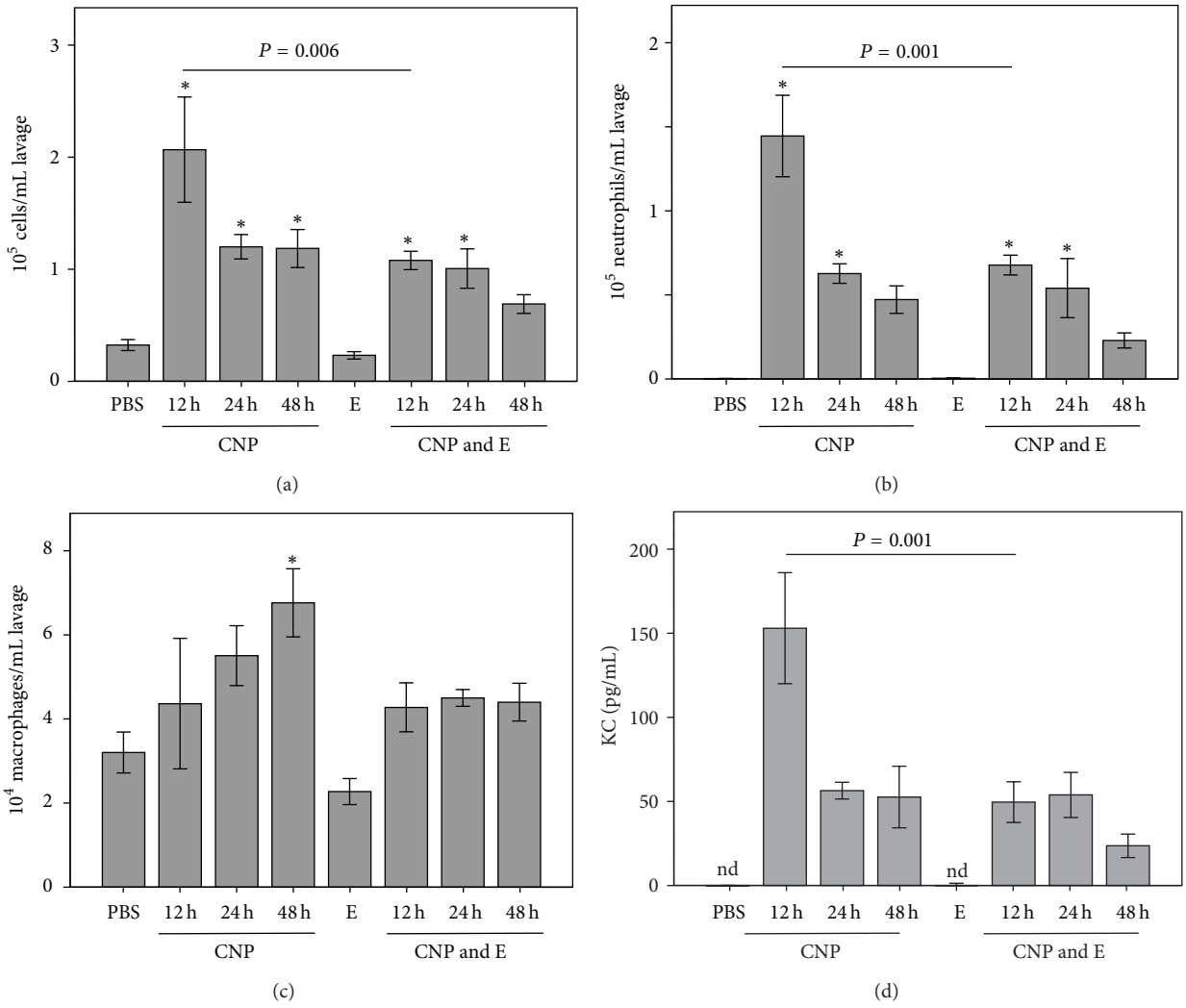

Figure 2: Time course of lung inflammation after single application of 5 mg/kg CNP in the presence or absence of ectoine (1 mM). Animals ($n = 5$) were analysed after the indicated time points. (a) Total number of cells per mL BAL; (b) total number of neutrophilic granulocytes per mL BAL; (c) total number of macrophages and monocytes per mL BAL; (d) pg/mL of KC in BAL. *, significantly different to the respective control (PBS or ectoine) after Tukey's HSD post hoc testing considering multiple testing; E, ectoine; nd, not detectable.

result may be an indication that ectoine has also beneficial effects with respect to the allergic sensitization which might be boosted by environmental pollution.

The potential of ectoine to reduce the exacerbation of the immune reaction by the neutrophilic lung inflammation was then tested in animals which were sensitized for 12 weeks by repetitive injection of the model allergen ovalbumin (OVA). Animals were challenged 12 h after the application of particles in the presence or absence of ectoine (Figure 4(a)). Inflammatory parameters in the lung of the animals were investigated 24 h after the provocation. At this time point, all sensitized animals exhibited elevated cell numbers in BAL which were highest in CNP-exposed animals (Figure 4(b)). This reaction proved to be significantly reduced when ectoine was applied together with the particles. The differential analysis of the cells revealed that the exacerbating effect of the particles as well as the preventive effect is mostly due to the changes in neutrophilic granulocytes. Remarkably, the same effects are observed at the level of lymphocytes and eosinophils which are considered relevant for the allergic response (Figure 4(e)). Due to the very low number of these cells and the overwhelming number of neutrophils, these cell types were analysed together (other cells). Although exhibiting high heterogeneity, macrophage numbers were elevated in all challenged animals irrespective of an existing inflammation during the provocation. As observed in earlier experiments, this effect was not influenced by the ectoine treatment (Figure 4(d)).

Considering that ectoine itself has no antioxidant capacity the current data may give an indication for the mechanisms by which environmental particles contribute to asthma exacerbation. In this scenario, ectoine significantly reduces the neutrophilic inflammation induced by the particles at the time point of the onset of the antigen provocation. Ectoine is known not to interact with the particles and it does not scavenge reactive oxygen species. It is therefore plausible that

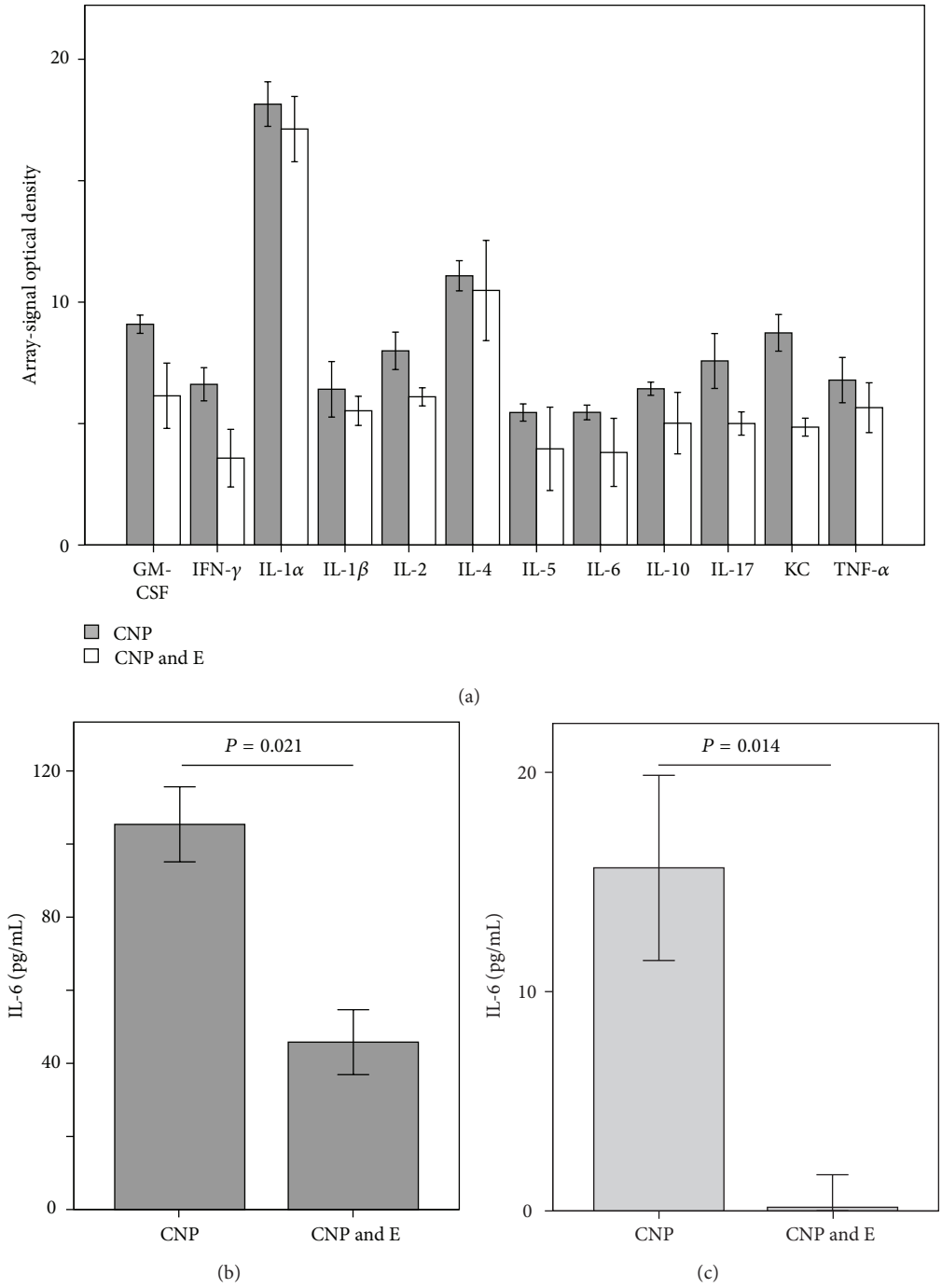

Figure 3: Effect of ectoine treatment on inflammatory mediators 12 h after exposure. (a) BAL analyses of two animals from each group. Given error bars are based on duplicates from both measurements. Significant values cannot be calculated. (b) IL-6 in BAL of 5 animals; (c) IL-6 in serum of 5 animals. CNP, 5 mg/kg carbon nanoparticles; E, 1 mM ectoine. Significant values (two sided) in (b) and (c) were calculated by Mann-Whitney U-test.

the exacerbating effect of combustion-derived nanoparticles is mediated by the proinflammatory trigger which can be observed at the level of neutrophil influx and the respective proinflammatory mediators.

Due to the molecular mechanisms by which ectoine acts, the substance in a first line was suggested to be used as a preventive agent against environmental particulate air pollution. Ectoine in epithelial cells has been shown to prevent typical effects of cell stress triggered by combustion-derived nanoparticles [16]. This strategy however was not designed to replace attempts to improve ambient air quality but was rather considered for predisposed persons who may suffer from chronic lung inflammation or from allergic diseases of the airways. It was therefore important to test

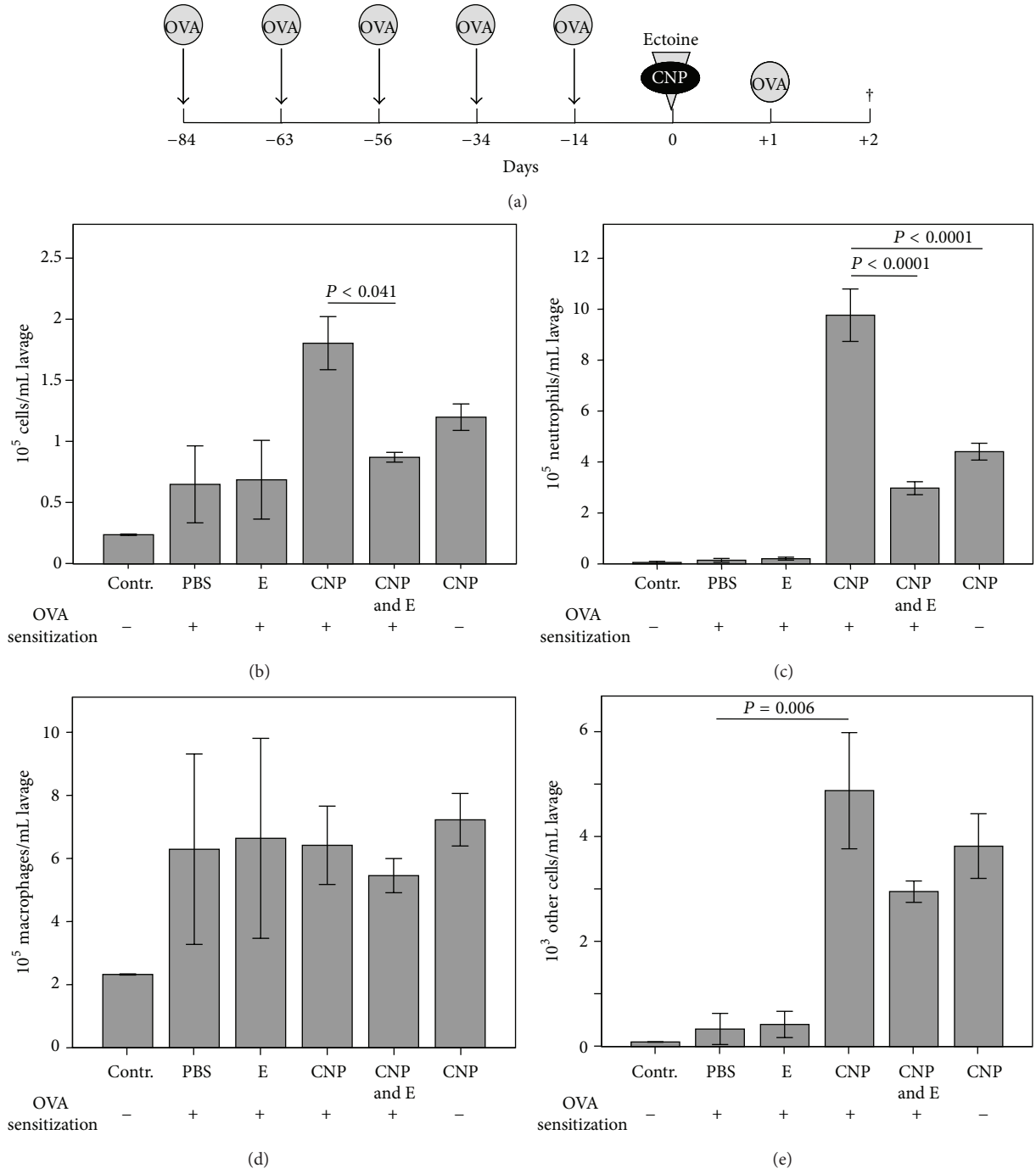

Figure 4: Effect of CNP and ectoine on OVA provocation-induced lung inflammation in sensitized animals (control groups $n = 3$and exposure groups $n = 5$). (a) Experimental design. Animals were sensitized by repetitive intraperitoneal OVA application. At day 0, 5 mg/kg CNP in the presence or absence of 1 mM ectoine was applied to the animals. OVA provocation by inhalation (1%, 30 min) was performed 12 h after treatment. Measurements were made 24 h after challenge. (b) Total number of cells per mL BAL; (c) total number of neutrophilic granulocytes per mL BAL; (d) total number of macrophages and monocytes per mL BAL; (e) total number of other cells including lymphocytes and eosinophilic granulocytes.

whether in the situation of an immune response which is exacerbated by the presence of environmental model particles the inflammatory outcome can be reduced by ectoine. The data show significant differences in neutrophil numbers 36 h after the challenge, indicating that the number of neutrophils recruited by the immune response on OVA provocation can be reduced. At this time point eosinophilic infiltration has just started. Other studies observe elevated levels of these cell types later after the challenge [17]. Therefore the analysis of other cell types in our system has to deal with very low

absolute numbers. Although statistically not significant, a trend in the reduction of these cell types by ectoine can also be observed. In order to investigate the effect of ectoine on this particular immune reaction, long-term experiments have to performed. It has to be tested whether one single ectoine application is sufficient to reduce the accumulation of eosinophils in the lung or whether repetitive treatments are necessary.

In humans different immunological phenotypes of asthma are described. Besides asthma which is characterised by eosinophilic granulocytes, neutrophilic asthma as probably environmentally induced disease is observed [18]. Like COPD, this disease is dominated by a stable chronic neutrophilic lung inflammation which often is not sensitive to the treatment with glucocorticoids [19]. Together with our earlier studies, which demonstrate an effect of ectoine on neutrophils in vivo and ex vivo [14], the current data suggest that ectoine might also be efficient in the treatment of neutrophilic asthma and should be tested in this context.

4. Conclusions

From the data presented here, we conclude that ectoine has beneficial effects on the exacerbation of airway immune responses by environmental particulate air pollution. After having revealed deeper insight into the value and mechanisms of ectoine in chronic neutrophilic lung inflammation, the recent data can be considered the first approach to apply this preventive strategy to predisposed persons like asthmatics. Furthermore, the study indicates the mechanistic importance of neutrophilic lung inflammation in the exacerbation of allergic airway diseases.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

Treatment of Rhinitis Sicca Anterior with Ectoine Containing Nasal Spray

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Objectives. The safety and efficacy of ectoine nasal spray and ectoine nasal spray with dexpanthenol in the treatment of rhinitis sicca were evaluated in two studies. *Design and Methods.* Two noninterventional observational studies were performed to evaluate the efficacy and safety of a nasal spray containing ectoine (study 1) and ectoine/dexpanthenol (study 2) over a period of two weeks including comparable numbers of patients suffering from rhinitis sicca anterior. Patients and physicians were asked to rate the efficacy in reducing symptoms and the tolerability over the treatment phase. *Results.* The treatment in both studies resulted in a clinical and statistical significant reduction of the main diagnosis parameters, nasal airway obstruction, and crust formation. There was also a significant reduction in the secondary diagnosis parameters in both studies. Importantly, the tolerability was very good. During the whole observational study, neither patients nor doctors stopped the medication due to unwanted effects. *Conclusion.* Rhinitis sicca could be successfully treated with a nasal spray containing ectoine and a nasal spray combining ectoine with dexpanthenol. The combination of both substances led to slight advantages.

1. Introduction

Rhinitis sicca or generally speaking dry nose is a rather frequent problem involving many people. The term "dry nose" has not yet been uniformly defined [1]. Otolaryngologists often use the terms "rhinitis sicca" or "dry rhinitis," although no clear definition exists. Many symptoms during dry nose could be encountered ranging from subjective sensation of the dry nose and itching up to mild burning, nasal obstruction, crusting associated with unpleasant smell, epistaxis, and diminished sense of smell. Rhinitis sicca anterior means a chronic inflammation in the region of the anterior part of the nose, affecting the anterior and caudal septum and/or the corresponding lateral nasal vestibule. Mechanical as well as environmental irritations lead to crust formation. In rare cases, patients suffer from a slight stench due to bacterial colonization of the crust formations. The treatment of rhinitis sicca involves mainly elimination of promoting factors, moistening, sufficient daily drinking amount, cleansing of the crusts, care of the mucosa and inhibition of possible infections, or in rare cases the elimination of overlarge endonasal space [1]. The main treatment for rhinitis sicca consists of humidification of the nose, especially the mucus, focusing in a real wash-out of possible inflammatory triggers and application of a protective layer on the mucus. The market offers a huge number of different devices involving saline, oils, moisturizers, sprays, and ointments for this purpose. Nasal irrigation and nasal saline sprays wash out inflammatory triggers directly [2, 3] and achieve an improvement of mucociliary clearance by improving the ciliary beat frequency [4, 5]. Nasal ointments mostly including glycerol develop a protective moistening effect and protect the nose from water loss [6]. Low concentrated oils also have beneficial effects on nasal ciliary beat frequency [7]. The efficacy of dexpanthenol, the alcohol analog of pantothenic acid, in the treatment of rhinitis sicca is widely spread in the OTC use and has been shown clinically [8]. In addition, use of dexpanthenol has a strong tradition in the treatment of various skin diseases in which dexpanthenol is used as humidifier/moisturizer. Also, use in wound healing has been reported [9]. Besides these different options, patients ask for alternative treatments as the current treatments often leave patients

unsatisfied and a demand for other nonpharmacological treatment options exists.

Ectoine is an extremolyte, a compatible solute which is produced by microorganism living under extreme environmental conditions such as extreme salinity or dryness [10]. In those microorganisms, ectoine serves as natural cell protectant [11, 12]. Different in vitro, ex vivo, and in vivo studies have shown that ectoine can be used to protect epithelial tissues and moisturize and reduce inflammations [13–15]. Ectoine acts physically via a mechanism called "preferential exclusion." In the presence of ectoine, membranes and lipids are protected indirectly: as ectoine is expelled from the surface of proteins and lipids, those are protected by a water shell, thereby increasing the fluidity of membranes and resulting in the preferential formation of the native conformation of proteins [11]. This might stabilize mucous membranes such as lining epithelia of the nose, thereby protecting those cells from invading allergens or pathogens [16]. Recent developments have demonstrated that these cell protective attributes could be transferred into medical devices including ectoine containing creams, nasal sprays, or eye drops which can be used for human use, for example, the treatment of atopic dermatitis, allergic rhinitis, and rhinosinusitis [17–20].

The use of ectoine in a saline based nasal spray could be a useful therapeutic approach for patients suffering from dry nose syndrome. Additionally a combinatory approach could be applied, for example, of ectoine and dexpanthenol. The combined effects of ectoine and/or dexpanthenol are already used in the field of dermatology and promise a useful combination effect for the treatment of rhinitis sicca. By using an ectoine and dexpanthenol nasal spray, the moisturizing and regeneration supporting effects of both compounds could assist a possible healing of ulcers and prevent nasal obstruction in addition to the reduction of primary symptoms.

2. Materials and Methods

The current paper describes two prospective, open-label, noninterventional trials (studies 1 and 2). Restricted inclusion of patients study based on the diagnosis of rhinitis sicca and strict adherence to the principle of nonintervention allowed data to be collected for a very unselective patient population. As study designs for both studies were very similar, data are summarized and differences are only outlined where applicable.

2.1. Medication. Patients in study 1 were treated with an ectoine containing nasal spray with 0.5% ectoine and further ingredients were sodium chloride, sodium-di-hydrogen-phosphate dihydrate, di-sodium-hydrogen-phosphate, and water.

Patients in study 2 used a 0.5% ectoine nasal spray which contained 1.0% dexpanthenol, sodium chloride, sodium-di-hydrogen-phosphate dihydrate, di-sodium-hydrogen-phosphate, and water.

2.2. Treatment and Study Design. Both studies were open for all patients from 18 years on, who were identified by ENT specialist with symptoms of dry nose. Following confirmation of the diagnosis of rhinitis sicca, patients were asked by the ENT specialist whether they were interested to participate in the current trials. Upon signing a patient information and consent form, patients had to attend two more site visits: V2 on day 7 ± 2 and V3 on day 14 ± 2. During the entire treatment period of 2 weeks, patients were asked to use the nasal sprays at least five times daily.

2.3. Scoring of Symptoms. Clinical symptoms were assessed on a 12-point scale ranging from 0 (no symptoms) to 12 (very severe symptoms).

During the visits, the physician assessed the main symptoms of nasal obstruction and crusting of the nose as well as the following secondary symptoms: endonasal blood deposits, concomitant pharyngitis, cacosmia, rhinorrhea, exudate viscosity, and turbinate hyperplasia.

On days 3, 6, 9, and 12 after start of the study, patients were asked to document the severity of the following symptoms in a patient diary: nasal obstruction, dryness of the nose, nose bleeding, sore throat, cacosmia, and exudate from the nose. In addition, patients were asked to describe the consistency of exudate on a 12-point scale from 0 (fluid) to 12 (crusted).

3. Scoring of Efficacy, Tolerability, and Compliance

Both efficacy and tolerability were assessed by physicians (during V2 and V3) and by the patients (days 3, 6, 9, and 12) on a scale from 0 (very good) to 12 (none/bad).

3.1. Statistics. The statistical analysis was carried out with SPSS version 15 (study 1) or 17 (study 2), respectively. Both efficacy and safety analyses were performed on the entire study population. Descriptive statistics were used for a quantitative report of the main study population features. Continuous variables were tested for normal distribution via Kolmogorov-Smirnov test. Further analysis was carried out with the Mann-Whitney U test, Wilcoxon test, or Friedman test. The level of significance was set to $P < 0.05$ in all tests. Unavailable data were treated as "missing values" or substituted by the "last value carried forward" method.

4. Results

Both studies were conducted in accordance with the Declaration of Helsinki. All investigations were carried out with informed consent of all participants.

Study 1 was a noninterventional trial taking place from April to July 2008; study 2 took place from March to April 2009. Both studies were carried out at a German ear nose throat (ENT) practice. Distribution and demographics of patients are shown in Figures 1 and 2.

4.1. Development of Symptoms

4.1.1. Nasal Obstruction. In both studies, the investigators assessment revealed that the symptom nasal obstruction decreased significantly from V1 to V2 as well as further to V3.

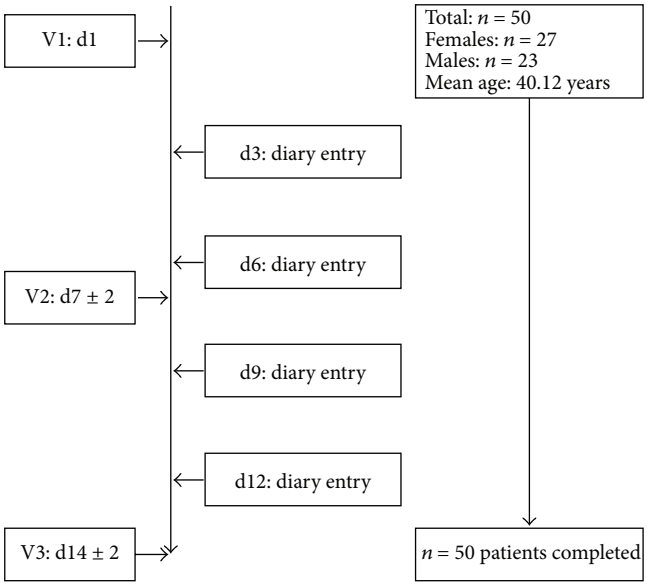

Figure 1: Patient flow and characteristics of demographic data in study 1.

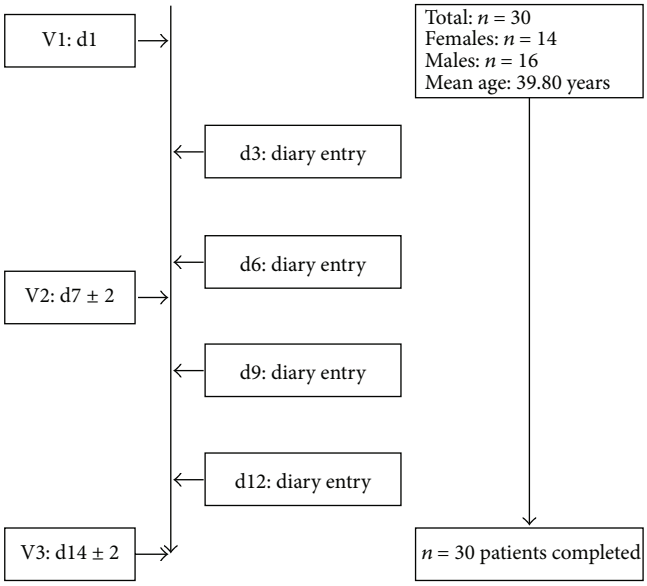

Figure 2: Patient flow and characteristics of demographic data in study 2.

In study 1, symptom scores decreased from baseline values 4.60 ± 2.23 at V1 to 2.74 ± 1.95 at V2 and then to 1.54 ± 1.52 at V3. Values in study 2 decreased comparably from 5.43 ± 1.46 at V1 to 2.23 ± 2.05 at V2 and further to 1.73 ± 1.89 at V3 (Figures 3 and 4).

Decreases of the symptom nasal obstruction were similar and in accordance with the patients' assessments. Values are listed in Table 1.

4.1.2. Crust Formation/Nasal Dryness. The symptom nasal crust formation decreased significantly from V1 to V2 and further to V3 in both studies in the investigators assessment. Values in study 1 decreased from baseline values of 6.20 ± 1.99 to 2.16 ± 2.26 at V2 and further to 1.52 ± 1.85 at V3. Values in study 2 decreased comparably from baseline values of 6.43 ± 2.08 to 2.40 ± 1.81 at V2 and to 1.30 ± 1.24 at V3. Results are depicted in Figures 5 and 6.

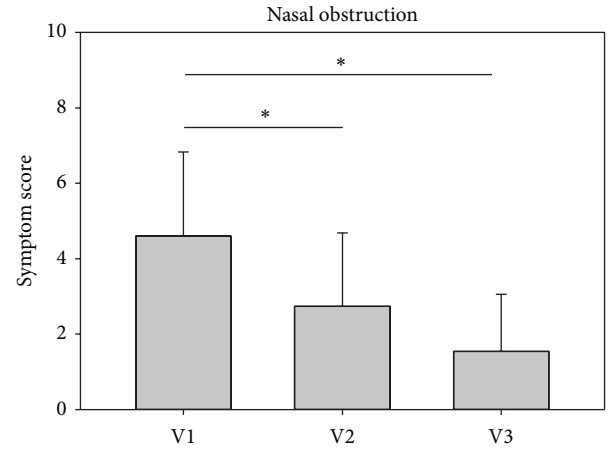

Figure 3: Development of nasal obstruction from V1 to V3 assessed by the investigator (study 1). The asterisks mark a statistical significance; the whiskers mark the standard deviation.

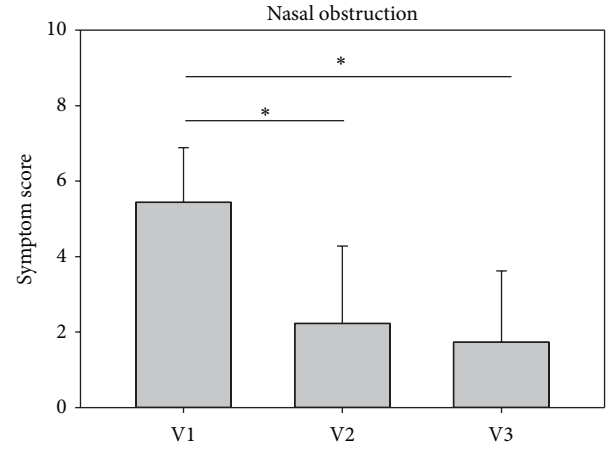

Figure 4: Development of nasal obstruction from V1 to V3 assessed by the investigator (study 2). The asterisks mark a statistical significance; the whiskers mark the standard deviation.

Patients evaluated the decrease of the symptom dry nose in a similar way to the physician's assessments as listed in Table 2. The symptom nasal dryness decreased significantly from day 3 to day 12 in both studies.

4.1.3. Secondary Symptom Scores. In addition to the symptoms nasal obstruction and crust formation/nasal dryness, further symptoms were assessed by both investigators and patients. As depicted in Figures 7 and 8, there was a similarity between the results of both studies in the investigators assessment. The symptoms blood deposits, pharyngitis, turbinate hyperplasia, and exudate viscosity improved significantly

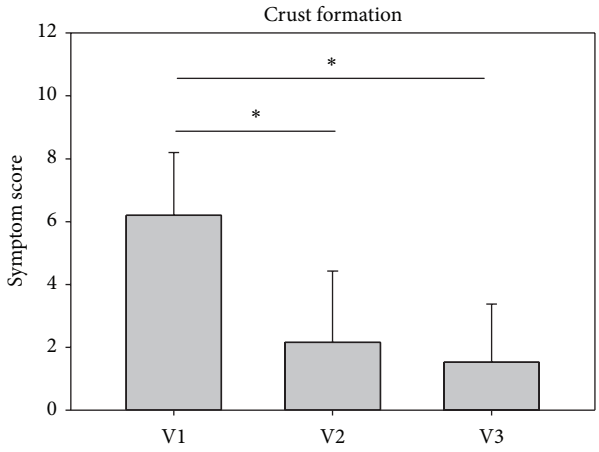

Figure 5: Development of crust formation from visit 1 (V1) to visit 3 (V3) in study 1. $^{*}P < 0.001$. The asterisks mark a statistical significance; the whiskers mark the standard deviation.

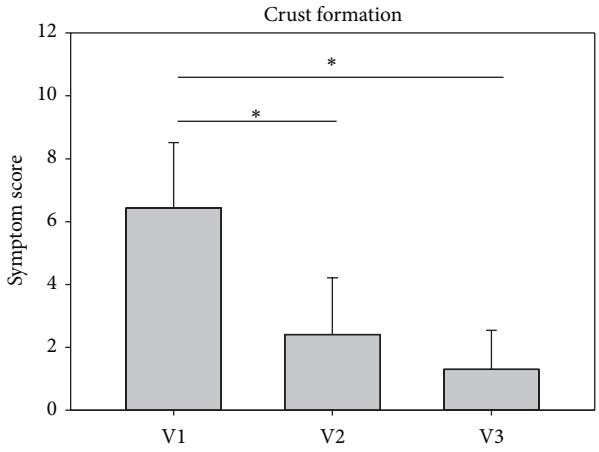

Figure 6: Development of crust formation from visit 1 (V1) to visit 3 (V3) in study 2. $^{*}P < 0.001$.

from baseline values at V1 to the final visit V3. The symptom rhinorrhea only improved significantly in study 2, whereas decreases in this symptom were nonsignificant in study 1. As only very few patients complained about the symptom cacosmia ($n = 2$ in study 1 and $n = 5$ in study 2), decreases in this symptom were negligible.

Patients' scores of secondary symptom evaluation are listed in Tables 3 and 4. In study 1, the symptom rhinorrhea improved significantly from d3 to d12. In study 2, nose bleeding, rhinorrhea, cacosmia, and exudate viscosity improved significantly over this time frame.

5. Efficacy, Tolerability, and Compliance

The physician judged both efficacy and tolerability of treatment after 7 days (V2) and after 14 days (V3). As shown in Figures 9 and 10, ectoine nasal spray treatment was considered to be both efficient and well tolerable. Mean values for efficacy at V3 were 3.5 ± 2.06 (study 1) and 1.83 ± 1.39 (study 2) meaning good to very good efficacy. Mean values for tolerability were 2.08 ± 1.21 (study 1) and 0.57 ± 0.97 (study 2), which also means good to very good tolerability.

Patients' assessments of tolerability and efficacy of treatment are depicted in Figures 11 and 12. Mean efficacy values were 3.12±3.08 at day 12 of treatment in study 1 and 2.43±2.24 in study 2 corresponding to good efficacy. Tolerability was judged as very good in both studies with mean values on day 12 of 1.40 ± 1.80 in study 1 and 1.17 ± 1.21 in study 2.

5.1. Adverse Events (AEs). In study 1, no AE occurred. In study 2, 1 AE occurred (acute rhinitis). The correlation with the treatment was judged as unlikely by the investigator. No SAE occurred in either of the two studies.

6. Conclusions

The aim of these observational studies was to gain insight into the tolerability and the extent to which the treatments influenced the severity of the patients' symptoms. An ectoine nasal spray (study 1) or an ectoine and dexpanthenol nasal spray (study 2) was tested in patients with rhinitis sicca under practical conditions. A total of 80 patients (50 patients in study 1, 30 patients in study 2) with a wide variety of disease severities participated in this postmarketing surveillance study. However, the potential flaw of these studies is their noncontrolled character, the missing randomization, or placebo control. Therefore the evidence grade of the results needs to be reduced at least to IIb.

Both nasal spray formulations showed a good tolerability and safety in the studies. No drop-out was recorded. The studies showed a significant decrease of the main symptoms nasal obstruction and crust formation from V1 to V2 as well as further to V3. The decrease of nasal obstructions assessed by the physicians was confirmed by patients in both studies, with a stronger decrease of symptoms assessed by the physicians. This is likely to be due to the timing of the patient's diary, as this was started at day three of treatment, when the first positive effect of the respective treatments had occurred already.

Apart from main symptoms, the secondary symptom scores also decreased similarly in both studies. In the investigators assessment, the symptoms blood deposits, pharyngitis, turbinate hyperplasia, and exudate viscosity improved significantly from starting values at V1 to the final visit V3. Differences in symptom reduction between both studies occurred only with respect to rhinorrhea. Treatment with the nasal spray with ectoine only did not lead to a significant improvement of rhinorrhea, whereas the improvement in with the ectoine nasal spray alone was not statistically significant. The degree of symptom reduction in the main parameter nasal obstruction seemed to be reduced more efficiently in the study with the combined nasal spray, as the score started with a higher value and dropped faster and to a higher degree as in the study with the ectoine nasal spray. In the patient assessment of study 2 symptom improvement was significant for nose bleeding, exudate viscosity, rhinorrhea, and cacosmia, whereas the patient assessment in study 1 showed only a significant reduction of the symptoms in nose

Table 1: Development of nasal obstruction assessed by patients on days 3, 6, 9, and 12 following treatment with ectoine nasal spray.

	d3	d6	d9	d12	P (d3 versus d12)
Study 1	2.74 ± 2.31	2.52 ± 2.18	2.10 ± 2.00	1.64 ± 1.68	<0.001
Study 2	3.67 ± 2.28	2.80 ± 1.99	2.33 ± 1.65	1.87 ± 1.33	<0.001

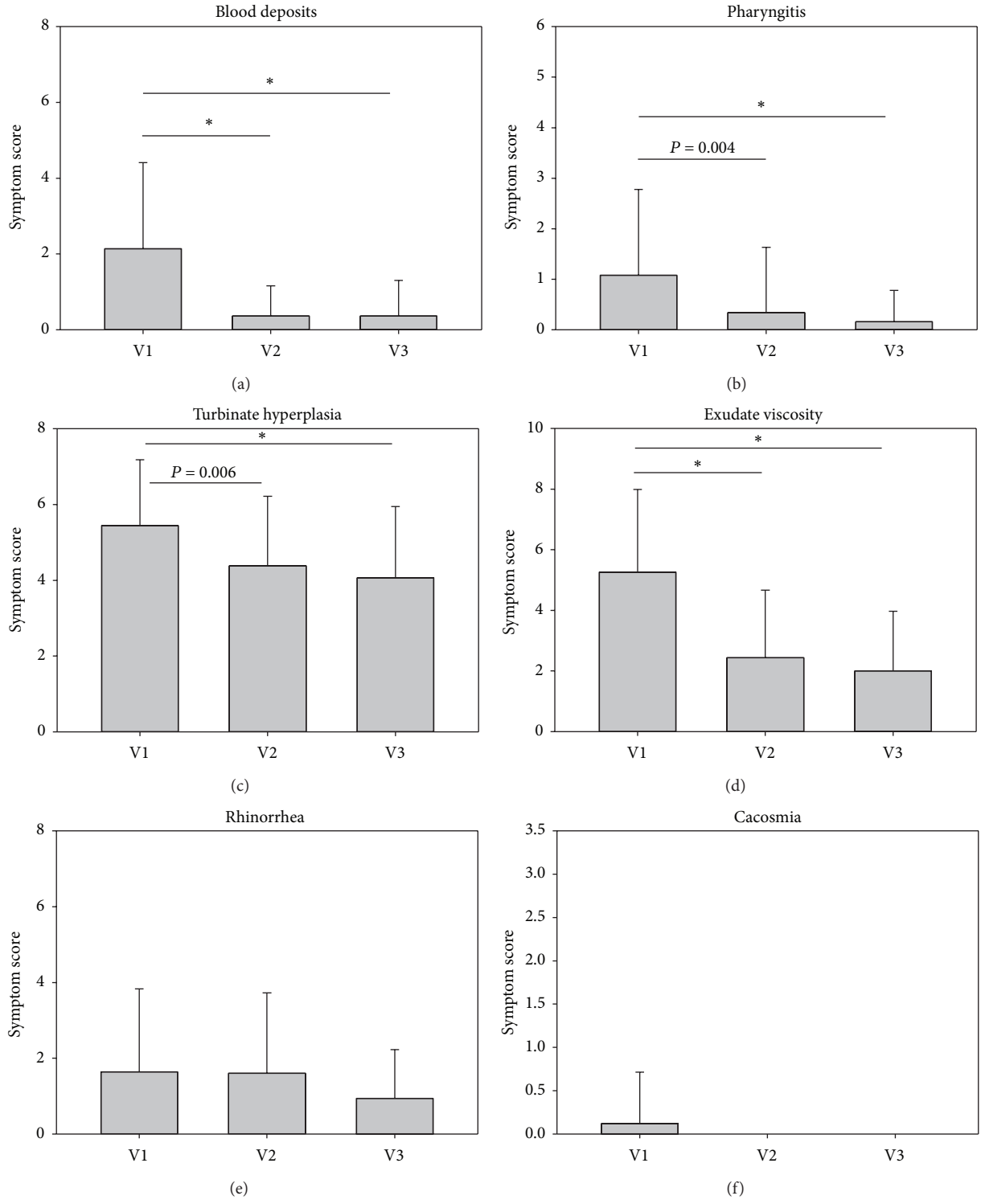

Figure 7: Development of secondary symptoms (ENT evaluation) during study 1. $^{*}P < 0.001$. The asterisks mark a statistical significance; the whiskers mark the standard deviation.

Table 2: Development of nasal dryness assessed by patients on days 3, 6, 9, and 12 following treatment with ectoine nasal spray.

	d3	d6	d9	d12	P (d3 versus d12)
Study 1	4.64 ± 2.40	3.76 ± 2.53	2.90 ± 2.48	2.42 ± 2.37	<0.001
Study 2	4.43 ± 2.49	3.30 ± 2.12	2.33 ± 1.97	1.83 ± 1.49	<0.001

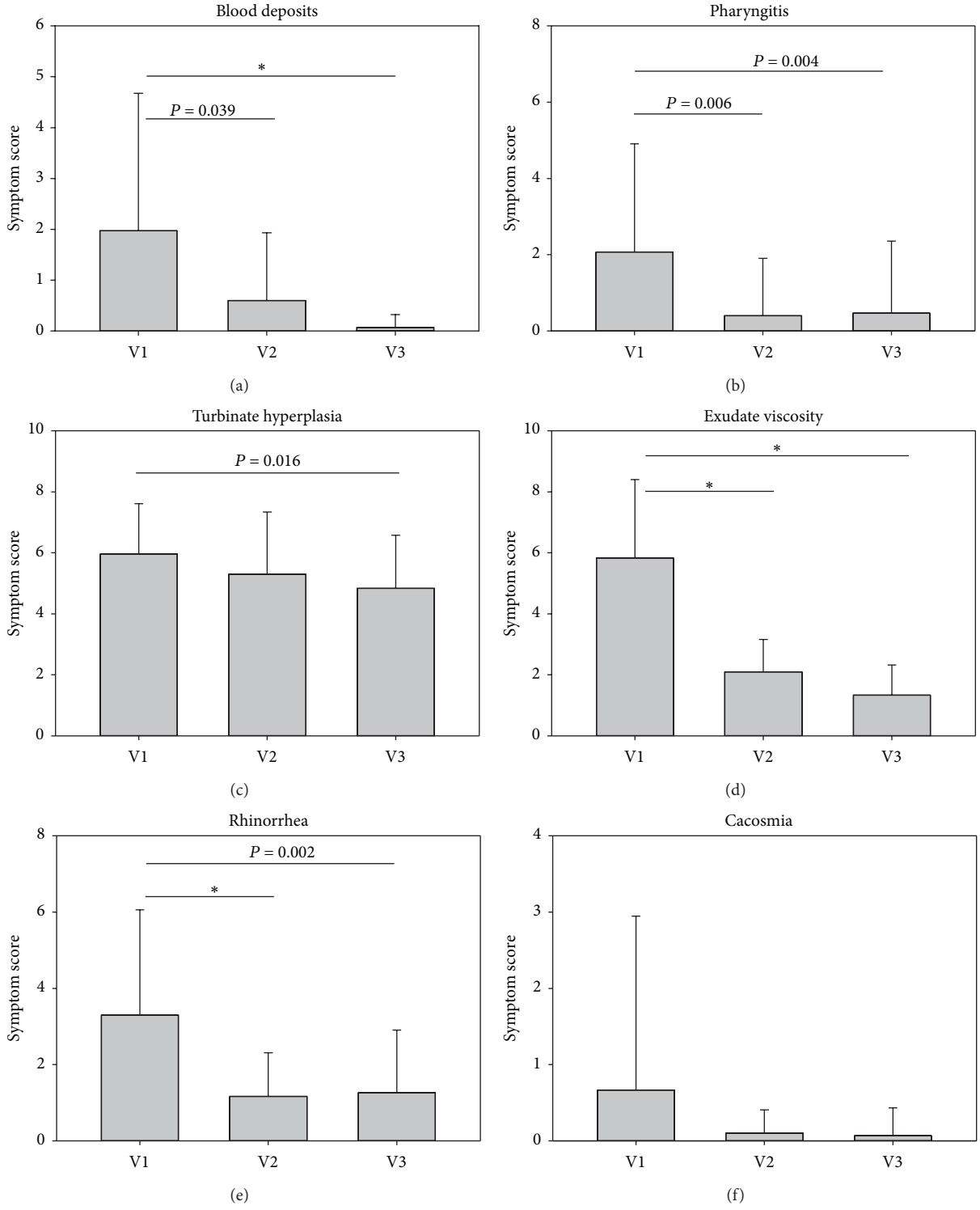

Figure 8: Development of secondary symptoms during study 2. $^{*}P < 0.001$.

Table 3: Patients' assessments of secondary symptom scores at days 3, 6, 9, and 12 following treatment start of study 1.

Symptoms	d3	d6	d9	d12	*P* value (d3 versus d12)
Nose bleeding	0.36 ± 0.85	0.46 ± 1.07	0.36 ± 0.85	0.30 ± 0.74	0.787
Pharyngitis	0.64 ± 1.77	0.62 ± 1.74	0.46 ± 1.20	0.42 ± 1.13	0.754
Exudate viscosity	3.36 ± 3.50	2.90 ± 3.03	2.38 ± 2.86	2.40 ± 2.93	0.108
Rhinorrhea	2.44 ± 2.16	2.10 ± 2.14	1.74 ± 1.88	1.42 ± 1.54	0.021
Cacosmia	0.60 ± 1.53	0.48 ± 1.31	0.42 ± 1.57	0.26 ± 0.69	0.388

Table 4: Patients' assessments of secondary symptom scores at days 3, 6, 9, and 12 following treatment start of study 2.

Symptoms	d3	d6	d9	d12	*P* value (d3 versus d12)
Nose bleeding	0.70 ± 1.60	0.73 ± 1.72	0.50 ± 1.01	0.17 ± 0.59	0.0019
Pharyngitis	1.07 ± 2.26	0.67 ± 1.63	0.57 ± 1.19	0.60 ± 1.00	0.719
Exudate viscosity	2.67 ± 2.38	2.50 ± 2.64	2.10 ± 2.23	1.67 ± 2.19	0.027
Rhinorrhea	2.57 ± 1.99	2.00 ± 1.72	1.90 ± 1.83	1.57 ± 1.45	0.025
Cacosmia	0.90 ± 2.26	0.70 ± 1.82	0.33 ± 0.99	0.30 ± 0.99	0.005

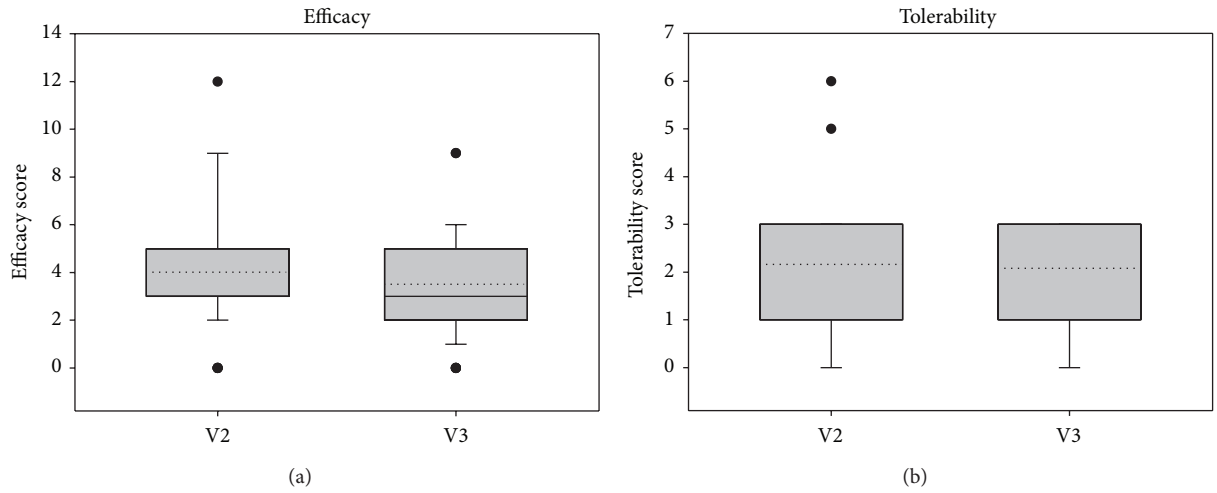

Figure 9: Judgment of efficacy and tolerability according to the physician's assessment in study 1.

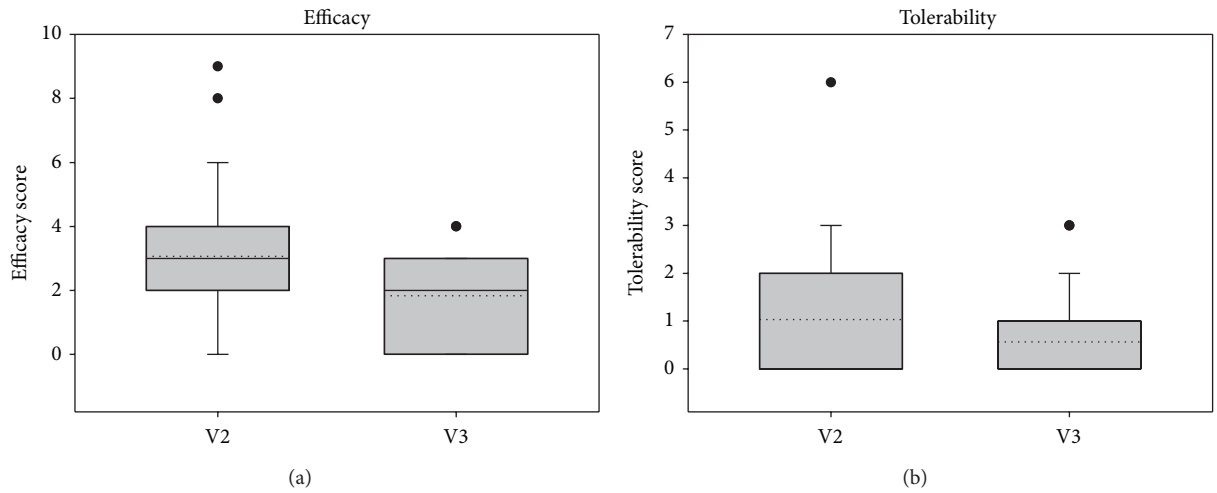

Figure 10: Judgment of efficacy and tolerability according to the physician's assessment in study 2.

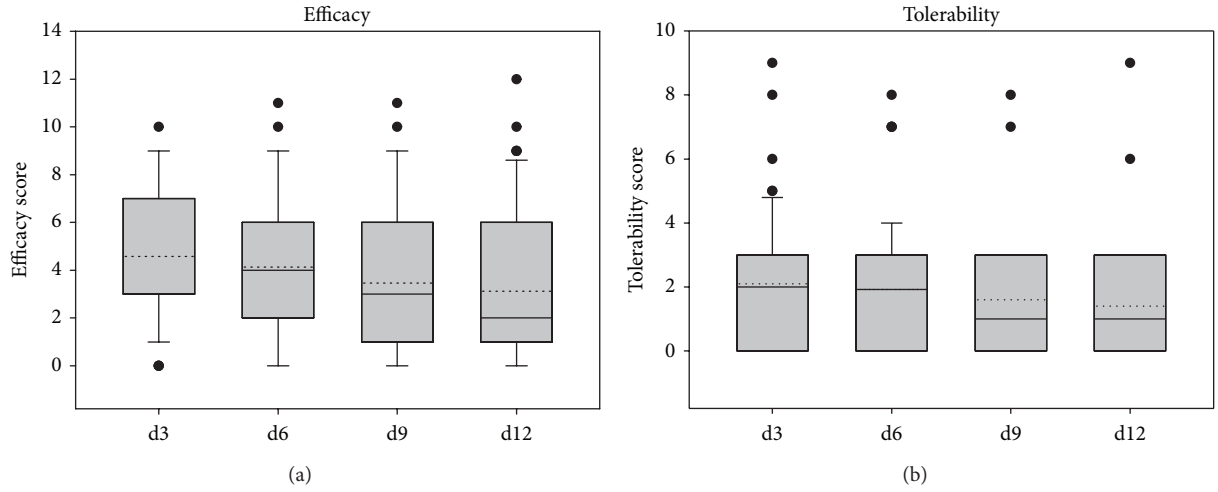

Figure 11: Judgment of efficacy and tolerability according to the patients' assessment in study 1.

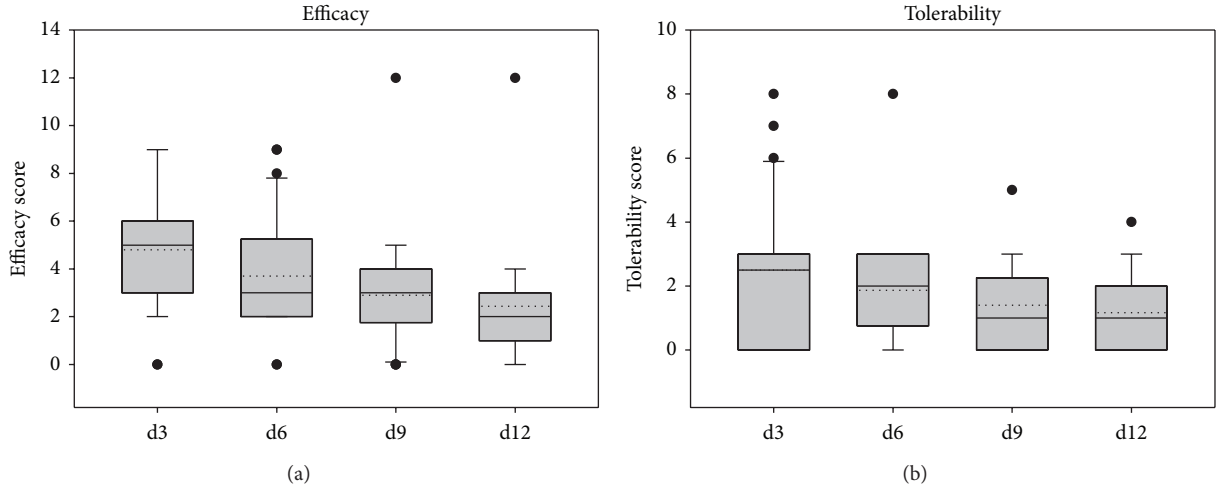

Figure 12: Judgment of efficacy and tolerability according to the patients' assessment in study 2.

bleeding. It can be mentioned that only a few patients in both studies suffered from cacosmia and the decreases in these symptoms were negligible for both of them.

As a summary, the ectoine nasal spray achieved in study 1 treatment success similar to that of the combination of ectoine and dexpanthenol in study 2 with respect to the main symptom scores of both studies, crust formation and nasal obstruction. Differences in treatment effect between both nasal sprays and studies were observable in the secondary parameters, both in physicians and in patients assessment, tending towards an additional effect if ectoine and dexpanthenol are combined in one product compared to ectoine alone. Both natural nonpharmacological nasal sprays showed efficacy in treatment of rhinitis sicca, which is comparable to the reported outcome for other products [21]. Data from preclinical studies also support the combination of ectoine and dexpanthenol (data not shown).

The mode of action and subsequent effect in treatment of rhinitis sicca of dexpanthenol is understood from the literature [22]. The treatment effects of the ectoine nasal spray can be attributed to its physical action. By increasing the fluidity of the nasal epithelia, the barrier function of this membrane is increased, therefore inhibiting the potential loss of water. Experiments with ectoine on biological and artificial membranes support this thesis further, including the reduction of mechanical stress induced membrane damage [15–17]. The additional hydrating effect of ectoine is well described in the literature [15, 23, 24] as well as the capacity of reduction of inflammation in skin and respiratory epithelium [14, 17, 18, 25].

Taken together, rhinitis sicca anterior or dry nose could be successfully treated with an ectoine containing nasal spray. Therefore an interesting option of a new and safe nonpharmacological treatment of rhinitis sicca will be available in the future. The addition of the well-known and accepted dexpanthenol did not enhance the treatment regarding the major symptoms scores. The decrease of symptom over the 14-day treatment period was more pronounced for the

combination of ectoine and dexpanthenol, but this difference was not significant. Slightly better improvement in different secondary symptoms revealed synergistic characteristics of the two substances in combination when compared to the ectoine nasal spray alone. However, these findings came from two independent noncontrolled trials. Therefore additional controlled trials are suggested to further prove the efficacy of ectoine nasal spray with or without dexpanthenol.

Disclosure

Dr. O. Scherner and Nina Werkhäuser are employees of Bitop AG, a company where medical devices, including the ectoine nasal spray, were developed and registered. Bitop AG sponsored the trials discussed in this paper. Dr. Uwe Sonnemann received sponsorship by Bitop AG to conduct the studies.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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