Effects of ASM-024, a modulator of acetylcholine receptor function, on airway responsiveness and allergen-induced responses in patients with mild asthma

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OBJECTIVES: To evaluate the safety, tolerability and clinical activity of ASM-024, a new cholinergic compound with dual nicotinic and muscarinic activity, in mild asthma.

METHODS: The present study involved 24 stable, mild allergic asthmatic subjects. In a cross-over design, ASM-024 (50 mg or 200 mg) or placebo were administered once daily by nebulization over three periods of nine consecutive days separated by a three-week washout. The effect of each treatment on the forced expiratory volume in 1 s (FEV₁), provocative concentration of methacholine causing a 20% decline in FEV₁ (PC₂₀), early and late asthmatic responses, and allergen-induced inflammation were measured.

RESULTS: Seventeen subjects completed the study. During treatment with ASM-024 at 50 mg or 200 mg, the PC₂₀ value increased respectively from a mean (± SD) 2.56±3.86 mg/mL to 4.11 mg/mL (P=0.007), and from 3.12±4.37 mg/mL to 5.23 mg/mL (P=0.005) (no change with placebo). On day 7 (day preceding allergen challenge), post-dosing FEV₁ increased by 2.0% with 50 mg (P=0.005) and 1.9% with 200 mg (P=0.008) (placebo -1.1%). ASM-24 had no inhibitory effect on early and late asthmatic responses, nor on sputum eosinophil or neutrophil levels. ASM-024 induced no serious adverse events, but caused cough in 22% and 48% of the subjects with 50 mg and 200 mg, respectively, compared with 10% who were on placebo.

CONCLUSIONS: ASM-024 did not inhibit allergen-induced asthmatic response and related airway inflammation, but reduced methacholine airway responsiveness and slightly improved lung function. The mechanism by which ASM-024 improves these outcomes requires further study.

Key Words: Allergen challenge; Asthma; Nicotinic receptor agonists
Florida, Florida, USA) but rather blocks the activation of the a3β4 and a7 nicotinic receptor ion channel function by ACh or nicotine.

ASM-024 is, however, able to activate the a7 nAChR channel opening in the presence of the positive allosteric modulator (PNU-120596), indicating that ASM-024 behaves as a ‘silent agonist’ that places the receptor in a desensitized state. Compounds with similar properties have been shown to induce signal transduction pathways independently of ion channel activation (11). Moreover, ASM-024 has demonstrated an antagonist effect on ACh-evoked activation at the M1, M2 and M3 muscarinic receptors expressed in Xenopus oocytes (12). It has shown a very good safety profile when administered by inhalation to healthy volunteers, the principal effects being local irritation and cough, mainly at high doses (13).

The primary objective of the present study was to evaluate the potential inhibitory effects of ASM-024 on airway responsiveness, airflow limitation and allergen-induced asthmatic responses in mild steroid-naive asthmatic subjects. We also obtained preliminary information regarding the dose-response profile of ASM-024 in terms of safety, tolerability and pharmacokinetic profile in this subject population.

**METHODS**

**Subjects**

The present study involved 24 stable, steroid-naive, mild allergic non-smoking asthmatic subjects with previously documented allergen-induced early and late asthmatic responses, respectively defined as an acute fall in forced inspiratory volume in 1 s (FEV1) ≥20% within 2 h following allergen challenge and a fall in FEV1 ≥15% between 3 h and 7 h following allergen challenge.

Subjects included men and women ≥18 and ≤50 years of age with mild allergic asthma using inhaled short-acting β2-agonists on demand as the only asthma medication. Female subjects of childbearing potential were required to use adequate contraception. All subjects were required to have an FEV1 ≥19 kg/m² and ≤≤20% fall in FEV1 was achieved 10 min post allergen inhalation. The same dose of allergen had to be administered on subsequent visits unless there was a safety issue, in which case the investigator could give a lower dose.

**Study medication**

The study medication was supplied as 5 mL vials containing an isotonic saline solution for the placebo as well as for the doses of 50 mg (12.5 mg/mL) and 200 mg (50 mg/mL) of ASM-024. Doses were administered by nebulization over a period of 15 min.

**Methacholine challenges**

Methacholine inhalation challenge was performed as described by Cockcroft et al (14), using tidal breathing, the aerosol being produced by a calibrated Wright nebulizer. The test was ended when a fall in FEV1 of at least 20% of the baseline value was recorded, after which the methacholine PC20 was calculated.

**Allergen bronchoprovocation**

Allergen challenges were performed as described by Boulet et al (15). The concentration of allergen extract required for inhalation was determined from a formula described by Cockcroft et al (16).

**Blood analyses**

Blood samples were collected before the administration of ASM-024 and period, controlling for possible carry over effects and followed with differential counts and stained with Diff Quik (American Scientific Products, USA). One of the investigative sites was designated to perform standardized inflammatory cell counting for all of the sites. All sputum samples were used regardless of squamous cell contamination; however, the cell percentage was based only on the inflammatory cells and airway epithelial cells.

**RESULTS**

Seventeen subjects completed the study per protocol. Seven subjects had to be withdrawn from the study due to study medication-induced cough (n=2), respiratory infection (n=1), asthma exacerbation (n=2), prestudy condition (n=1) and unrelated personal reason (n=1).
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Baseline airway calibre and airway responsiveness

On day 7, there was a slight but statistically significant increase in FEV1 of 2.0% following the administration of 50 mg of ASM-024 (P=0.005 versus placebo) and of 1.9% with the 200 mg dose (P=0.008), while with placebo the change was −1.1% (Figure 2). The FEV1/forced vital capacity ratio displayed a similar magnitude of change on day 7 (P<0.05 [data not shown]). Such effects were not observed on the first day of treatment or on day 9 (ie, following the last allergen challenge).

There was a significant improvement in methacholine PC20 on day 7 of treatment, before the allergen challenge on day 8, with both doses of ASM-024 (Figure 3). The mean PC20 value was 3.97 mg/mL (range 0.66 to 39.0 mg/mL) before and 4.29 mg/mL (range 0.63 to 57.0 mg/mL) after placebo period (P=0.008). During treatment with ASM-024 50 mg, PC20 increased from 2.98 mg/mL (range 0.35 to 20.16 mg/mL) to 5.24 mg/mL (range 0.5 to 32.4 mg/mL) (P=0.006) and during treatment with ASM-024 200 mg from 3.87 mg/mL (range 0.35 to 20.16 mg/mL) to 6.55 mg/mL (range 1.21 to 36.33 mg/mL) (P=0.003).

Allergen challenges

ASM-24 had no inhibitory effect on the allergen-induced change in methacholine PC20, or early and late asthmatic responses (Figures 3 and 4).

Airway inflammation

ASM-024 had no significant effect on the mean numbers of induced sputum total cell, eosinophil or neutrophil counts (or percentages) following the allergen challenge. No changes in white blood cell counts were observed following treatment. At the end of the treatment period, a statistically significant decrease in the blood lymphocyte count was observed for the dose of 50 mg compared with the placebo (P=0.009), with a similar trend observed for the dose of 200 mg (P=0.09).

Side effects

ASM-024 induced no serious adverse events but coughing was reported in 22% and 48% of the subjects at the doses of 50 mg and 200 mg, respectively, as compared with 10% on placebo, and bad taste was reported in 78% and 70% of the subjects at the doses of 50 mg and 200 mg, respectively, compared with 5% on placebo (Table 1).

Pharmacokinetics

ASM-024 was detected in plasma in all subjects who received it at either 50 mg or 200 mg. Individual postdosing plasma concentrations ranged between 0.7 ng/mL and 79 ng/mL at the 50 mg dose, and between 1.9 ng/mL and 311 ng/mL at the 200 mg dose. On the whole, systemic exposure appeared to be proportional between the two dose levels, with mean (± SD) values of 19±18 (median = 16 [n=20]) on dose 50 mg and 62±58 (median = 44 [n=20]) on dose 200 mg.
day 1 and 24±16 (median = 23 [n=17]) on day 9 at the 50 mg dose, and 88±88 (median = 52 [n=21]) on day 1 and 87±9 (median = 44 [n=18]) ng/mL on day 9 at the 200 mg dose. Residual levels of ASM-024 were observed predosing in some subjects in the morning of day 9 following repeat administration at both doses of 50 mg or 200 mg, with levels up to 4 ng/mL and 7 ng/mL, respectively. On the whole, there was no evidence of a clear relationship between the individual extent of systemic exposure to ASM-024 and the parameters associated with safety or clinical activity.

DISCUSSION

The present study showed that although ASM-024 had no significant effect on allergen-induced allergic asthmatic responses and induced sputum cell differential, it decreased methacholine response over time.

ASM-024 development was based on the hypothesis of a role for the cholinergic system in the regulation of airway bronchomotor tone and inflammation. Recent studies have demonstrated that ACH is also synthesized by non-neuronal cells, including inflammatory and epithelial cells, and is involved in the regulation of inflammation through binding to nicotinic receptors and, in particular, with the α7 subunit (α7nAChR), and also with other subtypes (18). Nicotine and other nicotinic receptor agonists have demonstrated similar anti-inflammatory properties (19). However, the additive properties of nicotine strongly limits its therapeutic potential. ASM-024 was developed to modulate the function of α7nAChRs, but without addictive properties. In the present study, we could not find significant effects of the drug on inflammatory features, except for a mild change in lymphocyte count of uncertain significance.

DMPP (1,1-dimethyl-4-phenyl piperazinium), a nicotinic receptor agonist that does not cross the blood brain barrier, was initially studied. It demonstrated both anti-inflammatory and smooth muscle relaxant properties (20-22). ASM-024, a quaternary ammonium compound, is an analogue of DMPP, has anti-inflammatory, smooth muscle relaxant and bronchoprotective properties in various in vitro and in vivo models (23). ASM-024 was also shown to have a potential anti-muscarinic effect. However, the precise mechanism of action of ASM-024 remains to be further established.

The allergen bronchoprovocation model has been used to explore potential usefulness of anti-asthma agents (15). Drugs that inhibited late asthmatic allergic responses were universally effective in treating asthma, although the magnitude of this effect could not be predicted. We used this method to test ASM-024 but found no significant effect on induced sputum cellular airway inflammation, nor on allergen-induced asthmatic responses. However, we observed a significant effect of ASM-024 on methacholine responses and airway calibre, suggesting that it may have benefits in the treatment of asthma, particularly with regard to its bronchoprotective effects.

In standard radioligand receptor binding competition assays, ASM-024 showed inhibition at the low micromolar range (half-maximal inhibitory concentration [IC50] 19 µM and a Ki 13 µM) for nonselective nAChR subtypes, and low binding affinity for most of the nAChR subtypes tested, except for the human α3β4 receptor, for which Ki was 0.88 µM. Recent observations from whole-cell voltage-clamp experiments on human α3β4 and α7 nicotinic receptor subtypes expressed in Xenopus oocytes have revealed that ASM-024 does not activate the ion channel opening when used alone, but inhibits ACH-evoked responses, indicating an antagonist effect on ion channel activation. However, when co-applied with the type II α7 positive allosteric modulator, PNU-120596, which elicits a conformational change of the receptor, ASM-024 appears to function as an agonist and effectively activates the α7 ion channel (12). Compounds with similar properties are defined as ‘silent agonists’ that could mediate signal transduction pathways independently of ion channel activation (11).

Similarly, receptor binding assays for the various muscarinic receptor subtypes indicated low binding affinity, but the capacity to decrease muscarinic responses to ACH of M1, M2 and M3 AChR expressed in Xenopus oocytes. These observations indicate a complex multifunctional mechanism of action, which remains under investigation. The bronchoprotective effect is, however, unlikely to be solely due to its influence on airway calibre because this last effect was small compared with the change in airway response.

Finally, with regard to airway responsiveness, some subjects had a PC25 methacholine >16 mg/mL. This can be explained by the fact that their study was performed outside their allergen season. All had previously been diagnosed as having mild allergic asthma.

With regard to side effects of the drug, the most commonly reported was a cough. Although uncertain, explanations for this may include the bitter taste of the compound, a local irritation by the formulation, with a relatively long duration of administration.

CONCLUSION

In a population of mild asthmatic subjects, ASM-024 did not inhibit allergen-induced asthmatic response and related airway inflammation, but significantly reduced methacholine airway responsiveness and slightly improved baseline lung function. The mechanism by which ASM-024 improves these outcomes requires further study. We believe this drug has sufficient therapeutic potential to warrant exploration of its effects in obstructive airways diseases.

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