

ANALGESIC DRUGS COMBINATIONS IN THE TREATMENT OF DIFFERENT TYPES OF PAIN

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Pain Research and Treatment

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Editorial

Analgesic Drugs Combinations in the Treatment of Different Types of Pain

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Pain relief can be achieved by a diversity of methods, with drug use being the basis of analgesic treatment. Clinical use of combinations of analgesic drugs has augmented considerably in the last few years. The purpose of combining two or more drugs with different mechanisms of action is to achieve a synergistic interaction [1], yielding a sufficient analgesic effect with lower doses, and, therefore, reduce the intensity and incidence of untoward effects. At present, many diverse classes of drugs serve as an efficient complement to nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen or opioids, in the management of pain. But we emphasize that the success of a drug combination depends on the type of pain that is targeted (acute/chronic, inflammatory, neuropathic, cancer). Thus, opioids have frequently been used in combination with acetaminophen or NSAIDs for the clinical management of both acute and chronic pain. Likewise, the NSAIDs-acetaminophen combination has been administered to patients to relieve the pain. At the end, the use of these combinations limits the doses of medication that a patient can receive. However, not all the opioid-NSAID, opioid-acetaminophen, or NSAID-acetaminophen combinations are clinically successful in all cases. For example, the association of weak opioids, such as dextropropoxyphene, to acetaminophen does not significantly increase pain relief compared to acetaminophen alone [2]. The administration of rectal acetaminophen combined with ibuprofen does not

improve analgesia after adenoidectomy in the immediate postoperative period compared with either drug alone [3]. Likewise, the combination of codeine with paracetamol results in additional pain relief but may be accompanied by an increase in nausea, dizziness, vomiting, and constipation [4]. Therefore, several other combinations of analgesic agents must be evaluated experimentally or clinically to gain insight into their potential clinical use. In this sense, different combinations have been suggested. In the present special issue, a study realized by H. A. Ponce-Monter et al. showed that the diclofenac plus B vitamins combination was more effective to reduce the pain than diclofenac alone. Authors conclude that the combination of diclofenac plus B vitamins could be a safe and inexpensive postsurgical analgesic strategy.

In the present issue, A. Porwal and coworkers showed how different drug combinations may not be equally effective in an acute pain model; in a large study population, they in fact compared diclofenac and dicyclomine injection to a combination of dexketoprofen and dicyclomine for the treatment of acute renal colic and provided evidence that the latter was significantly more effective and tolerable than the former drug combination. On the other hand, A. Hama and J. Sagen, in a comprehensive review of the available preclinical and clinical studies, illustrate the pharmacological and physiological mechanisms that justify the use of a

combined drug therapy for the treatment of neuropathic pain due to spinal cord injury. The authors point out how a combination drug treatment strategy, wherein several pain-related mechanisms are simultaneously engaged, may be more efficacious than treatment against individual mechanisms alone, being possible to reduce the doses of the individual drugs, thereby minimizing the potential for adverse side-effects.

Clinicians should be conscious about the benefits and risks of the drugs combination in the management of pain. Also, physicians must be aware that NSAIDs can cause potentially serious adverse effects when used in combination with other common medications such as anticoagulants, corticosteroids, or antihypertensive agents. Finally, patients should be properly counseled on the appropriate and safe use of the combination of analgesics.

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Review Article

Antineuropathic and Antinociceptive Drugs Combination in Patients with Chronic Low Back Pain: A Systematic Review

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Purpose. Chronic low back pain (LBP) is often characterized by both nociceptive and neuropathic components. While various monotherapies have been reported of only limited efficacy, combining drugs with different mechanisms of action and targets appears a rational approach. Aim of this systematic review is to assess the efficacy and safety of different combined pharmacological treatments, compared to monotherapy or placebo, for the pharmacological treatment of chronic LBP. **Methods.** Published papers, written or abstracted in English from 1990 through 2011, comparing combined pharmacological treatments of chronic LBP to monotherapy or placebo were reviewed. **Results.** Six articles met the inclusion criteria. Pregabalin combined with celecoxib or opioids was shown to be more effective than either monotherapy. Oxycodone-paracetamol versus previous treatments and tramadol-paracetamol versus placebo were also reported as effective, while morphine-nortriptyline did not show any benefit over any single agent. **Conclusions.** In spite of theoretical advantages of combined pharmacological treatments of chronic LBP, clinical studies are remarkably few. Available data show that combined therapy, including antinociceptive and antineuropathic agents is more effective than monotherapy, with similar side effects.

1. Introduction

Successful treatment of chronic pain depends on identification of the involved mechanism and use of appropriate therapeutic approaches. Woolf et al. [1] proposed that pain symptoms and syndromes should be classified into two broad mechanism-based pain categories: tissue-injury pain (nociceptive) or nervous-system-injury pain (neuropathic).

Even if there is increasing knowledge that different mechanisms of pain require appropriate treatments and often polypharmacotherapy, and although drug combination is frequently empirically adopted in the clinical practice [2–5], prospective studies concerning the relative efficacy and safety of therapeutical drug associations to treat various painful conditions are still remarkably few [6–10] and, as recently reported, “*more preclinical, clinical, and translational studies are needed to improve the efficacy of combination drug therapy that is an integral part of a comprehensive approach to the management of chronic pain*” [11].

Although many patients have self-limited episodes of acute low-back pain (LBP) and do not seek medical care [12], this condition is among the five most common reasons for all physician visits in the USA [13, 14]. Among those who do seek medical care, pain, disability, and return to work typically improve rapidly in the first month [15]; however, up to one-third of patients report persistent back pain of at least moderate intensity one year after an acute episode [16, 17].

Medications are the most frequently recommended intervention for low back pain [14, 18]. In one study, 80% of primary care patients with low back pain were prescribed at least one medication at their initial office visit, and more than one-third were prescribed two or more drugs [5]. The most commonly prescribed medications for low back pain are nonsteroidal anti-inflammatory drugs (NSAIDs), skeletal muscle relaxants, and opioid analgesics [5, 19, 20]. Benzodiazepines, systemic corticosteroids, antidepressant medications, and antiepileptic drugs are also prescribed [21]. Monotherapies of chronic LBP with NSAIDs, acetaminophen and tricyclic

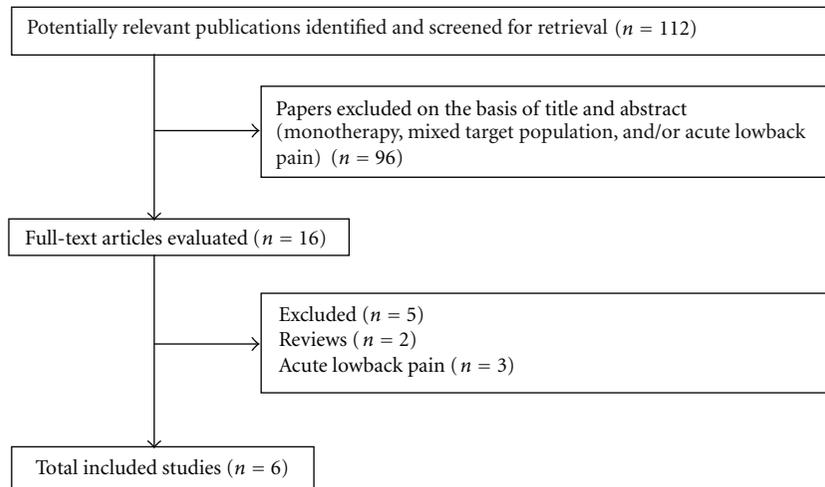


FIGURE 1: Flow diagram of inclusion and exclusion of articles for combined pharmacological interventions for chronic low back pain.

antidepressants, opioids, tramadol, benzodiazepines, and gabapentin (for radiculopathy) have all been found to provide only a limited pain relief, ranging from 10 to 20 points on a 100-point visual analogue pain scale [22].

Chronic LBP has been shown to be the result of neuropathic as well as nociceptive pain mechanisms and has therefore been classified as a mixed pain syndrome [23–25]. Nonspecific nociceptive pain is the result of an inflammatory response to tissue injury, while neuropathic pain describes cutaneous projected pain arising from the lumbar spine and/or nerve roots (radicular pain or radiculopathy) [3, 4]. The multifactorial nature of chronic LBP has often been underrecognized and undertreated. Thus, recent studies have demonstrated that approximately 20–55% of patients with chronic LBP have a >90% likelihood of a neuropathic pain component and, in an additional 28% of patients, a neuropathic pain component is suspected [5, 26, 27]. The presence of a neuropathic pain component is associated with more severe pain symptoms and higher healthcare utilization costs [28].

Based on this evidence, it has been suggested that antidepressants and/or anticonvulsants in combination with either opioids, traditional nonsteroidal anti-inflammatory drugs, or muscle relaxants could be useful in the treatment of this condition [27, 29, 30]. The aim of this systematic review is to evaluate evidence for the effectiveness of pharmacological combination therapy in chronic LBP, with specific reference to the management of nociceptive and neuropathic pain components.

2. Materials and Methods

Published papers written in English or including an English abstract, published from 1990 through 2011 and reporting the results of a combined pharmacological treatment of chronic lowback pain (LPB), compared with monotherapy or placebo, were reviewed. To this aim, we searched international databases, including EMBASE, PubMed/Medline, Google Scholar, SCOPUS, CINAHL, Cochrane Central Re-

gister of Controlled Trials, Cochrane Database of Systematic Reviews, <http://www.google.com/>, and <http://www.yahoo.com/>. Inclusion criteria were the following:

- papers written or with an abstract in English;
- papers concerning the results of management of chronic low-back pain (symptoms duration >6 months);
- treatment using a combination of two or more drugs, versus monotherapy or placebo.

Two investigators, CR and ML, searched and reviewed independently the literature and classified the references found in terms of whether they should be included on basis of the title and the abstract of the paper. In addition to original study reports, review articles were also included and the reference lists from all reviewed articles were assessed to complete the literature search. At the end of the reviewing process, the two reviewers' lists of papers were compared and if any discrepancy occurred, reclassification was performed according to the consensus reached.

This strategy identified 112 articles, the abstracts of which were hand searched to identify a subset with the specific focus of pharmacological treatment of chronic LBP of relevance to the current review. Six studies on pharmacological management of chronic LBP (irrespective of the cause) were identified as relevant and were included in this paper (Figure 1).

3. Results

Table 1 summarizes the included studies examining combination pharmacotherapy of chronic LBP. Three studies evaluated paracetamol in combination with tramadol [35, 36] or oxycodone [32].

In the first study ($n = 318$), three-month treatment with tramadol 37.5 mg/paracetamol 325 mg yielded significantly greater improvements in pain VAS score ($P < 0.015$) and Pain Relief Rating Scale score ($P < 0.001$) than placebo.

TABLE 1: Table 1 Clinical trials on combination pharmacological therapy of chronic low back pain.

Reference	Trial design	Duration	Main inclusion/exclusion criteria	Pain type	Intervention(s) and dose	Principal outcome
L. Romanò et al. [31]	Prospective, randomized, 3-way cross-over study 4-weeks treatment with each therapy	12 week	18–75 years Chronic LBP for >6 months due to disc prolapse, lumbar spondylosis, and/or spinal stenosis Minimum VAS >40 mm (on a scale of 0–100 mm) Patients with neurological disease excluded	Mixed	Celecoxib 3–6 mg/kg/die + placebo (<i>n</i> = 36) and Pregabalin 1 mg/kg/die for the first week, then 2–4 mg/kg/die + placebo (<i>n</i> = 36) and Celecoxib 3–6 mg/kg/die + pregabalin 1 mg/kg/die for the first week, then 2–4 mg/kg/die (<i>n</i> = 36)	Combination therapy was more effective than either monotherapy for mean pain reduction (assessing using 0–100 mm VAS)
Gatti et al. [32]	Prospective, observational, and open-label study	6 week	Chronic LBP (46 months) Moderate to severe (>3 on a 0–10 VAS) Pain not responsive to previous systemic or local analgesic treatment	Osteoarticular, nociceptive pain (Group A) or neuropathic pain (Group B)	Group A Previous treatment discontinued: Oxycodone 5 mg + paracetamol 325 mg/8 hours (<i>n</i> = 78) Group B Previous treatment (except gabapentin). Fixed combination of oxycodone 5mg + paracetamol 325 mg/8 hours (<i>n</i> = 72)	Group A 73.9% and 78.3% (assessed using 0–10 VAS), respectively Group B All patients reported improved or stable neuropathic pain symptoms except pain preventing sleep
Pota et al. [33]	Prospective, observational, and open-label study	2 month	Chronic LBP (33 months)	Mixed	(<i>n</i> = 22) Month 1: Buprenorphine TDS 35 µg/ml Month 2: Buprenorphine TDS 35 µg/ml + pregabalin 150 mg or Buprenorphine TDS 35 µg/ml + placebo	Significant reductions in pain(assessed using 0–100 VAS) were observed after month 1 (<i>P</i> < 0.01) Significant reductions in painafter month 2 were only observed in the combination group (<i>P</i> < 0.01)

TABLE 1: Continued.

Reference	Trial design	Duration	Main inclusion/exclusion criteria	Pain type	Intervention(s) and dose	Principal outcome
Khoromi et al., [34]	Single-centre, cross-over, randomized trial	9 week	18–65 years		Morphine 15–90 mg and Nortriptyline 25–100 mg and Morphine 15–90 mg nortriptyline 25–100 mg	No significant reductions in mean leg pain (assessed using 0–10 VAS) or other leg or back pain were observed in any treatment group Pain reduction relative to placebo was 14% for nortriptyline, 7% for morphine, and 7% for combination therapy
			Lumbar radiculopathy Average leg pain score >4 (0–10 cm VAS) Patients with polyneuropathy and peripheral vascular disease associated with symptoms of numbness, or patients with burning pain in the lower extremities, were excluded	Neuropathic		
Peloso et al., [35]	Multi-centre, randomized, double-blind study	21-day washout period, 91-day double-blind treatment period	> 18 years		Tramadol 37.5–300 mg + paracetamol 325–2600 mg (<i>n</i> = 167) or Placebo (<i>n</i> = 169)	Mean final pain intensity scores (assessed using 0–100 mm VAS) were significantly lower with combination therapy (47.4) than with placebo (62.9; <i>P</i> < 0.001), as were mean final pain relief scores (assessed on 6-point Likertscale: 1.8 and 0.7, resp., <i>P</i> < 0.001)
			Chronic LBP Pain intensity >40 (0–100 mm VAS) Patients with neurologic deficits in lower extremities, symptomatic disk herniation, severe spinal stenosis, or spondylolisthesis excluded	Noiceptive		
Ruoff et al., [36]	Multi-centre, randomized, double-blind, parallel group study	21-day washout period, 10-day titration period, 81-day treatment period	25–75 years Chronic LBP Pain intensity >40 (0–100 mm VAS)	Mixed	Tramadol 37.5–300 mg + paracetamol 325–2600 mg (<i>n</i> = 161) OR Placebo (<i>n</i> = 157)	Significantly lower final meanpain score (assessed by 0–100 mm VAS) with combination therapy than with placebo (<i>P</i> < 0.015)

CR=controlled release; LBP: low back pain; Mixed: mixed nociceptive and neuropathic pain; TDS: Transdermal delivery system; VAS: visual analogue scale.

Significant improvements were also observed for Roland Disability Questionnaire (RDQ) scores, several of the sensory Short-Form McGill Pain Questionnaire (SF-MPQ) items, and the Role-Physical, Bodily Pain, Role-Emotional, Mental Health, Reported Health Transition and Mental Component items of the Short Form 36 (SF 36; all $P < 0.05$). The rates of discontinuation due to insufficient pain relief were significantly lower with tramadol plus paracetamol (22.1%) than for placebo (41.0%; $P < 0.001$), and the proportion of patients and investigators rating treatment as “good” or “very good” was higher with combination therapy than with placebo ($P < 0.001$ for patients; $P = 0.002$ for investigators). Adverse events were more common with the combination (68.9%) than with placebo (46.5%), as were adverse drug reactions (23.6% versus 3.8%) and rates of discontinuation due to adverse events (18.6% versus 5.7%). Nausea, somnolence, and constipation were significantly more frequent with combination treatment than with placebo ($P < 0.05$) [36].

In the second study, patients with at least moderate chronic LBP received tramadol 37.5 mg/paracetamol 325 mg in a fixed combination tablet; VAS scores after 3 months were significantly lower with tramadol/paracetamol than with placebo ($P < 0.001$). Combination therapy was also associated with significantly improved scores on several measures, including RDQ score and physical-related items on the SF-MPQ and SF-36 ($P < 0.05$). Similar results to those reported above by Ruoff et al. [36] were observed for discontinuation due to insufficient pain relief, the proportion of patients rating treatment as “good” or “very good” and the incidence of adverse events [35].

Gatti et al., in a prospective observational study [32] examined the efficacy of a fixed-dose combination of oxycodone plus paracetamol using the Pain Management Index. Patients were stratified according to the presence of prevalent osteoarticular pain ($n = 78$) or prevalent neuropathic pain ($n = 72$). Combination therapy was associated with an improvement in pain in the majority of compliant patients, although its benefit in patients with neuropathic pain was less marked.

Two papers reported on the efficacy of pregabalin with, respectively, celecoxib [31] or transdermal (TDS) buprenorphine [33]. In our previously published prospective, single-blind, randomized study [31], the safety and efficacy of the association of celecoxib and pregabalin with either monotherapy for treatment of chronic low back pain of various origin, were compared; data were also analyzed on the basis of pain quality assessed with the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) pain scale [37, 38]. Our study showed that the association pregabalin/celecoxib resulted in a statistically significant reduction of self-reported pain when considering either all the recruited patients or the subpopulations divided according to LANSS score. On the contrary, celecoxib/placebo and pregabalin/placebo only produced a statistically significant reduction of reported pain in, respectively, patients with LANSS score < 12 ($P = 0.01$) (nociceptive pain) and in patients with LANSS score > 12 ($P = 0.03$) (neuropathic pain), but not when including all the patients. The drug combination also proved to be more effective than pregabalin alone or than celecoxib alone,

except for patients with LANSS score < 12 , in which treatment combination or monotherapy provided similar results. When all patients were considered, celecoxib alone provided 12.4% pain reduction, pregabalin alone 10.4%, and their combination 38.2%. The largest pain reduction (51.8%) was observed with the association pregabalin/celecoxib in patients with LANSS score > 12 . Pregabalin drug consumption, when used in association with celecoxib, was significantly lower ($P < 0.05$) compared to monotherapy. The occurrence of side effects was similar during either monotherapy or combination treatment [31].

Similarly, Pota and coworkers [33] found that the combination of pregabalin and buprenorphine, TDS yielded significantly greater reductions in VAS scores than buprenorphine monotherapy ($P < 0.01$). In the first month of therapy buprenorphine TDS alone provided a meaningful pain reduction (VAS 82.75 ± 15 versus 38.25 ± 5 , $P < 0.01$); at the end of the first month, patients were then divided in two groups: Group A receiving one-month therapy with buprenorphine 35 $\mu\text{g}/\text{mL}$ plus pregabalin 150 mg and Group B buprenorphine plus placebo. At the end of the treatment, only Group A presented a further reduction of the VAS ($P < 0.01$). The authors concluded that “*buprenorphine TDS determines a notable relief from pain. Moreover the association of low doses of pregabalin allowed a further relief.*”

The unique study evaluating the combination of morphine with nortriptyline [34], failed to provide sufficient data as to regard the efficacy of this free opioid-antidepressant combination for the treatment of chronic LBP. In this study, performed on 61 patients with sciatica, the combination of morphine and nortriptyline did not reduce average leg pain scores or any other leg or back pain scores, while 89% of patients receiving combination treatment reported an adverse event, most commonly constipation.

4. Discussion

This systematic review shows that, in spite chronic LBP is thought to be commonly the result of both nociceptive and neuropathic mechanisms [23] and hence a rationale approach would be targeting the different mechanisms of pain by combining specific drug agents, remarkably few clinical trials are currently available to validate this hypothesis.

This may due to different reasons including

- (i) the difficulty in designing/performing clinical trials involving more treatments at the same time;
- (ii) potential drugs' interactions and possible adverse effects. Any specific combination of agents need to be first evaluated on the basis of the respective pharmacokinetic profile and possible interactions and then clinically tested. In free dose combinations, the onset of adverse events can, to some extent, be overcome by initiating treatment at low doses and slowly escalating the dose until maximum analgesia or intolerable side effects arise; drug combination may also provide reduced consumption of any single drug and adverse events comparable to monotherapy [31];

- (iii) unpredictable dosing regimen. At variance with that reported above concerning the possible advantage of free dose combinations, fixed dose are easier to study and to market, being also likely associated with greater patient's compliance than free combinations; however, identifying the best dose ratio for all the patients, balancing the efficacy and tolerability of any single drug within a fixed combination may be a challenging exercise;
- (iv) scarce economical interest of drug companies.

All of these potential drawbacks may, to a different extent, concur to explain the limited research on drug combinations, in spite of theoretical positive considerations and notwithstanding the empirical widespread use of drug associations in the clinical practice [5].

Among the six studies that were included in the present review, two examined a fixed-dose regimen of paracetamol and tramadol combination against placebo [35, 36]. These studies appear much similar, in their design and outcomes, to a "traditional" monotherapy versus placebo study [39] and do not really seem to add any insight as to concern the control of different types of pain.

On the contrary, the association of pregabalin plus celecoxib [31] or of pregabalin and an opioid agent [33] seem more focused on targeting different pain components of chronic LBP.

Gabapentinoids have already been successfully used in combination with other analgesic drugs to improve neuro-pathic pain control. Gilron et al. [7] first reported on the efficacy and safety of a combination of gabapentin and morphine compared with that of each as a single agent in patients with painful diabetic neuropathy or postherpetic neuralgia. In 41 patients, gabapentin-morphine combination showed significantly better pain control ($P < 0.05$) versus placebo, gabapentin, and morphine. More recently, Gatti et al. reported the Multicenter Italian Study, which compared the efficacy, safety, and quality of life of combination therapy with controlled release (CR) oxycodone plus pregabalin versus monotherapy in patients with neuropathic pain of various origins [40]. This study showed in 409 patients that the combination of CR oxycodone plus pregabalin was more effective than monotherapy for alleviating neuropathic pain ($P = 0.003$) and to improve quality of life ($P = 0.0009$), while combination therapy also allowed dose reduction of both agents (22% for CR oxycodone and 51% for pregabalin).

Interestingly, in our reported study, celecoxib or pregabalin when used alone were shown to be not effective in patients with, respectively, neuropathic or nociceptive low back pain type, as evaluated with the LANSS pain scale [31]. This is not surprising, given the specific ability of pregabalin to control neuropathic pain [2, 41, 42], while celecoxib is a selective COX-2 inhibitor that has been proved to be effective in the treatment of different pain models that are considered predominantly of nociceptive origin [43, 44]. However, this finding also supports the hypothesis of a better efficacy of a combined approach to the mixed pain conditions and points

out the importance of patient selection when evaluating the analgesic efficacy of any specific treatment.

A recent systematic review of pharmacological monotherapies for chronic nonspecific low back pain [45] showed no effects of different types of antidepressants, compared to placebo, on any of the primary investigated outcomes, including pain intensity, depression and functional status. The study from Khoromi et al. [34], in a mixed pain population, suffering from low back pain with lumbar radiculopathy, seems to confirm, with the limitation imposed by the small sample size, that even nortriptyline alone or in combination with morphine has limited effectiveness.

Other frequently prescribed medications, like muscle relaxants [19, 20], have not been investigated in randomized clinical trials for the treatment of chronic low-back pain [45] and we could not find any study regarding their use in a combined pharmacological therapy of this condition.

It is worth noting how published comprehensive reviews of clinical trials [46] and even the most recently reported guidelines concerning the treatment of chronic low back pain fail to address the use of combined pharmacological treatments [47, 48]. While, in fact, several drugs are compared and recommended as monotherapy, associations are not mentioned. The paucity of the available data may well explain, in our opinion, the lack of indications in this regard and points out the need for further research and well designed clinical trials.

5. Conclusions

Pain treatment should be guided by the underlying mechanisms and should take into consideration pain quality as well as pain intensity. Chronic LBP often comprises both nociceptive and neuropathic components, and various monotherapies have been repeatedly reported as only partially effective. Therefore, an individualized, multimodal therapy, combining drugs with different mechanisms of action represents a rational approach. However, available studies investigating drug combinations are remarkably few. In particular, combination of pregabalin and celecoxib or buprenorphine has been demonstrated to be more effective than either monotherapy and relatively safe. The association of paracetamol with tramadol or oxycodone has also been shown to be effective for reducing chronic low-back pain, even if not evaluated against respective monotherapy. Further research in combined pharmacological treatment of chronic LBP with well-designed studies may offer valuable tools for the clinical practice and is strongly suggested.

Conflict of Interests

The authors declare that they have no conflict of interest related to the publication of this paper.

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Clinical Study

Efficacy and Tolerability of Fixed-Dose Combination of Dexketoprofen and Dicyclomine Injection in Acute Renal Colic

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Objective. To evaluate the efficacy and tolerability of a fixed-dose combination of dexketoprofen and dicyclomine (DXD) injection in patients with acute renal colic. **Patients and Methods.** Two hundred and seventeen patients were randomized to receive either DXD ($n = 109$) or fixed-dose combination of diclofenac and dicyclomine injection (DLD; $n = 108$), intramuscularly. Pain intensity (PI) was self-evaluated by patients on visual analogue scale (VAS) at baseline and at 1, 2, 4, 6, and 8 hours. Efficacy parameters were proportion of responders, difference in PI (PID) at 8 hours, and sum of analogue of pain intensity differences (SAPID). Tolerability was assessed by patients and physicians. **Results.** DXD showed superior efficacy in terms of proportion of responders (98.17% versus 81.48; $P < 0.0001$), PID at 8 hours ($P = 0.002$), and SAPID_{0-8 hours} ($P = 0.004$). The clinical global impression for change in pain was significantly better for DXD than DLD. The incidence of adverse events was comparable in both groups. However, global assessment of tolerability was rated significantly better for DXD. **Conclusion.** DXD showed superior efficacy and tolerability than DLD in patients clinically diagnosed to be suffering from acute renal colic.

1. Introduction

Acute renal colic (ARC) is a common emergency condition mimicking acute abdominal or pelvic condition. About

12% of the population is likely to suffer from ureteric colic sometime in their lifetime and recurrence rates can approach about 50% [1]. It is extremely important to relieve the excruciating pain associated with this condition and

establish a confirmatory radiological diagnosis at the earliest onset.

The severe pain of ARC is due to increasing wall tension in the urinary tract as a result of obstruction of the urinary flow. The rising pressure in renal pelvis stimulates release of prostaglandins that cause vasodilatation. This leads to diuresis and thus further increase in the intrapelvic pressure. Prostaglandins also lead to ureteric spasm that further amounts to pain [2, 3].

Parenteral nonsteroidal anti-inflammatory drugs (NSAIDs) have been used widely for the treatment of ARC and have been shown to achieve greater reduction in pain scores than opioids. The use of NSAIDs has reduced the requirement for further analgesia beyond short term [4]. Unlike opioids, NSAIDs not just symptomatically relieve pain but also inhibit synthesis of prostaglandins, which are involved in the etiopathogenesis.

Spasmolytics are traditionally used in renal colic, biliary colic, or dysmenorrhoea for relief of smooth muscle spasm. As spasmolytics relieve the pain associated with smooth muscle spasm, the combination of NSAIDs with spasmolytics is likely to be synergistic. Fixed dose of combinations (FDC) of mefenamic acid, aceclofenac with spasmolytics such as dicyclomine or drotaverine have been demonstrated to be highly effective in relief of acute spasmodic pain [5, 6]. In the study performed by Pareek et al., addition of spasmolytic such as drotaverine to aceclofenac was found to provide significant therapeutic benefit as compared to monotherapy with aceclofenac [6].

The parenteral formulation of dexketoprofen trometamol, the S-enantiomer of ketoprofen, has shown good safety and efficacy in the treatment of ARC in previous studies [3, 7]. The present study was planned to evaluate the efficacy and tolerability of FDC of an NSAID, dexketoprofen with dicyclomine (DXD) injection in the treatment of clinically diagnosed ARC when administered as an intramuscular (IM) injection. To our knowledge, this is the first clinical study reported for this FDC.

2. Patients and Methods

2.1. Objective. The objective of this study was to compare the efficacy and tolerability of FDC of dexketoprofen and dicyclomine IM injection (DXD) with FDC of diclofenac and dicyclomine IM injection (DLD) in the treatment of patients clinically diagnosed to be suffering from ARC.

2.2. Study Design. This was a randomised controlled, multicentric, open-label, parallel group study conducted at different centres across India. The study was approved by institutional review board or independent ethics committee for each centre. Written informed consent was provided by each participant prior to any study-related procedure. The execution and monitoring of the study were done in accordance with the requirements of Good Clinical Practice.

2.3. Study Population. The study population involved male and female patients between 18 and 65 years of age

presenting with acute colicky pain in the flank and/or radiating to the abdomen or genitalia. Patients with moderate to severe pain on visual analogue scale (VAS ≥ 40 mm) and willing to provide written informed consent were included in the study. The important exclusion criteria included hypersensitivity to the study medications or intolerance to NSAIDs or any anesthetic medication; active or suspected gastrointestinal ulcer, chronic dyspepsia, or gastrointestinal bleeding; Crohn's disease or ulcerative colitis; history of bronchial asthma, severe heart failure/moderate-to-severe renal dysfunction (creatinine clearance < 50 mL/min.), or severely impaired hepatic function (Child-Pugh score 10–15); hemorrhagic diathesis and other coagulation disorders; contraindication to use of NSAIDs; diagnosed gastrointestinal obstruction; myasthenia gravis; glaucoma.

2.4. Treatment Procedure. Patients presenting with acute colicky pain in the flank region were screened based on complete medical history and examination. Patients satisfying the selection criteria were randomised to receive either FDC of dexketoprofen (as trometamol) 50 mg and dicyclomine 20 mg IM injection (DXD) [manufactured by Emcure Pharmaceuticals Ltd., Pune] or FDC of diclofenac (as sodium) 50 mg and dicyclomine 20 mg IM injection (DLD) [from commercial source]. Patients were randomized in 1:1 ratio to "DXD" or "DLD" using blocks of 10 through online randomization software available at <http://www.randomization.com/>. Any concomitant therapy deemed necessary was provided for the patients as per investigator's discretion. However, any other analgesic, anti-inflammatory, or muscle-relaxant therapy, and products from alternative system of medicine with analgesic, anti-inflammatory action were not allowed. The patients were simultaneously investigated radiologically for renal pathology.

2.5. Efficacy Variables. The intensity of pain was assessed from VAS at baseline and at the end of 1, 2, 4, 6, and 8 hours after administration of study medication. At least 50% improvement in pain score at 8 hours was considered as the responder's criterion. Proportion of responders in each study group was considered as primary efficacy variable.

The secondary variables included pain intensity difference (PID) after 8 hours of injection and sum analogue of pain intensity difference (SAPID) over 8 hours.

PID was calculated for each observation by subtracting the present PI from the baseline value. $SAPID_{0-8 \text{ hours}}$ was calculated as the weighted sum of the PIDs obtained from $t = +1$ hour (hr) to $t = 8$ hours (hr) on VAS using the following equation: $SAPID = \sum [PID_t \times \text{time (hr) elapsed since previous observation}]$. The secondary efficacy variables also included assessment for patient's clinical global impression for change in pain.

2.6. Tolerability Variables. Assessment of tolerability was done by recording patient's and physicians' global assessment on tolerability of the drug and proportion of the patients experiencing any drug-related adverse events.

TABLE 1: Demographic and baseline data.

	FDC of dexketoprofen and dicyclomine injection (DXD)	FDC of diclofenac and dicyclomine injection (DLD)	<i>P</i> value*
No of patients (<i>n</i>)	109	108	—
Age, years (Mean ± SD)	34.54 ± 10.87	36.86 ± 12.22	0.14
Sex (M:F)	79:30	68:40	0.15
Systolic BP, mm Hg (Mean ± SD)	126.53 ± 10.95	128.06 ± 11.58	0.32
Diastolic BP, mm Hg (Mean ± SD)	82.22 ± 7.40	81.89 ± 7.51	0.74

* Fisher's test applied for proportions and unpaired *t*-test for numerical data; SD: standard deviation.

2.7. *Statistical Analysis.* Assuming responder rate of 0.7 in control group, a sample size of 108 in each group had 80% power to detect an increase of 0.16 with a significance level (α) of 0.05 (two-tailed; GraphPad StatMate 2.00). Fisher's exact test was applied to observe if there are significant differences between the responder rates. The decreases in PI (VAS score), PID, and SAPID were calculated (Mean ± SD) for each group and compared between the groups by using unpaired *t*-test. The within-group comparison of VAS scores was done using paired *t*-test. Tolerability was assessed by evaluating the percentage of patients reporting side effect. Analysis of adverse events and global assessment of safety and efficacy was done using Fisher's exact test. For all statistical tests, a *P* value of less than 0.05 was considered significant.

3. Results

3.1. *Patient Demography.* Total 217 patients were recruited and completed the study of which 109 patients received DXD and 108 patients received DLD. The baseline demographic data for both groups were comparable (Table 1). The clinical diagnosis of acute renal colic was found to be consistent with the radiological diagnosis of renal calculus in about 65% patients in both groups.

3.2. *Efficacy.* The baseline VAS scores were comparable between the two groups (Table 1). There was a significant decrease in VAS scores as compared to baseline in both the groups starting from 1 hour after administration of the study drugs. The VAS score at the end of 6 hours and 8 hours was significantly less in DXD group as compared to DLD group ($P = 0.02$ for 6 hrs, $P = 0.007$ for 8 hrs) (Figure 1, Table 2).

There were significantly more responders (at least 50% improvement in VAS) in DXD group (98.17%) compared to DLD group (81.48%; $P < 0.0001$). The SAPID and PID at 8th hour were significantly more in DXD group compared to DLD group ($P = 0.004$ and 0.002) (Table 2).

In the patient-reported clinical global impression for change in pain, significantly more (71.56%) patients in DXD group demonstrated a "much better" response as compared to DLD group (40.74%, $P < 0.0001$). On the other hand,

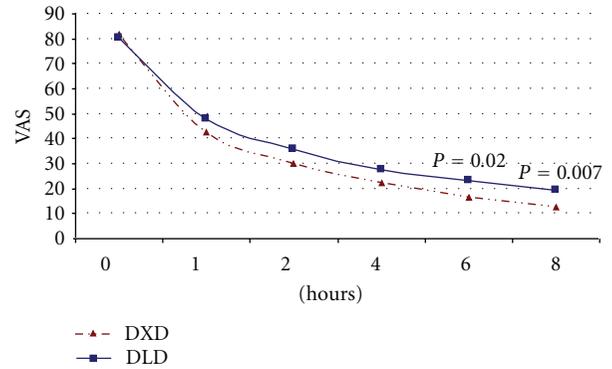


FIGURE 1: Improvements in VAS scores over 8 hours after DXD and DLD injections; unpaired *t*-test applied for between-group comparison.

TABLE 2: Efficacy parameters for DXD and DLD injections.

Variables	DXD (<i>n</i> = 109)	DLD (<i>n</i> = 108)	<i>P</i> value*
Responder rate (%)	98.17	81.48	<0.0001
Baseline VAS, (Mean ± SD)	81.97 ± 11.68	80.47 ± 12.44	0.36
VAS score at 8th hr, (Mean ± SD)	12.46 ± 15.18	19.35 ± 21.47	0.007
PID at 8th hr, (Mean ± SD)	69.51 ± 18.69	61.12 ± 20.00	0.002
SAPID, (Mean ± SD)	480.91 ± 156.67	420.35 ± 146.67	0.004

VAS: visual analogue scale, PID: pain intensity difference, SAPID: sum of pain intensity difference, * Fisher's test applied for proportions and unpaired *t*-test for numerical data, SD: standard deviation.

DLD group had more patients with "slightly better" response as compared to DLD group (20.37% versus 0.92, $P < 0.0001$). All patients in DXD group had improvement in pain, where as 2.78% patients in DLD group reported no change in pain (Figure 2).

3.3. *Tolerability.* The adverse events reported with DXD and DLD are depicted in Table 3. The incidence of vomiting and nausea occurred in relatively higher number of patients in DLD group. Incidence of all the other adverse events was comparable between the two groups.

On patients' global assessment of tolerability, significantly more patients in DXD group rated the tolerability as good or very good (98.17% versus 75.92%; $P < 0.0001$) (Figure 3(a)). Similarly, 98.17% physicians reported favourable tolerability of DXD as compared to 77.78% for DLD ($P < 0.0001$) (Figure 3(b)).

4. Discussion

The analgesic and anti-inflammatory activity of ketoprofen is limited to its S(+)enantiomer or dexketoprofen and the R(-)enantiomer is devoid of any such activity. Use of dexketoprofen in place of ketoprofen offers distinct benefits

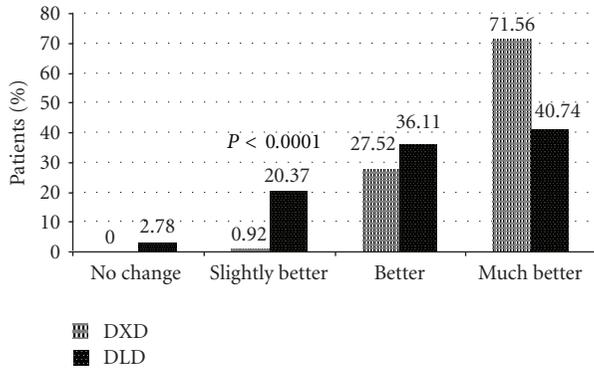


FIGURE 2: Patient-reported clinical global impression for change in pain. Fisher's exact test applied between proportion [(much worse + worse + slightly worse + no change) versus (slightly better + better + much better)].

TABLE 3: Adverse events (ITT analysis).

Adverse event	(DXD); % (n) (n = 109)	(DLD); % (n) (n = 108)	P value*
Total no. of patients	11.93 (13)	12.04 (13)	1.00
Burning micturition	2.75 (3)	0.93 (1)	0.62
Pain at injection site	2.75 (3)	0 (0)	0.25
Headache	1.83 (2)	0.93 (1)	1.00
Nausea	1.83 (2)	7.40 (8)	0.06
Dryness of mouth	1.83 (2)	0 (0)	0.5
Generalised weakness	1.83 (2)	0.93 (1)	1.00
Giddiness	0.92 (1)	0.93 (1)	1.00
Weakness	0.92 (1)	0 (0)	1.00
Cough	0 (0)	0.93 (1)	0.5
Vomiting	0 (0)	4.63 (5)	0.03

* Fisher's exact test ITT: Intention to treat.

such as same analgesic effect at lower doses, avoidance of excess metabolic load, and lack of adverse effects or drug interactions due to R-enantiomers [8, 9].

Oral dexketoprofen has been shown to have faster onset of analgesia than several other NSAIDs. Tromethamine salt of dexketoprofen is highly water soluble, which allows rapid and almost complete absorption of dexketoprofen [8]. Oral dexketoprofen is a first-line drug used for the treatment of mild-to-moderate acute pain and has shown its comparable efficacy as well as better tolerability than ketoprofen in several pain models such as dental pain, dysmenorrhea, and back pain [8, 10]. Parenteral administration of dexketoprofen has shown efficacy in reducing acute abdominal pain such as renal colic [3] and postoperative pain following hernia repair surgery [11]. Intramuscular dexketoprofen 50 mg was found to have faster, better, and longer analgesia than intramuscular diclofenac 50 mg [11].

NSAIDs are commonly used in clinical practice in combination with antispasmodics. Use of injectable NSAIDs and antispasmodics in ARC can subside the acute pain as well as reduce oedema and inflammation at the site of ureteric

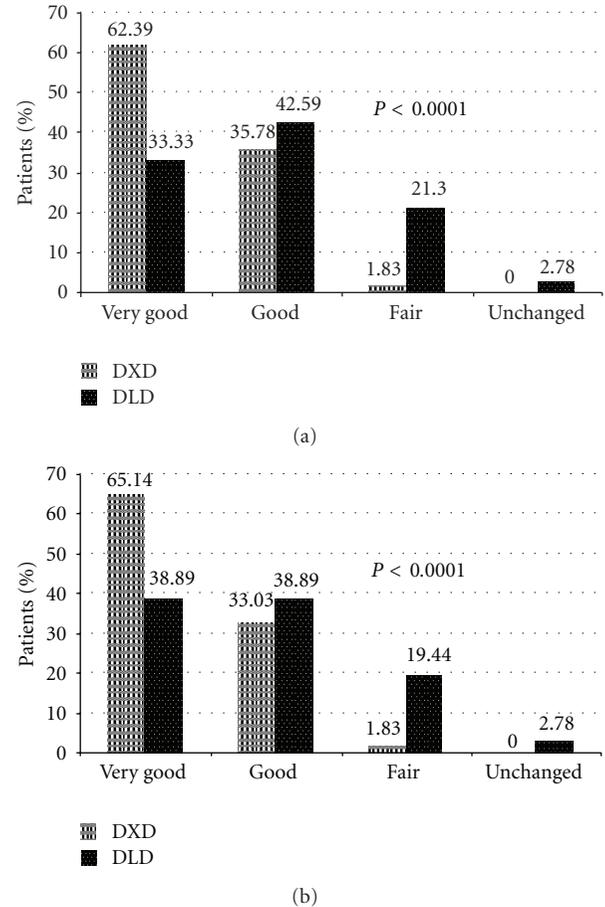


FIGURE 3: (a) Patient's global assessment of tolerability, (b) Physician's global assessment of tolerability. Fisher's exact test applied between proportions [(very good + good) versus (fair + unchanged)].

obstruction. It has been shown that addition of spasmolytic adds to the efficacy of NSAID in the treatment of acute spasmodic pain [6]. However, there are very few published studies assessing the safety and efficacy of such combinations and superiority of one combination over another. The results of the present study demonstrate that FDC of dexketoprofen and dicyclomine injection has better efficacy in reduction of ARC than FDC of diclofenac and dicyclomine injection, a commonly used FDC for acute spasmodic pain. The responder rate for DXD was more than 98% and the degree of analgesia achieved was significantly better than DLD. This was translated into significantly better patient-reported clinical global impression for change in pain. The results of this study were consistent with the results of a previous study on injectable dexketoprofen, which also showed better efficacy than diclofenac in the treatment of postoperative pain [11].

DXD was well tolerated as compared to DLD with more than 98% patients and physicians reporting good or very good tolerability for DXD as compared to 75–77% for DLD. DXD was also found to cause less incidence of vomiting

than DLD. However, the total incidence of adverse events was comparable for DXD and DLD.

This study had a potential limitation that it was open-label, which could introduce bias. However, patients were not aware of the specific medication in the injection syringe, assuring unbiased response. In this study, we used diclofenac 50 mg instead of 75 mg as the commercially available FDCs of diclofenac with dicyclomine in the country contain no more than 50 mg of diclofenac.

5. Conclusion

This first report on the fixed-dose combination of dexketoprofen and dicyclomine injection shows that this product has superior efficacy and tolerability than the fixed-dose combination of diclofenac and dicyclomine injection in patients clinically diagnosed to be suffering from acute renal colic.

Disclosure

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Research Article

Morphine and Clonidine Synergize to Ameliorate Low Back Pain in Mice

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Chronic low back pain (LBP) is a debilitating condition associated with signs of axial and radiating pain. In humans with chronic LBP, opioids are often prescribed with varying outcomes and a multitude of side effects. Combination therapies, in which multiple pharmacological agents synergize to ameliorate pain without similar potentiation of adverse reactions, may be useful in improving therapeutic outcome in these patients. The SPARC-null mouse model of low back pain due to disc degeneration was used to assess the effects of opioid (morphine) and α_2 -adrenergic agonist (clonidine) coadministration on measures of axial and radiating pain. The results indicate that systemic morphine and clonidine, coadministered at a fixed dose of 100 : 1 (morphine : clonidine), show a synergistic interaction in reversing signs of axial LBP, in addition to improving the therapeutic window for radiating LBP. Furthermore, these improvements were observed in the absence of synergy in assays of motor function which are indicative of side effects such as sedation and motor incoordination. These data show that the addition of low-dose systemic clonidine improves therapeutic outcome in measures of both axial and radiating pain. Combination therapy could be of enormous benefit to patients suffering from chronic LBP.

1. Introduction

Low back pain (LBP) is a common condition associated with disability, decrease in quality of life, and significant economic burden [1–3]. Chronic LBP can include both axial and non-axial symptoms [4]. Axial LBP is characterized by spontaneous or movement-evoked pain or soreness confined to the spine and low back region. Radiating, non-axial LBP is pain that radiates from the back down one or both legs. This condition is often referred to as radicular pain or sciatica, because the pain usually follows the course of the sciatic nerve [5–8]. In animal models, radiating pain can be measured in the hindpaw. Although the exact mechanisms of LBP remain unclear, evidence suggests that the degeneration of intervertebral discs (IVDs) is associated with an increased risk of chronic LBP [9–12].

Pharmacotherapy is the most common treatment option for patients suffering from LBP with or without radiating pain [13]. Although non-steroidal anti-inflammatory drugs are the first line of defense against LBP, they do not sufficiently treat chronic and severe LBP. Opioids are often prescribed with varying therapeutic outcome [1, 14, 15] and are associated with undesired effects that limit their use, such as constipation, nausea, somnolence, fatigue, and the development of tolerance [16]. Since opioids such as morphine remain the gold standard of chronic pain treatment, it is vital to investigate strategies that would decrease required doses without diminishing the therapeutic effects. One such strategy is the addition of a non-opioid analgesic that will potentiate the analgesic effects of morphine without potentiating the undesirable adverse reactions.

The addition of α_2 -adrenergic agonists (α_2 ARs) improves opioid-induced antinociception in rodents following both systemic and spinal administration [17–25]. Evidence from human studies suggests that the use of opioid- α_2 AR agonist combinations in clinical pain management could minimize the side effects associated with both α_2 AR and opioid therapeutics [26, 27]. Furthermore, combination therapy may be effective in the treatment of chronic, opioid-insensitive pain states [28], and the α_2 AR agonist clonidine is approved for use in chronic pain. To date, the therapeutic benefit of opioid- α_2 AR agonist co-administration in chronic axial and non-axial LBP has not been systematically explored in either humans or animal models.

In this study, we used the SPARC-null mouse model of LBP due to disc degeneration (DD) to examine the effects of opioid- α_2 AR agonist combinations. SPARC (secreted protein, acidic, rich in cysteine; aka osteonectin or BM-40) is an evolutionarily conserved collagen-binding protein present in IVDs. SPARC is known to influence bone remodeling, collagen fibrillogenesis, and wound repair. Decreased expression of SPARC has been associated with aging and DD in human IVDs [29], and targeted deletion of the SPARC gene results in accelerated disc degeneration in the aging mouse [30]. DD in these mice is also associated with behavioural signs of axial and radiating LBP [31, 32].

The aim of the current study is to use the SPARC-null mouse model of low back pain to study the interaction between the prototypic opioid (morphine) and alpha-2 adrenergic agonists (clonidine) in treating signs of chronic axial and radiating pain.

Our results support the hypothesis that combination therapy using morphine and clonidine has the potential to improve therapeutic outcome for the chronic back pain patient.

2. Materials and Methods

2.1. Mice. SPARC-null mice (backcrossed to the C57BL/6 background) and wild-type (WT) controls (C57BL/6, Charles River, QC, Canada) were used as in previous studies [31–34].

4–6 month old male SPARC-null and WT control mice were bred in-house. Animals were housed in groups of 2–5, had unrestricted access to food and water, and were on a 12 hr light-dark cycle. All drug administration was adjusted for weight. SPARC-null mice were slightly lighter than WT mice (SPARC-null: 24.3 ± 0.3 at 4 months and 27.9 ± 0.4 at 6 months; WT: 25.9 ± 0.5 at 4 months and 32.1 ± 0.6 at 6 months). All experiments were performed blind to genotype and treatment, using a randomized block design.

All experiments were approved by the Animal Care Committee at the McGill University and conformed to the ethical guidelines of the Canadian Council on Animal Care and the guidelines of the Committee for Research and Ethical Issues of IASP [35].

2.2. Behavioural Analysis

2.2.1. Tail Suspension Assay. Mice were suspended individually underneath a platform by the tail with adhesive tape

attached 0.5 to 1 cm from the base of the tail and were videotaped for 180 s. The duration of time spent in (a) immobility (not moving but stretched out) and (b) escape behaviours (rearing to reach the underside of the platform, extending to reach the floor, or self-supported at the base of the tail or the suspension tape) were determined. The duration of immobility reflects the animal's willingness to stretch its main body axis. Decreased immobility is indicative of axial discomfort. This test is adapted from a traditional assay used to measure depression [36], and we have shown that it reliably measures signs of axial pain in mice [31, 32]. A cutoff of 180 seconds was applied when interpreting the data.

2.2.2. Sensitivity to Cold Stimuli. A modified version of the acetone drop test was used [37], where the total duration of acetone-evoked behaviours (AEBs: flinching, licking, or biting) were measured in seconds for 1 minute after a drop of acetone ($\sim 25 \mu\text{L}$) was applied to the plantar surface of the hindpaw. An increased behavioural response to acetone suggests the development of cold allodynia and decreased reactivity is suggestive of antiallodynic efficacy. A cutoff of 4 s was applied when interpreting the data to facilitate isobolographic analysis.

2.2.3. Rotarod Assay. The accelerating rotarod assay was used to monitor animals for motor function (IITC Life Science Inc., Woodland Hills, CA, USA) with the mouse adapter (rod diameter, 3.2 cm) [38]. The task includes a speed ramp from 0 to 30 rotations per minute over 60 s, followed by an additional 240 s at the maximal speed. A decline in the latency to fall off the rotarod reflects motor incoordination. Mice were not trained prior to testing sessions. A cutoff of 200 s was used when interpreting the data.

2.2.4. Open Field Assay. A transparent open field apparatus ($24 \times 24 \text{ cm}^2$) was placed in a quiet room illuminated with white light. The floor of the apparatus was equally divided into nine squares ($8 \times 8 \text{ cm}^2$). Mice were individually placed into the open field on the central square, and their spontaneous behaviour was videotaped for 5 min. Subsequent analysis of the total number of squares visited was used to assess general motor activity [39]. An increase in the number of peripheral squares covered reflects hyperactivity, while a decrease is indicative of sedation. Following drug administration, animals underwent tail suspension just before being placed in the open field.

2.2.5. Timeline. The schedule of testing was as follows: 16 weeks of age: habituation to tail suspension; 20 weeks: baseline open field and tail suspension assays; 22 and 26 weeks: baseline and after drug administration for acetone and rotarod assays; 24 and 28 weeks: tail suspension and open field after drug administration. A wash-out period of 2 weeks was included between drug exposures to ensure that only the acute effects of each drug were studied.

2.3. Pharmacological Treatment. Analgesic agents or saline control were administered to SPARC-null and WT mice by

TABLE 1: Effect of combination therapy on drug potency.

Assay	Strain	Morphine ED ₅₀	Clonidine ED ₅₀	Observed combination ED ₅₀	Theoretical combination ED ₅₀	Interaction
Tail suspension (Axial pain)	SPARC-null	10 (±4.0)	0.05 (±0.04)	0.08 (±0.23)	3.3 (±2.1)	Synergistic
	WT	18 (±6.0)	8.2 (±21)	NA	17 (±5.7)	NA
Acetone (cold allodynia)	SPARC-null	~35 (±50)	0.08 (±0.09)	3.5 (±6.3)	6.6 (±6.0)	Additive
	WT	6 (±2.0)	0.1 (±0.2)	2.7 (±8.9)	4.2 (±1.8)	Additive
Rotarod (motor incoordination)	SPARC-null	8 (±6.1)	0.3 (±0.3)	~56 (±85)	6.5 (±4.5)	Additive
	WT	~17 (±14)	0.1(±0.2)	No efficacy	7.3 (±7.4)	NA
Open field (overall activity)	SPARC-null	*0.2 (±0.1)	0.2 (±0.2)	No efficacy	NA	NA
	WT	*0.6 (±0.3)	0.14(± 0.16)	*0.13 (± 0.08)	NA	NA

Morphine and clonidine ED₅₀ values (mg/kg, i.p.) either alone or in combination at a dose ratio of 100:1 (observed combination ED₅₀). The Theoretical Combination ED₅₀ is the predicted ED₅₀ for the combination in the absence of any interaction. The interaction indicates if the observed combination ED₅₀ was statistically different from the theoretical combination ED₅₀. ~ indicates that the ED₅₀ value was determined by extrapolation if maximum efficacy was less than 50%. *In the open field assay, morphine had no potency as a sedative but caused hyperactivity. A drug or drug combination was considered to exhibit no efficacy if maximum efficacy was under 30%. NA = not available (it is not possible to calculate these values when one drug lacks efficacy).

i.p. injection (5 mL/kg injected directly in the intra-peritoneal cavity). Morphine (Medisca Inc., Montreal, QC, Canada) and clonidine (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) were dissolved in 0.9% saline either alone or in combination at a constant dose ratio of 100:1 (morphine : clonidine). Animals were tested 60 minutes after drug administration.

2.4. Data Analysis

2.4.1. Behavioural Phenotype of LBP. Comparisons between saline-treated SPARC-null and WT mice were performed for each assay by 2-tailed, unpaired *t*-test. Welch's correction was used when the condition of equal variances was not met. Sample size ranged between 35 and 48 mice/group of saline-treated mice.

2.4.2. Dose-Response Analysis (Table 1). Individual dose points are reported as raw data for both strains and all pharmacological treatments as means with standard error of the mean (SEM). In order to calculate ED₅₀ values, individual dose points were first converted to % maximum possible effect (%MPE) according to the following equations

Tail suspension:

$$\% \text{ MPE} = \frac{\text{drug} - \text{saline}}{\text{maximum} - \text{saline}} \times 100,$$

maximum effect = 180 seconds in immobility. (1)

Acetone:

$$\% \text{ MPE} = \frac{\text{saline} - \text{drug}}{\text{saline} - \text{maximum}} \times 100,$$

maximum effect = 0 seconds of AEB-induced behaviour. (2)

Rotarod:

$$\% \text{ MPE} = \frac{\text{saline} - \text{drug}}{\text{saline} - \text{maximum}} \times 100, \quad (3)$$

maximum effect = 0 seconds latency to fall.

Open field:

$$\% \text{ MPE} = \frac{\text{saline} - \text{drug}}{\text{saline} - \text{maximum}} \times 100, \quad (4)$$

maximum effect = 0 squares crossed.

ED₅₀ values and confidence limits were calculated according to the graded dose-response method of Tallarida and Murray [40] on the linear portion of each dose-response curve. ED₅₀ values were determined by extrapolation in cases where maximum efficacy was between 30 and 50%. If 30% efficacy was not reached, ED₅₀ values were not calculated and was considered to lack efficacy. A minimum of three doses were used for each drug or combination of drugs.

2.4.3. Isobolographic Analysis (Table 1). Isobolographic analysis is the "gold standard" for evaluating drug interactions [40, 41]. Dose-response curves were constructed for each agonist administered alone, and the ED₅₀ values were calculated. The two drugs were then coadministered at a constant dose-ratio approximately equal to their potency ratio, a third dose-response curve was constructed, and an experimentally derived combination ED₅₀ was calculated.

To test for interactions between agonists, the ED₅₀ values and standard error of all dose-response curves were arithmetically arranged around the ED₅₀ value using the following equation: $(\ln(10) \times \text{ED}_{50}) \times (\text{SEM of log ED}_{50})$ [41]. Isobolographic analysis necessitates this manipulation. When testing an interaction between two drugs, a theoretical additive ED₅₀ value is calculated for the combination based on the dose-response curves of each drug administered separately. This theoretical value is then compared by a *t*-test with the observed experimental ED₅₀ value of the combination. An interaction is considered synergistic if the experimental ED₅₀

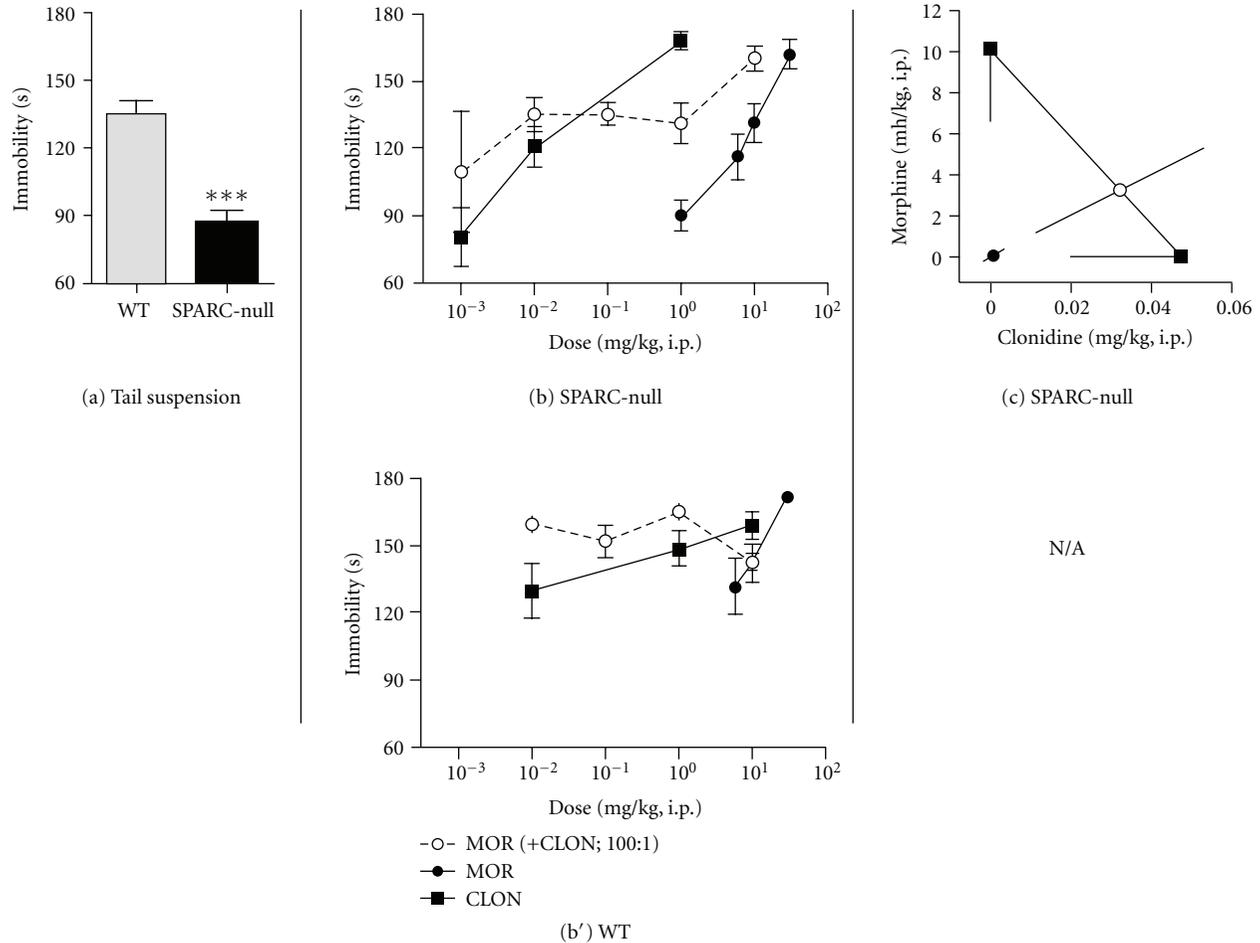


FIGURE 1: Morphine and clonidine synergize to attenuate axial pain in SPARC-null mice. (a) Saline-treated SPARC-null animals spend less time in immobility compared to WT mice in the tail suspension assay, indicative of axial pain. (b), (b') In SPARC-null mice (b), morphine (●) and clonidine (■) dose-dependently inhibited axial pain when administered systemically either alone or coadministered (i.p.) at a constant dose ratio of 100 : 1 (morphine : clonidine). In WT mice (b'), morphine (●) and clonidine (■) dose-dependently inhibited axial pain when administered systemically, but the combination lacked efficacy. (c) Isobolographic analysis applied to the data from (b). The y -axis represents the ED₅₀ for morphine, and the x -axis represents the ED₅₀ for clonidine. The lines directed from each ED₅₀ value toward zero are the lower 95% confidence limits of each ED₅₀. The line connecting these two points is the theoretical additive line. The open circle on the theoretical additive line represents the calculated theoretical ED₅₀ value of the combination if the interaction is additive. The observed combination ED₅₀ (●) was significantly ($P < 0.05$; t -test) lower than the theoretical additive ED₅₀ (○), indicating that the interaction is synergistic. An isobolograph was not plotted for WT mice, since the combination lacked efficacy in this assay. Error bars represent \pm SEM for each dose point ($n = 5-11$ animals/dose). See Table 1 for ED₅₀ values. *** $P < 0.0001$.

is significantly less ($P < 0.05$) than the calculated theoretical additive ED₅₀.

Visualization of drug interactions can be facilitated and enhanced by graphical representation of isobolographic analysis (Figures 1, 2, and 3, c-c'). This representation depicts the ED₅₀ of each agent on the x - or y -axis. For example, Figure 1(c) presents the ED₅₀ of morphine on the y -axis and the ED₅₀ of clonidine on the x -axis. The line connecting these two points depicts the dose combinations expected to yield 50% efficacy if the interaction is purely additive and is called the theoretical additive line. The theoretical additive ED₅₀ and its confidence interval are determined mathematically and plotted spanning this line. The observed ED₅₀ for the combination is plotted at the corresponding x, y coordinates

along with its 95% confidence interval for comparison to the theoretical additive ED₅₀. Isobolographs were plotted only when both drugs alone and the combination showed efficacy.

All dose-response and isobolographic analyses were performed with the FlashCalc pharmacological statistics software package generously supplied by Dr. Michael Ossipov.

2.4.4. Therapeutic Window (Table 2). Therapeutic window (TW) is a measure of the amount of an agent required to produce the desired effect (i.e., analgesia) compared to the amount that produces the undesired effect (i.e., motor impairment). In this study we define the TW as the ED₅₀ (undesired effect)/ED₅₀ (desired effect). A TW < 1 indicates the drug is more potent in the production of the undesired effect

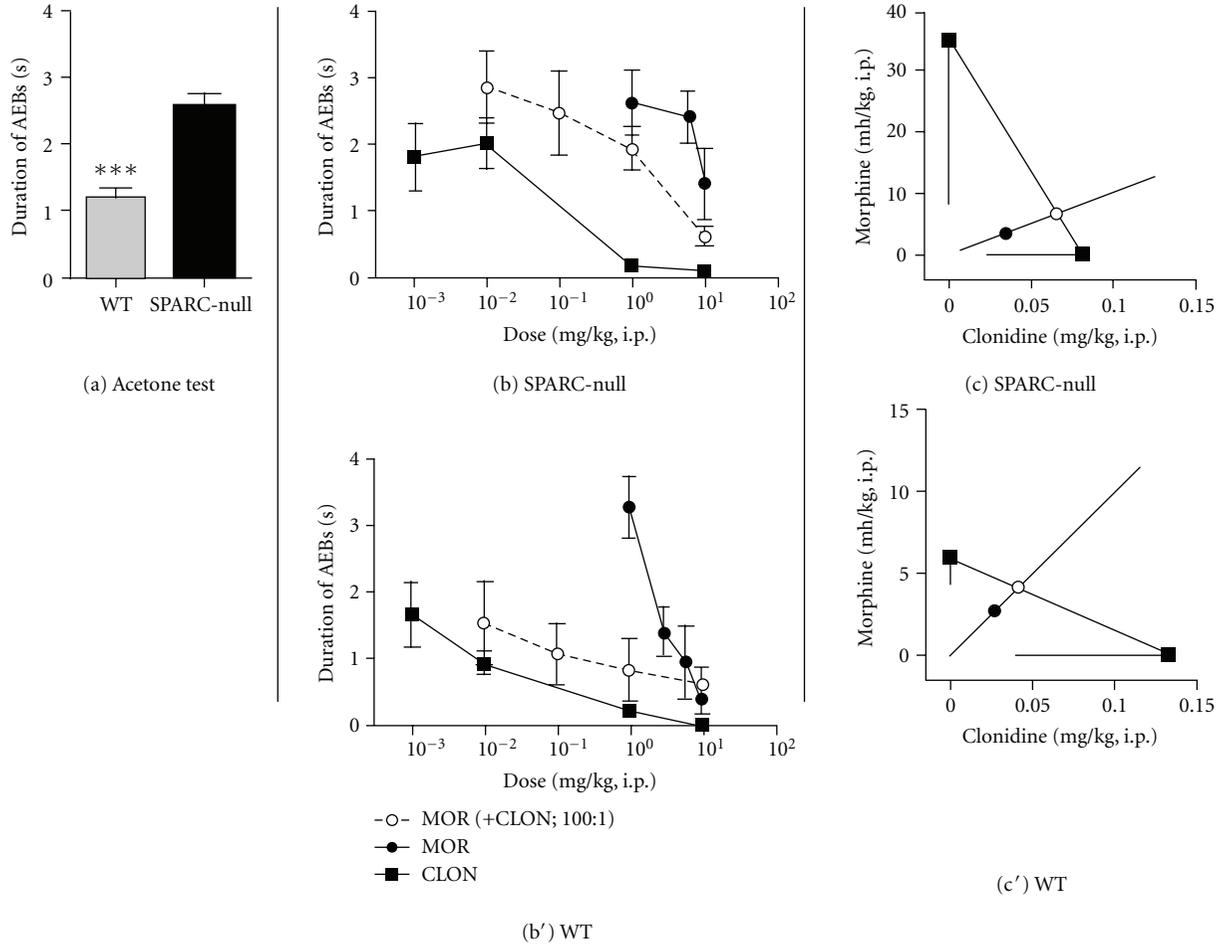


FIGURE 2: Effect of coadministration of morphine and clonidine on cold allodynia. (a) Saline-treated SPARC-null animals exhibit more acetone-evoked behaviours compared to WT mice in the acetone assay, indicative of cold hypersensitivity on the hindpaw. (b), (b'). In both SPARC-null (b) and WT (b') mice, morphine (●) and clonidine (■) dose-dependently inhibited cold allodynia when administered systemically either alone or coadministered (i.p.) at a constant dose ratio of 100 : 1 (morphine : clonidine). (c), (c') Isobolographic analysis applied to the data from (b), (b'). The y-axis represents the ED₅₀ for morphine, and the x-axis represents the ED₅₀ for clonidine. The lines directed from each ED₅₀ value toward zero represent the respective lower 95% confidence limits of each ED₅₀. The line connecting these two points is the theoretical additive line. The open circle on the theoretical additive line represents the calculated theoretical ED₅₀ value of the combination if the interaction is additive. The observed combination ED₅₀ (●) was not significantly different (*t*-test) from the theoretical additive ED₅₀ (○) in either strain, indicating that the interaction is additive in both cases. Error bars represent ±SEM for each dose point (*n* = 5–11 animals/dose). See Table 1 for ED₅₀ values. ****P* < 0.0001.

than the desired effect. A TW > 1 indicates that the desired effect can be achieved in the absence of the side effect. Higher indices are more advantageous therapeutically.

3. Results

3.1. Morphine and Clonidine Synergize to Improve Axial Pain in the Tail Suspension Assay. SPARC-null mice show signs of axial pain compared to WT mice as shown in the tail suspension assay (135.4 ± 5.2 s in WT versus 86.8 ± 5.7 s in SPARC-null, *P* < 0.0001, 2-tailed *t*-test, Figure 1(a)). Both in SPARC-null and WT mice, systemic administration of either morphine or clonidine produced dose-dependent increases in immobility, indicative of reduced axial discomfort, 60 minutes after injection (Figures 1(b), 1(b')).

The dose-response data from Figure 1(b) is represented graphically as an isobogram in Figure 1(c). As shown in Figure 1(c), the ED₅₀ of the combination (closed circle) in SPARC-null mice is lower than the theoretical additive ED₅₀ (open circle), indicating that this interaction is synergistic. This synergistic interaction was confirmed by statistical comparison between the observed combined ED₅₀ value and the theoretical additive ED₅₀ value.

In WT mice, all morphine + clonidine coadministration doses showed similar efficacy in the range tested (Figure 1(b')). Additional doses of this combination need to be explored to resolve the dose-response relationship necessary for isobolographic analysis (Table 1).

3.2. Morphine and Clonidine Are Additive in the Acetone Test of Cold Allodynia. SPARC-null mice show signs of cold

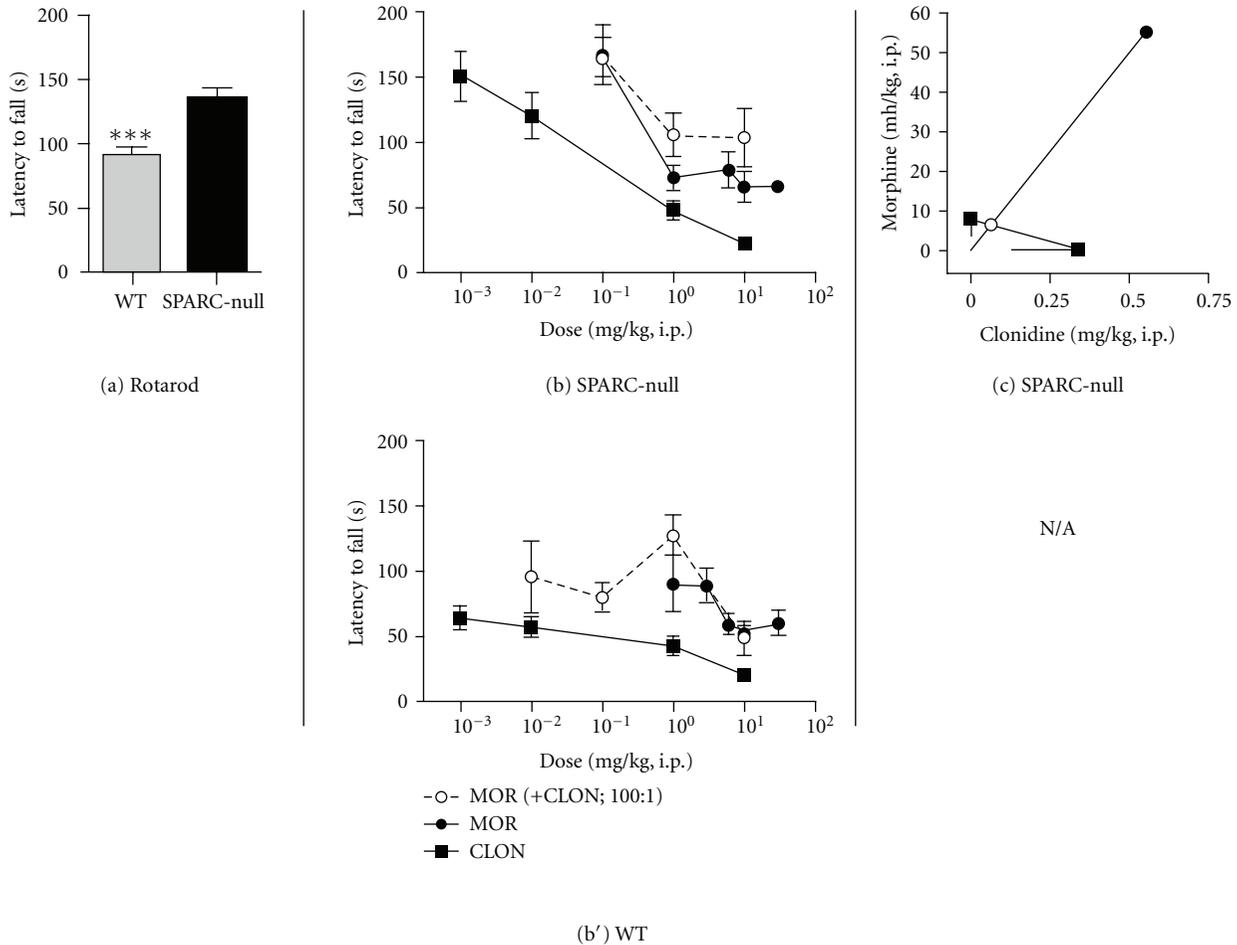


FIGURE 3: Effect of coadministration of morphine and clonidine on motor function. (a) Saline-treated SPARC-null animals perform better on the rotarod assay compared to WT mice, indicative of an absence of motor impairment in SPARC-null mice. (b), (b') In SPARC-null mice (b), morphine (●) and clonidine (■) dose-dependently caused motor impairment when administered systemically either alone or coadministered (i.p.) at a constant dose ratio of 100:1 (morphine:clonidine). In WT mice (b'), morphine (●) and clonidine (■) dose-dependently caused motor incoordination when administered systemically, but the combination lacked efficacy. (c) Isobolographic analysis applied to the data from Figure 1(b). The y-axis represents the ED₅₀ for morphine, and the x-axis represents the ED₅₀ for clonidine. The lines directed from each ED₅₀ value toward zero represent the respective lower 95% confidence limits of each ED₅₀. The line connecting these two points is the theoretical additive line. The open circle on the theoretical additive line represents the calculated theoretical ED₅₀ value of the combination if the interaction is additive. The observed combination ED₅₀ (●) was not significantly different (*t*-test) from the theoretical additive ED₅₀ (○), indicating that the interaction is additive. Isobolographic analysis was not performed in WT mice since the combination lacked efficacy in this assay. Error bars represent ±SEM for each dose point (*n* = 5–11 animals/dose). See Table 1 for ED₅₀ values. ****P* < 0.0001.

allodynia on the hindpaw compared to WT mice, as shown in the acetone assay (1.2 ± 0.1 s in WT versus 2.6 ± 0.2 s in SPARC-null, *P* < 0.0001, 2-tailed *t*-test, Figure 2(a)). In SPARC-null mice, systemic administration of clonidine produced dose-dependent analgesia in the acetone assay at 60 minutes after injection, while morphine failed to reach 50% MPE but was of sufficient maximum efficacy (45%) to extrapolate an ED₅₀ value (Figure 2(b)).

In WT mice, the administration of either morphine or clonidine alone produced dose-dependent antinociception in the acetone assay (Figure 2(b')). This interaction was tested statistically by comparing the observed combined ED₅₀ value and the theoretical additive ED₅₀ value and was

shown to be additive. The dose-response data from Figures 2(b), 2(b') are represented graphically as isobolograms in Figures 2(c), 2(c'). As shown in Figures 2(c), 2(c'), the ED₅₀ of the combination (closed circle) in both strains is not significantly different from the theoretical additive ED₅₀ (open circle), indicating that this interaction is additive (Table 1).

3.3. Morphine and Clonidine Are Additive in the Rotarod Test of Motor Impairment. SPARC-null mice do not show signs of motor impairment at 6 months of age. Rather, they perform better than WT mice in the rotarod assay at this

TABLE 2: Combination therapy improves therapeutic window.

Strain	Drug(s)	ED ₅₀ value (±SE; mg/kg, i.p.)			Therapeutic window	
		Motor	Axial	Non-axial	Motor/axial	Motor/non-axial
SPARC-null	Morphine	8 (±6.1)	10 (±4.0)	~35 (±50)	0.8	0.2
	Clonidine	0.3 (±0.3)	0.05 (±0.04)	0.08 (±0.09)	6.8	4.3
	Morphine (+ CLON; 100 : 1)	~56 (±85)	0.08 (±0.23)	3.5 (±6.3)	700	16
WT	Morphine	~17 (±14)	18 (±6.0)	6.0 (±2.0)	0.9	2.8
	Clonidine	0.1 (±0.2)	8.2 (±21)	0.1 (±0.2)	0.01	1.0
	Morphine (+ CLON; 100 : 1)	<i>No efficacy</i>	<i>No efficacy</i>	2.7 (±8.9)	N/A	N/A

The Therapeutic window is the ratio of the ED₅₀ value (mg/kg, i.p.) of the undesired effect (motor impairment) to the desired effect (inhibition of axial or non-axial pain). A larger therapeutic window suggests the drug or drug combination will be analgesic at doses that do not produce motor impairment. ~ indicates that the ED₅₀ value was determined by extrapolation if maximum efficacy was less than 50%. NA = not available (the combination lacked efficacy in the rotarod assay in WT mice). Note the much larger therapeutic window achieved with the addition of clonidine to morphine.

age (92.1 ± 5.9 s in WT versus 136.2 ± 6.2 s in SPARC-null, $P < 0.0001$, 2-tailed t -test, Figure 3(a)). In SPARC-null mice, systemic administration of either morphine or clonidine produced dose-dependent motor impairment in the rotarod assay at 60 minutes after injection (Figure 3(b)). Only clonidine produced a dose-dependent effect in WTs (Figure 3(b')).

The SPARC-null dose-response data from Figure 3(b) is represented graphically as an isobologram in Figure 3(c). As shown in Figure 3(c), the ED₅₀ of the combination (closed circle) is not significantly different from the theoretical additive ED₅₀ (open circle), indicating that this interaction is additive. In WT mice, morphine + clonidine coadministration lacked efficacy, and thus it was not possible to perform isobolographic analysis in this assay (Table 1).

3.4. Opposing Effects of Morphine and Clonidine in the Open Field Test of Voluntary Activity. SPARC-null mice do not differ from WTs in the number of peripheral squares covered in the open field, indicative of no overall change motor activity (49.1 ± 5.9 squares in WT versus 45.7 ± 3.8 squares in SPARC-null, $P = 0.6$, 2-tailed t -test, Figure 4(a)). In both SPARC-null and WT mice, systemic administration of morphine produced dose-dependent hyperactivity, while clonidine produced dose-dependent sedation in the open field assay at 60 minutes after injection in (Figure 4(b), 4(b')). Since the two agonists exert opposite effects on overall activity, isobolographic analysis was not performed for the open field test.

3.5. Effect of Morphine and Clonidine Coadministration on Therapeutic Window. The data presented above demonstrate that coadministration of morphine and clonidine produces antinociceptive but not sedative synergy following i.p. administration. We therefore examined the impact of coadministration on the therapeutic window (TW) between sedation and antinociception. In Table 2, the TW has been calculated for morphine and clonidine alone and in combination following systemic administration for both axial pain and cold allodynia. In SPARC-null mice, the window for each drug given alone ranged from 0.2 and 6.8, indicating little separation between the antinociceptive and sedative effective dose ranges. In contrast, the addition of a small amount

of clonidine to morphine increased these values to 700 for axial LBP and 16 for non-axial LBP. These changes reflect the fact that analgesia is reached before sedation when the drugs are coadministered. These increases in therapeutic window are the result of potentiation in the antinociceptive assays in parallel with an additive interaction in the undesired side effect (motor impairment).

4. Discussion

4.1. Morphine and Clonidine Synergy Improves Therapeutic Outcome for Axial Pain. SPARC-null mice develop behavioural signs of axial pain by 4–6 months of age concurrent with disc degeneration [31, 32, 42]. In the current study, we show that while morphine and clonidine dose-dependently attenuate axial pain, the side effects of motor impairment, sedation (clonidine), and hyperactivity (morphine) develop in a similar dose range. Systemic coadministration of morphine and clonidine not only resulted in synergy in SPARC-null but also the therapeutic window of the combination was greater than for either drug administered alone. The pharmacological effects observed in SPARC-null animals are not likely due to motor impairment or sedation, since the morphine + clonidine combination lacked efficacy in our tests of motor function. Furthermore, while morphine produced increases in overall activity, morphine-treated animals spent more time in immobility in the tail suspension assay, indicative of antinociception.

The majority of preclinical studies examining opioid- α_2 AR interactions to date have been carried out in naïve rodents, where the measured endpoint is antinociception to cutaneous noxious stimuli [21–25, 43] or inhibition of chemically-evoked behaviours [44, 45]. In contrast, the current study focused on pharmacological reversal of pathological signs of axial LBP in a preclinical model of intervertebral disc degeneration-related pain. To our knowledge this is the first demonstration of an opioid-adrenergic antinociceptive synergy in LBP in preclinical studies.

In patients suffering from axial LBP, pain management remains inadequate. Patients with mild or severe LBP are often prescribed two or more medications in addition to opioids, reflecting the challenging nature of LBP [46]. Currently the primary use of clonidine as a pain management tool is as

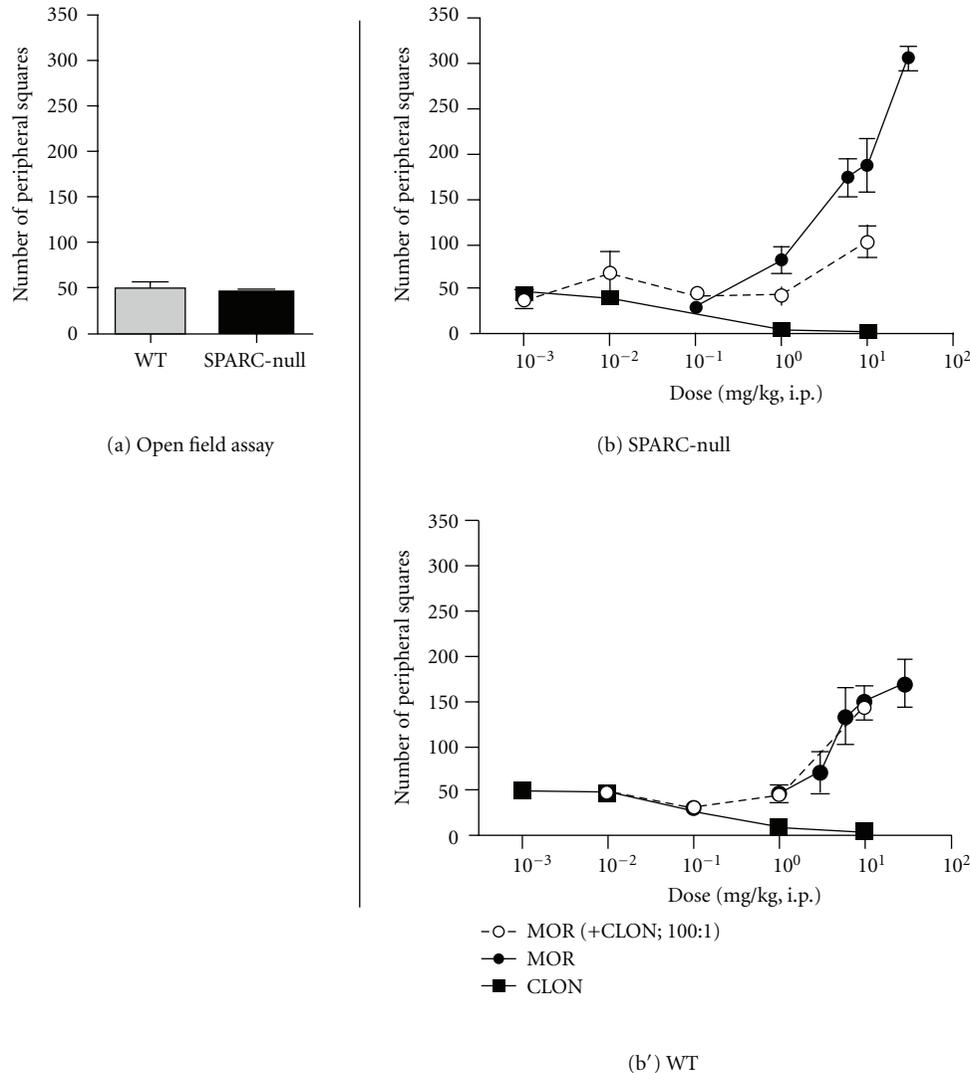


FIGURE 4: Effect of coadministration of morphine and clonidine on overall activity. (a) Saline-treated SPARC-null animals do not differ from WT mice in the number of peripheral squares covered in the open field, indicative of comparable overall activity between the two strains. (b), (b'). Both in SPARC-null (b) and WT (b') mice, morphine (●) caused an increase in activity, while clonidine (■) dose-dependently caused sedation. When coadministered (i.p.) at a constant dose ratio of 100 : 1 (morphine : clonidine), the combination showed no efficacy in SPARC-null mice and produced hyperactivity in WT mice. No isobolographs were plotted for either strain as the drugs had opposing effects. Error bars represent \pm SEM for each dose point ($n = 5-11$ animals/dose). See Table 1 for ED₅₀ values.

a spinal adjuvant for opioids in intractable cancer pain [47]. Although not currently indicated for patients with chronic axial LBP, our results suggest that low doses of systemic clonidine may be a useful addition to opioid therapy.

4.2. Coadministration of Morphine and Clonidine Increases the Therapeutic Window for Radiating Pain. Cold allodynia in the hindpaw of SPARC-null mice is a behavioural measure of non-axial, radiating pain. While cold allodynia is reversed by systemic clonidine, that efficacy is associated with side effects including motor impairment and sedation. Although the coadministration of morphine and clonidine was additive in our model, we did observe an improvement in the therapeutic window, such that therapeutic effects were

observed at doses associated with minimal side effects. We therefore believe that suppression of cold allodynia by the combination of morphine and clonidine is independent of motor impairment.

Radiating pain, which may accompany axial pain in patients suffering from LBP [5–8], is thought to have a mainly neuropathic mechanism [48]. As a result, anti-neuropathic agents and not opioids are the treatment of choice in these patients. Consistent with the reduced opioid efficacy commonly associated with neuropathic pain conditions, morphine failed to reach 50% efficacy in cold hypersensitivity in SPARC-null mice in the current study. Furthermore, while the ED₅₀ values for morphine were between 8 and 10 mg/kg in the tail suspension and rotarod assays, the extrapolated

ED₅₀ value for morphine in non-axial pain was >30 mg/kg. These observations support the predictive validity of the current model.

Studies evaluating opioid- α_2 AR agonist interactions in rodent models of neuropathic pain have demonstrated synergistic interactions between morphine and the α_2 AR agonists clonidine and moxonidine [17, 49]. While morphine and clonidine coadministration did not result in synergy in radiating pain in the current study, it did improve the therapeutic window in this modality. Previous work demonstrating that opioid- α_2 AR synergy is sensitive to both route of administration and the behavioral endpoint could explain this seeming discrepancy [22], as could the use of chronic pain models with different etiologies.

These results, together with the synergy observed in axial analgesia, demonstrate that combinations of morphine and clonidine target both the axial and radiating pain aspects observed in SPARC-null mice. In humans, the ability to obtain sufficient relief of both axial and radiating pain with the combination of morphine and a low dose of clonidine could result in less adverse drug reactions, fewer undesired or unanticipated drug interactions, increased patient compliance, and improved quality of life.

4.3. Opioid- α_2 AR Agonist Interactions. In humans, only a few studies have examined the interaction between opioid- α_2 AR agonists in chronic pain conditions. In one study, the addition of epidural clonidine benefited patients with intractable cancer pain, particularly those with a significant neuropathic component [47], and the combination of intrathecal morphine + clonidine is useful for the management of chronic pain after spinal cord injury [50, 51]. In order to maximize the clinical relevance of the current study, systemic administration was selected; spinal delivery requires invasive procedures that add additional risks. A variety of systemically delivered adrenergic agonists (i.e., clonidine, dexmedetomidine, moxonidine, tizanidine) are currently available for use in humans and could be utilized as adjuvants in patients not receiving sufficient efficacy from opioids.

Although there are many studies reporting functional interactions between opioids and α_2 AR agonists (for review see [52]), the molecular mechanisms underlying these interactions are not clear. Depending on the agonists used, analgesic synergy may be mediated by α_{2A} -, α_{2B} -, or α_{2C} -adrenergic receptor subtypes and mu- or delta-opioid receptors [44, 53–55]. Evidence from immunohistochemical studies suggests that opioid receptors are coexpressed in the same population of sensory neurons as α_2 ARs [56] and that antinociceptive synergy requires activation of calcium channels [57, 58] and protein kinase C [45, 59]. Physical association between G protein-coupled receptors such as the opioid and adrenergic receptors has been proposed to account for the synergistic effects observed [56, 60, 61]. It is well established that coexpression of GPCRs results in the formation of heteromeric complexes with altered functional and ligand binding properties [62]. Such interactions could occur at the level of the primary afferent neurons, the spinal cord and other sites in the CNS (i.e., locus coeruleus [63]), as well as in the periphery.

5. Future Directions

We have studied the acute effects of morphine, clonidine, and their combination 60 minutes after systemic administration. However, in clinical situations most patients undergo chronic pharmacotherapy. It is therefore critical to study these interactions using a chronic dosing paradigm. The use of multimodal therapy may be of even greater therapeutic benefit if chronic studies reveal protective effects of the combination against the development of tolerance or opioid-induced hyperalgesia. Clonidine is also known to reduce opioid withdrawal symptoms, a property that may be beneficial in long-term management of chronic noncancer pain [64].

Our study was carried out in a transgenic mouse model of LBP due to disc degeneration. While this model incorporates pharmacologically reversible behavioral measures of both axial and radiating pain associated with progressive, age-dependent intervertebral disc degeneration [31, 32, 42], it is unlikely to fully parallel patients suffering from LBP. Ultimately further studies in both preclinical models and human subjects are required to fully understand the therapeutic benefit of adrenergic adjuvant therapy.

6. Conclusions

We have used a mouse model of chronic LBP due to progressive disc degeneration to explore the effects of morphine and clonidine coadministration on measures of axial and radiating pain. Side effects including motor impairment and overall change in activity were also assessed. This is the first study to report a synergistic interaction between clinically used analgesics in a rodent model of chronic low back pain and to include the measurement of both axial and radiating pain. The results indicate that the addition of low-dose systemic clonidine can improve therapeutic outcomes both in axial and radiating pain measures, which could be of enormous benefit to patients suffering from chronic LBP.

Disclosure

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Conflict of Interests

The authors have no conflicts of interest.

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Review Article

Effects of Combined Opioids on Pain and Mood in Mammals

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The authors review the opioid literature for evidence of increased analgesia and reduced adverse side effects by combining mu-opioid-receptor (MOR) agonists, kappa-opioid-receptor (KOR) agonists, and nonselective low-dose-opioid antagonists (LD-Ant). We tested fentanyl (MOR agonist) and spiradoline (KOR agonist), singly and combined, against somatic and visceral pain models. Combined agonists induced additive analgesia in somatic pain and synergistic analgesia in visceral pain. Other investigators report similar effects and reduced tolerance and dependence with combined MOR agonist and KOR agonist. LD-Ant added to either a MOR agonist or KOR agonist markedly enhanced analgesia of either agonist. In accordance with other place-conditioning (PC) studies, our PC investigations showed fentanyl-induced place preference (CPP) and spiradoline-induced place aversion (CPA). We reduced fentanyl CPP with a low dose of spiradoline and reduced spiradoline CPA with a low dose of fentanyl. We propose combined MOR agonist, KOR agonist, and LD-Ant to produce superior analgesia with reduced adverse side effects, particularly for visceral pain.

1. Introduction

This paper supports, with scientific references, the hypothesis of a clinical utility of combinations of moderate doses of (a) a selective mu opioid receptor (MOR) agonist, (b) a selective kappa opioid agonist (KOR), and (c) ultralow doses of a nonselective opioid antagonist. The authors propose this triple opioid combination to produce a superior analgesic profile while reducing adverse and possibly lethal side effects of MOR and KOR agonists. Whereas somatic and neurogenic pain of short and long terms may be controlled with use of the proposed combination, the treatment should be most effective in allaying chronic visceral pain.

2. The Need for Improved Opioid Analgesic Drug Regimens

MOR agonists such as morphine, methadone, fentanyl, hydrocodone, and oxycodone are very effective analgesics, and about 23 million prescriptions are dispensed each year

for extended-release and long-acting opioids alone, which represented about 10 percent of the opioid market in 2009 (April 19, 2011, teleconference with Janet Woodcock, M.D., Director, Center for Drug Evaluation and Research, U.S. Food and Drug Administration). The beneficial effects of the opioids are frequently compromised by development of tolerance, dependence, hyperalgesia, addiction, and respiratory, and cardiovascular toxicities, the latter two leading too often to fatal consequences (White and Irvine [1]; “The Hill”: Pecquet (4/19/11): “Healthwatch” blog reported, “As a first step, the FDA sent letters to opioid manufacturers on Tuesday requiring that they provide a plan for training and educating patients about the safe use, storage and disposal of opioids. They have 120 days to respond, setting in place a regulatory process that officials hope to have in place within 12 months. ‘We have determined that a Medication Guide Communication plan is not sufficient to mitigate the serious risks,’ the letters state. ‘Your (strategy) must include tools to manage these risks.’ The FDA missive was sent to producers of Dolophine (methadone); ms Contin, Kadian, Avinza,

Embeda, Oramorph (morphines), Oxycontin (oxycodone); Exalco (hydromorphone); Duragesic (transdermal fentanyl); Butrans (buprenorphine); and Opana ER (oxymorphone)"; Hardman et al. [2]; Smith et al. [3]). Coop, who served for years as US Surgeon General, and his colleague MacKerell [4], urged the medical community to devise more effective and safer drug combinations of opioids. More recently, the FDA has now imposed new risk evaluation and mitigation strategy (REMS) requirements on marketers of extended release and long-acting opioids. This agency interaction thus supports the need for improvements in the way that opioid analgesics are prescribed and used.

Smith also called for improved analgesics, indicating that there were no ideal opioid preparations [5]. He pressed for the study of combinations to enhance analgesia while reducing unwanted side effects in 6 categories: (a) to prolong analgesic duration, (b) to increase analgesic efficacy (synergy), (c) to diminish or minimize adverse side effects, (d) to reduce nonbeneficial effects, (e) to reduce tolerance and development of hyperalgesia, and (f) to decrease dependency and addiction liability. Piercefield et al. [6] cited many overdose deaths in the United States that were related to methadone and other MOR agonists, mainly among males 35–54 years of age. In addition to significant opioid abuse, lethal outcomes occur due to provider and patient unfamiliarity with proper dosing regimens to ameliorate these problems with opioid dosing. Williamson et al. [7] indicated that many preventable overdose deaths occurred with methadone use in Australia, both prescribed and illegally diverted. Indeed, globally, risk of serious medical consequences of opioid use has not decreased and there remain specific therapeutic needs for safer and more effective opioid preparations.

3. Initial Studies with Mixed Opioid Agonists

The staff at Dr. Rech's Michigan State University neuropsychopharmacology research laboratory began animal studies with mixed opioid agonists in the 1980s, seeking an improved opioid analgesic agent against colorectal distension (CRD) nociception (visceral pain model) in feline subjects (Sawyer et al. [8], Sawyer & Rech [9], Sawyer et al. [10]). Feline subjects react to MOR agonists with a manic-like disoriented excitation, having dominant brain excitatory opioid receptors (Robertson and Taylor [11]). This prompted us to seek a calmer, sedating analgesic response with KOR-agonist activity. While these cats reacted to oxymorphone with agitated excitement, a different behavior was seen when they received the mixed action MOR/KOR agonist butorphanol subcutaneously (s.c.). The subjects remained quiet and even purred when petted over the first postdrug hour, with a moderate antinociceptive response that showed a ceiling effect. During the second postdrug hour, as the butorphanol antinociception waned, the cats became irritable. They flinched when touched and startled to a sharp noise. Nalbuphine and pentazocine, agonist-antagonist KOR agents, had less effective antinociception and exhibited a second-hour phase of irritation similar to that seen with butorphanol.

Canine subjects were also tested for butorphanol antinociception in the CRD procedure (Houghton et al. [12]; Sawyer et al. [13]). This species, which possesses dominant brain inhibitory opioid receptors, was calm and sedated during the first hour after butorphanol or oxymorphone injection. During the second hour butorphanol-treated dogs reacted with irritability similar to that phase observed in the cat. Pain relief was similar to that in feline subjects, but was accompanied by a slight respiratory depression and reduced heart rate. Thus, in both species, butorphanol's MOR agonist component was evident during the first postdrug hour, whereas KOR-agonist signs of agitation emerged during the second postdrug hour.

In a later study, Dr. Briggs et al., as a graduate student, examined the interactions of butorphanol combined with oxymorphone in the cat [14]. The combined drugs exhibited synergistic antinociception in the CRD over the response to each drug administered separately, but without the initial phase of excitement seen with oxymorphone alone.

4. Studies of Selective MOR and KOR Agonist Antinociception, Alone and Combined

These experiments were performed with Dr. Briggs and supported her thesis dissertation under Dr. Rech's mentorship (Briggs, S.L.: Interactions of mu- and kappa-opioid agonists, Michigan State University, 1996). In these experiments, the selective KOR-1 agonists spiradoline and enadoline, as well as the selective MOR agonist fentanyl, were tested for antinociception in the cold-water tail-flick (CWTF) assay (Briggs et al. [15]). The CWTF assay, a somatic pain nociceptive test, was chosen since Pizziketti et al. [16] found it to be efficient and sensitive to both opioid agonists. The opioids tested in these experiments were shown to be full agonists for maximal antinociception. Both spiradoline and enadoline were as efficacious, but less potent analgesics than fentanyl. Furthermore, naloxone (NLX), a nonselective opioid antagonist, attenuated both fentanyl antinociception, at 0.1 mg/kg, and KOR-agonists antinociception, at 0.5 mg/kg. Fentanyl antinociception was markedly reduced in methadone-tolerant animals, whereas spiradoline antinociception was unchanged. Spiradoline antinociception was nullified by pretreatment with n-binaltorphimine (n-BNI, KOR-1-specific antagonist). Fentanyl antinociception was abolished by beta-funaltrexamine (b-FNA, MOR-specific antagonist). And, as expected, b-FNA pretreatment did not alter spiradoline antinociception, nor did n-BNI pretreatment alter fentanyl antinociception.

Fentanyl and spiradoline were also tested in rats for pain relief in the CRD procedure, a visceral pain model, along with oxymorphone and enadoline (Briggs and Rech [17]). All showed fully effective antinociception when administered separately. Combining fentanyl and spiradoline produced additive (low doses) or supra-additive (high doses) effects. The supra-additive combination was attenuated by either b-FNA or n-BNI (greater with the latter). When b-FNA and n-BNI were tested against the antinociception of single doses in

CRD, paradoxical effects again occurred: the fentanyl effect was not antagonized by b-FNA, whereas the spiradoline effect was. Thus, complex paradoxical interactions took place in the CRD test, as opposed to the expected results as seen using the CWTF procedure.

Rech combined fentanyl and spiradoline in the CWTF (see Briggs et al. [15] above), to test for an additive antinociceptive response in rats (not previously published). In this last test, respiratory depression to fentanyl alone (0.008 mg/kg) was reduced when fentanyl (0.004 mg/kg) and spiradoline (0.56 mg/kg) were combined in ED50 doses to yield comparable antinociceptive levels for agonists given singly. This result resembled those respiratory effects reported by Verborgh et al. in rats [18] and Houghton et al. in dogs [12], both of which showed reduced respiratory depression to a MOR agonist by combining it with a KOR agonist.

An article by Negus et al. [19], which described results somewhat similar to the CRD and CWTF studies in rats by Briggs and Rech [17] and Briggs et al. [15], is reviewed here for comparison and contrast. Negus et al. tested fentanyl and U69593 (KOR-1 agonist) interactions in monkeys in three behavioral assays: (a) schedule-controlled responding for food (fixed ratio 30), (b) thermal nociception (50°C) for tail-withdrawal latencies (somatic pain model), and (c) schedule-controlled self-administration of both agonists, alone and combined. In the food assay both agents reduced rate of responding, and combined drugs produced subadditive effects. Both drugs alone induced dose-dependent antinociception, and combined drugs yielded additive antinociception. In the self-administration assay, fentanyl maintained responding for the drug, whereas U69593 did not. Combined drugs caused reduced self-administration levels with increasing fixed-ratio values. Thus, activation of both mu and kappa receptors with combined drugs appeared to reduce addiction liability while maintaining the additive decrease in pain.

A conventional wisdom indicating that combined MOR and KOR opioids had no role in pain relief is likely to have been related to interactions with the early KOR agonist-antagonists, pentazocine, and nalbuphine. After development of selective KOR-1 agonists by The Upjohn Company, some studies that were performed by non-Upjohn researchers with U-50,488H continued to report antagonism of MOR-agonist antinociception by U-50,488H, as follows. Pan et al. [20], Pan [21], Bie and Pan [22], and Tershner et al. [23] studied microinjections of the agents into brainstem nuclei. They showed KOR agonists to antagonize MOR-agonist antinociception using a somatic pain (tail-flick) test. The same group (Meng et al., [24]) tested rats with U69593 microinjected into the brain stem-rostral-ventromedial medulla (RVM), using tail-flick latency and RVM activity. The KOR agonist was proposed to be either pronociceptive (direct effect on "OFF cells") or antianalgesic by presynaptic and postsynaptic inhibition of glutamate inputs to RVM OFF cells.

In 2002, McNally and Akil authored a book chapter [25] on opioid pain modulation, emphasizing that KOR agonists antagonized MOR-agonist analgesia. In contrast to that emphasis on antagonism of MOR-agonist activity by

KOR agonists, there are many references (presented below) which support the utility of combined MOR- and KOR-agonists for synergistic action in the relief of pain. But prior to presentation of this listing, a review of the role of ultralow-doses of nonselective opioid antagonists is provided below. Ultralow doses of nonselective opioid antagonists, in combination with MOR and KOR agonists, are proposed here as representing a potentially superior clinical treatment to reduce pain, especially of the visceral type.

5. Ultralow-Dose Effects of Nonselective Opioid Antagonists

Naloxone (NLX) and naltrexone (NTX), in doses 50 to 150 times less than those used to antagonize antinociception of MOR and KOR agonists, have induced surprising effects in experimental models. Shen and Crain found these doses of antagonists to markedly enhance mu-opioid agonists' antinociception. Tolerance, physical dependence, and opioid-induced hyperalgesia were reversed to marked analgesia, along with reduced side effects [26–30]. These paradoxical results were defined more fully by Angst and Clark in a review [31], presenting the concept of competing opioid excitatory and inhibitory receptors in mammalian nervous systems, expressing the activation of excitatory mu receptors as opioid-induced hyperalgesia (OIH).

Tilson et al. [32] originally described hyperalgesia in rats following 3 days of s.c. morphine administration, followed by withdrawal. The morphine antinociceptive threshold in an electrical nociceptive tail-flick test was found to be reduced to 30 percent below the control (saline s.c.) nociceptive response. The authors offered the results as a measure of the intensity of morphine withdrawal. Low-dose nonselective antagonist effects on MOR excitatory opioid receptor mechanisms have been reported by many other researchers (see Christrup [33], Chu et al. [34], Field et al. [35], Powell et al. [36], Juni et al. [37], Abul-Husn et al. [38], McNaull et al. [39], and Tsai et al. [40]). Similar interactions between low-dose antagonists and KOR agonists occur, though less dramatically, in enhanced KOR-1-agonist effects on excitatory KOR opioid receptors. Examples are reports by Clemens and Mikes [41], Largent-Milnes et al. [42], Sloan and Hamann [43], and Webster et al. [44].

6. Other Antinociceptive Interactions of KOR Agonists in Animals

Bhargava et al. [45] determined that KOR activation by U-50,488H did not modify the development of antinociceptive tolerance to morphine in rats. However, Bie and Pan [22], cited earlier, found KOR agonists injected into the brain stem nucleus raphé magnus to attenuate MOR-agonist antinociception (to tail-flick, somatic pain model). Withdrawal-induced hyperalgesia, presumably by inhibition of glutamate transmission, was also suppressed. Black and Trevethick [46] proposed that KOR activation was especially effective in suppressing visceral pain (also see Yaksh [47]). Disrupting the KOR gene in mice impaired KOR-agonist

antinociception of visceral pain and attenuated morphine withdrawal (Simonin et al. [48]).

U-50,488H antagonized respiratory depression of DAMGO (MOR-agonist peptide) and morphine, the effects being reversed by the antagonist n-BNI (Dosaka-Akita et al. [49]). Field et al. found enadoline (KOR-1 agonist) to reverse hyperalgesia and allodynia in a rat model of surgically induced pain [35]. The KOR agonist peptide Dynorphin A-(2–17) reduced morphine tolerance in mice (He and Lee [50]). KOR-agonist activity in rat periaqueductal gray was found to attenuate morphine tolerance and dependence (Herra'ez-Baranda et al. [51]). Jang et al. [52] used nalbuphine to block morphine tolerance and dependence in rats. Khotib et al. [53] injected U-50,488H s.c. for 7 days in mice, upregulating morphine receptor function and enhancing antinociception. Ko et al. [54] injected U-50,488H into monkeys to reduce morphine-provoked pruritis, while maintaining or enhancing the antinociception effect of morphine.

As described in a series of articles, Sutters et al. [55], Miaskowski et al. [56, 57], and Miaskowski and Levine [58] microinjected DAMGO and U-50,488H intracerebroventricularly (i.c.v.) and intrathecally (i.t.) to test antinociceptive interactions against mechanical nociception (visceral pain). They obtained antagonistic or enhanced effects, the latter with reduced side effects of both agonists. Most combinations resulted in synergistic antinociception, the greatest with i.c.v. DAMGO and i.t. U-50,488H. Mechanisms were proposed involving multiple brain-spinal ascending and descending neuronal loops, with mu and kappa receptors at junctions of shared components. Background evidence relating to these concepts was presented by Yaksh [47] and his colleague, Schmauss [59]. They had mapped MOR and KOR receptor sites with microinjections into brain stem and spinal-dorsal-horn sites, microinjecting agonists and testing for somatic (thermal tail-flick) and visceral antinociception (writhing). These studies demonstrated that somatic and visceral pain, along with their suppression, are mediated by distinctly different pathways.

Ren et al. [60] administered i.t. subanalgesic doses of morphine and dynorphin A (1–13) in combination, which resulted in marked antinociceptive synergy, assessed by tail-flick latency in rats. However, when dynorphin A (1–13) was injected i.c.v., the pain relief from i.c.v. morphine was markedly antagonized. Therefore, combined MOR- and KOR-agonist effects greatly depend upon sites of administration. Ross and Smith [61] and Nielsen et al. [62] determined that acute oxycodone antinociception was attenuated by pretreatment with n-BNI, and that oxycodone and morphine had distinctly different profiles of action, convincingly proving oxycodone to be a KOR agonist. With chronic use, however, oxymorphone, the major metabolite of oxycodone, accumulates, adding a MOR-type antinociception to the effects of oxycodone. In humans, however, oxycodone is metabolized to oxymorphone in too low amounts (10%) to affect pain relief (Chinalore et al. [63], Tompkins et al. [64], and Zwisler et al. [65]). However, Ross et al. [66] combined a low dose of oxycodone with morphine in rats, i.c.v., i.p., and s.c., to cause synergistic antinociception, along with reduced central nervous side effects.

Schepers et al. [67] described results of Harley and Hammond using acute microinjections of MOR and KOR agonists into rat brain-stem rostral-ventromedial medulla (RVM) to yield a thermal antinociception that was potentiated in the presence of an inflammatory condition. Schepers' group extended those studies by injecting rats with complete Freund's adjuvant (cFA) into a paw plantar region to promote inflammation (a chronic visceral pain process). Two weeks later antinociception was induced by infusion into RVM of U69593 or DAMGO over 4 hours. Paw withdrawals were assessed by Hargreave's method. Mechanical thresholds with von Frey and Randall-Sellito methods were obtained, after which infusion of each drug produced prominent antinociception. Millan [68] tested U-50,488H and U69593 in rats subjected to noxious pressure (visceral pain), thermal and electrical stimuli. Prominent antinociception occurred to pressure, a weak response was seen to thermal stimuli, and the agonists were inactive to electrical shock (somatic pain).

Vonvoigtlander and Lewis [69] attenuated U-50,488H antinociception in rats by pretreatment with reserpine and p-CPA (brain 5-HT depletors). Spiradoline (U-62,0676) antinociception, however, was little affected by the pretreatments. Ho and Takemori [70] determined that U-50,488H pain relief in rodents was also blocked by pretreatment with 5-HT antagonists. These results suggest that some U-50,488H effects may differ from those of spiradoline and other arylacetamide KOR-1 agonists. U-50,488H (3.2–10 mg/kg pretreatment) completely blocked development of tolerance to chronic morphine in rats (Yamamoto et al. [71]). U-50,488H (10 mg/kg) also restored antinociception in morphine-tolerant animals. Other KOR agonists (enadoline, dynorphin A-(2–17), and nalbuphine) also reversed or blocked morphine tolerance, hyperalgesia, and allodynia (see [35, 50–52] above). These results clearly support the combined treatment with chronic MOR and KOR agonists to maintain and enhance a persistent analgesia as compared to effects of chronic MOR-agonist treatment alone.

Systemic morphine and spiradoline were compared in hot-plate, tail-flick, and acetic acid writhing in mice by Kuniyama et al. [72]. Spiradoline was more potent than morphine, and tolerance developed to either agonist on chronic treatment. Spiradoline pretreatment did not inhibit the morphine antinociception in any test. Turner et al. [73] pretreated rats with an ultralow dose of NTX before injecting morphine in a thermal tail-flick paradigm. These studies demonstrated that the morphine antinociceptive response is enhanced after low-dose NTX pretreatment versus morphine control antinociceptive scores. Furthermore, they indicate that NTX reverses the development of tolerance to chronic morphine treatment.

Sounvoravong et al. [74] compared morphine and U-50,488H for tail-pinch antinociception in a neuropathic pain model (sciatic nerve ligation). The morphine response was weak, while the U-50,488H response was similar to that in control mice. In a dynamic allodynia test, only U-50,488H produced antinociception and a decreased hyperalgesia. These findings suggest that KOR agonists are superior to MOR agonists for control of these types of pain.

7. Antinociceptive Interactions of KOR Agonists in Human Subjects

Stahl et al. [75] also found that visceral pain in humans was often difficult to control with MOR agonists. They reported that oxycodone was superior to morphine for treatment of some types of visceral pain. Gear et al. [76] reported that nalbuphine increased postoperative dental pain in male patients, but not in females. Pretreatment with a subanalgesic dose of morphine reversed this response to analgesia by antagonizing this nalbuphine antianalgesic response in males.

8. Rewarding and Aversive Effects of Opioids and Other Drugs Reflecting Motivational (Mood) Influences

Early studies in motivational opioid effects were aggressively pursued by Shippenberg and colleagues. Shippenberg et al. [77] tested morphine and fentanyl for development of tolerance and cross-tolerance, and interaction with U69593 tolerance, in a place conditioning (PC) procedure. Noncontingent morphine for 4 days induced tolerance to the development of conditioned place preference (CPP) on training rats in an unbiased multicompartiment PC maze. Cross-tolerance to fentanyl was also established. Noncontingent injection of U69593 produced tolerance to the subsequent attempt to train subjects to the KOR agonist for conditioned place aversion (CPA). Pretreatment with non-contingent U69593 did not result in tolerance when morphine was subsequently trained for CPP, however. Shippenberg et al. [78] treated rats with complete Freund's adjuvant (cFA) for 7 days to provoke inflammation in a hind limb. Subjects were then trained for U69593 aversion (CPA), which failed to develop. Therefore, prolonged noxious inflammation by cFA interfered with the development of a CPA response to the KOR-1 agonist. These results were suggested as indicating potential clinical utility of the agonist for management of chronic pain states.

Bals-Kubik et al. [79, 80] determined that aversive effects of MOR antagonists and KOR agonists using PC were centrally mediated. NLX (nonselective opioid antagonist) and CTOP (MOR-selective antagonist) produced CPA after s.c. or i.c.v. injections in rats. n-BNI i.c.v. did not induce CPA, but U50,488H and E-2078 (a dynorphin derivative) did. The opioids showing CPA were active in much lower doses i.c.v. than with s.c. doses. The mechanism for drug-induced aversion appears to be a blockade of brain mu responses. Shippenberg et al. [81] sought more detailed neurochemical bases for these motivational effects, thought to involve mesolimbic DA neurons. The neurotoxin 6-OHDA was microinjected bilaterally into the NAcc to abolish both morphine CPP and U69593 CPA. Lesions with 6-OHDA in some other mesolimbic nuclei did not affect the PC scores. Microinjection of the D-1 DA antagonist SCH-23390 into NAcc attenuated the PC of both agonists. A D-2 DA antagonist (sulpiride) was without effect.

To continue their studies of aversive opioid mechanisms, Shippenberg and Bals-Kubik [82] microinjected NLX or

CTOP into either the ventral tegmental area (VTA) or NAcc of rats to induce CPA. Lesions of NAcc with 6-OHDA nullified the aversion from intra-VTA CTOP, without modifying aversions from intra-NAcc CTOP or systemic NLX. The authors submitted that aversive effects caused by systemically administered opioid receptor antagonists do not depend upon mesolimbic DA neurons. Compulsive drug use, even after prolonged abstinence, involves 80–90% relapse rates (Shippenberg et al. [83]). This suggests that repeated drug use induces long-term alterations involving reactions of brain motivational systems to support the compulsion. Brain KOR functions, interacting with central MOR sites, play an essential role in driving opposing mood states. Central neurochemical changes with repeated drug use underscore vulnerabilities for addiction to opioids, cocaine and amphetamines, and alcohol, as well as to their combinations. Potential drug therapies targeting these altered systems are suggested treatment for these addictions.

Pfeiffer et al. [84] indicated that KOR agonists are free of the undesirable side effects of MOR agonists, including euphoria. Dysphoric actions to KOR agonists were thought to be mediated via sigma/phencyclidine receptors. However, the benzomorphan KOR agonist MR 2033 was inactive at sigma/phencyclidine receptors. They studied MR 2033 in human males, finding that the drug elicited dose-dependent dysphoria and psychotomimetic effects that were antagonized by NLX. Thus, MR 2033 appears to exert these aversive effects by way of kappa receptors, implying the existence of opposing MOR/KOR motivational systems in mammalian brains.

Another article by Shippenberg's group is that by Acri et al. [85]. Along with a host of other investigators, they studied interactions between cocaine and KOR agonists. U69593, in repeated doses, was described as downregulating pre- and postsynaptic DA D-2 receptors in rat brain striatum. This effect led to the prevention of cocaine-induced behavioral sensitization, which may have clinical relevance for the treatment of cocaine addiction.

Olmstead and Burns [86] used PC to test the hypothesis that ultralow doses of NTX coadministered with MOR or KOR agonists would alter their rewarding or withdrawal-induced aversive effects. NTX doses (0.03–30 ng/kg) were tested against oxycodone CPP in female rats (more sensitive than males for PC). NTX, 5 ng/kg, blocked CPP of morphine, 5 mg/kg, as well as the CPA to withdrawal from chronic morphine, 5 mg/kg for 7 days. Coadministering NTX, 20 pg/kg, also blocked the CPA to withdrawal from chronic oxycodone (KOR agonist), 3 mg/kg for 7 days. NTX effects on CPP to oxycodone, 3 mg/kg, produced an altered dose response. The lowest doses of NTX (0.03 and 0.3 ng/kg) blocked the CPP, the middle dose (3 ng/kg) had no effect, and the highest dose (30 ng/kg) combined with oxycodone trended toward a CPP. Therefore, ultralow NTX blocked acute reward of morphine or oxycodone, in addition to blocking withdrawal-induced aversion by chronic treatment with each agonist. (Authors' comment: Low-dose NTX appears to act selectively on excitatory opioid receptors to mediate these motivational effects of interactions of the agonists.)

Bowen et al., [87] found that mixed MOR/KOR agonists decreased cocaine i.v. intake better than selective KOR agonists in rhesus monkeys. U-50,488H and spiradoline i. p. decreased morphine and cocaine intake in rats, these effects lasting for 5-6 days in some subjects (Glick et al. [88]). The KOR effects were reversed by s.c. n-BNI. Kim et al. [89] determined that rats, when first injected with cocaine, showed an enhanced CPP to morphine and CPA to U69593. The CPA was delayed and more persistent than the CPP. Both of these effects were blocked by microinjecting MK-801 (NMDA receptor antagonist) bilaterally into the VTA, just before cocaine injection. Thus, both opioids acted upon the VTA to induce CPP or CPA. Kuzmin et al. [90] administered U-50,488H to reduce cocaine and morphine self-administration. An inverted U-shaped dose-response curve was observed for the KOR agonist, low doses enhancing self-administration, and higher doses decreasing self-administration of both morphine and cocaine.

Negus et al. [91] described decreased cocaine self-administration by chronic administration of EKC and U-50,488H in rhesus monkeys. Cocaine self-administration interactions were also studied in rhesus monkeys by Mello and Negus [92]. Eight KOR agonists were involved, each infused over 10 days. Dose-dependent sustained reductions in cocaine self-administration were noted for EKC, Mr2033, bremazocine, U-50,488H, and enadoline, along with some decrease in food intake. Cyclazocine, PD117302, and spiradoline did not alter cocaine self-administration. EKC and U-50,488H effects were antagonized by n-BNI and NLX. Negus et al.'s [19] of testing fentanyl and spiradoline self-administration interactions was reviewed in page 4.

Soderman and Unterwald [93] reported that cocaine CPP was attenuated by a MOR antagonist microinjected into NAcc or caudal VTA, suggesting that cocaine reward was mediated through activation of MOR receptors in either of these two brain nuclei. Additional investigation of cocaine and opioid interactions was authored by Valdez et al. [94] and Thompson et al. [95]. Valdez et al. indicated that KOR-agonist treatment in squirrel monkeys reinstated effects of cocaine, which was then attenuated by pretreating with naltrexone but not by n-BNI, suggesting a subpopulation of KOR receptors activating stress mechanisms. Thompson et al. (Shippenberg's group) described repeated dosing with U69593 to modulate DA uptake in the NAcc of rats in a manner opposite to that of cocaine, whereas acute U69593 transiently increased DA uptake. The KOR agonist also altered the activity of the DA transporter function.

Narita et al. published a series of articles dealing with rewarding and anxiety interactions of MOR and KOR agonists in rodents (see Narita et al. [96, 97]). A chronic inflammatory state by formalin injection into rats suppressed morphine-induced reward. Pretreatment with n-BNI (KOR-1-selective antagonist) almost completely reversed this effect. Also, the morphine-induced increase in limbic forebrain DA turnover was attenuated by the inflammation, this effect being reversed by n-BNI. Therefore, inflammation may have induced a sustained activation of endogenous kappa opioid receptors in NAcc. In mice, injection of cFA or sciatic nerve ligation (SNL, neuropathic pain) produced an anxiogenic

effect 4 weeks after injection or surgery. DAMGO-(MOR agonist) stimulated [³⁵S]GTPgammaS binding in the amygdala was suppressed by cFA or SNL. The cFA group showed an increase in [³⁵S]GTPgammaS binding in membranes of the amygdala after injection of the KOR agonist ICI199,441, suggesting an increase in receptor activation of G proteins. The authors proposed that these states of chronic pain produce anxiogenic effects and suppress MOR-agonist reward in rodents.

Ultralow doses of NTX (1, 10, 100 pg/kg/i. v. infusion) and oxycodone interactions were examined in rats by Leri and Burns [98]. Only the lowest dose enhanced oxycodone self-administration (0.1 mg/kg/infusion), suggesting a reduced rewarding potency of the opioid agonist. During tests of reinstatement in an extinction phase, all NTX doses decreased drug seeking induced by priming injections of oxycodone (0.25 mg/kg, s.c.) or foot-shock stress. During self-administration on a progressive ratio schedule, the agonist (0.1 mg/kg/infusion) plus NTX (1 pg/kg/infusion) reached a break-point sooner compared to self-administration of oxycodone alone. Adding a NTX dose, 10 mg/kg s.c., enhanced acute stimulatory effects of the agonist (1 mg/kg, s.c.), along with increased locomotor activity by oxycodone, 7 × 1 mg/kg, s.c. So the ultralow dose NTX cotreatment augmented oxycodone locomotor activity and opioid analgesia, but reduced the agonist's rewarding potency and vulnerability to relapse. (Authors' comment: The latter two effects may have occurred through a blockade of brain excitatory opioid receptors by the ultra-low-dose NTX.)

Funada et al. [99] blocked morphine CPP with a low dose of U-50,488H or E-2078 (KOR agonists). CPA was seen with PC using higher doses of the KOR agonists, but not with the lower doses. Pretreatment with U-50,488H or E-2078 abolished CPA due to morphine withdrawal, and this effect was reversed by pretreatment with n-BNI. U-50,488H was inactive in altering the CPP of apomorphine (DA agonist). Similar interactions of MOR and KOR agonists were reported by Tao et al. [100], Tsuji et al. [101], and Bolanos et al. [102].

The main deterrent to the clinical application of KOR agonists as analgesics for control of chronic pain in human subjects is the disturbing side effect of dysphoria (Walker et al. [103]).

Sante et al. [104] observed a CPP in rats by microinjecting morphine into the brainstem dorsal periaqueductal gray. Microinjections of the peptide CTOP (selective MOR antagonist) or U-50,488H into dorsal periaqueductal gray induced a CPA. These results once more demonstrate mutually opposing motivational effects of brain MOR and KOR activations in specific brain nuclei (see also Koob and Le Moal [105]).

A study of PC interactions of fentanyl (Fn) and spiradoline (Sd) in our laboratory was prompted by the hypothesis that combined s.c. MOR and KOR agonists would reduce both CPP of the MOR agonist and CPA of the KOR agonist (Rech et al. [106]). Four groups of rats, 6 in each group (A, B, C, D) were trained over five 7-day sessions in a PC procedure to saline (control group A), or dose-response levels (L = low, M = medium, and H = high) of Fn and Sd (groups B,

C, and D). Shuttle responses of subjects between the two compartments were recorded by an automated recording system, avoiding potential subjective errors by an observer tabulating the subjects' movements.

A dose-dependent CPP was formed in animals treated with fentanyl. Medium and low doses of fentanyl (0.003 and 0.006 mg/kg) were also associated with CPP, while in the same group of animals low and medium doses of Sd were capable of producing a CPA. Interestingly, a low dose of Sd reduced the CPP of a high dose of fentanyl. In addition, a medium dose of fentanyl produced a reduction in the CPA produced by a medium dose of Sd. Thus, the hypothesis in question was confirmed, that is, the KOR agonist aversion was reversed by a low dose of the MOR agonist (see also [99]). Since a low dose of KOR agonist also reduced MOR agonist reward, our results support a reciprocal interaction between drug-induced preferential and aversive motivational states. To assure that the sedative effects of both drugs were not compromising this study, we also analyzed the number of shuttles per 15 min trial for each subject as an index of locomotor activity. There was no correlation between these shuttle results and the PC data.

Morales et al. [107] compared PC effects of morphine and U-50,488H in either a two- or three-compartment device. Morphine CPP was similar in the two instruments, but the U-50,488H CPA was better developed in the two-compartment device. They also employed an automatic recording system.

PC was also used by Hirakawa et al. [108] in rats, to study affective responses to combined methoxamine (alpha-1-adrenergic agonist) and U69593 microinjected into RVM. Methoxamine, 0.05 mg, plus U69593, 0.178 micrograms, produced hyperalgesia in the tail-flick assay as well as CPA. Adjusted single drug doses caused CPA, no PC effect, or CPP. PC effects and spinal nociceptive reactivity showed no correlation.

MOR- and KOR-agonist and antagonist subjective interactions in human volunteers were studied by Preston and Bigelow [109]. They administered hydromorphone (MOR agonist) and pentazocine (mixed MOR/KOR agonist) followed by NTX, 25 mg or 12.5 mg. Before NTX hydromorphone caused typical MOR effects ("liking", calming). Pentazocine showed less intense effects of this type, along with some restlessness. The high dose of NTX blocked the effects of both agents. The 12.5 mg of NTX also blocked hydromorphone effects, but uncovered more irritability and psychotomimetic effects (typical KOR-agonist responses) as pretreatment before pentazocine. Thus, the MOR activity of pentazocine in the absence of NTX appeared to keep the drug's KOR agonist actions in check. The lower dose of NTX, selectively blocking MOR receptors, allowed for the KOR agonist influences to emerge.

9. Conclusions

Combining moderate doses of a MOR agonist (fentanyl, methadone, oxymorphone, and hydromorphone) with low doses of a KOR agonist (spiradolone, enadolone, U69593, oxycodone) produced the following:

(i) additive analgesia in somatic pain assays and supra-additive analgesia in visceral pain paradigms, along with a reduction in adverse side effects such as respiratory depression, and tolerance, dependence and hyperalgesia from chronic MOR agonist treatment was attenuated by pretreatment with a KOR agonist ([14, 15, 17–19, 50, 53, 55–58], see also [67]);

(ii) analgesia of oxycodone, a KOR agonist, was superior to morphine in visceral pain states, in both animal and human subjects [41, 61–66, 75].

Combining ultralow doses of NLX or NTX with MOR and KOR agonists resulted in

(i) enhanced analgesia, reduced tolerance, and dependence for both agonists, and decreased hyperalgesia after chronic MOR agonists [26–29, 31, 34–36, 39–44];

(ii) MOR CPP, KOR CPA, and self-administration was altered by

(a) reduced rewarding potency and relapse vulnerability of oxycodone [88, 90, 98, 99];

(b) a KOR-agonist dose too low to cause CPA (n.s. trend), which attenuated a high-dose MOR-agonist CPP and self-administration (reducing addiction liability [102, 104, 106]);

(c) a MOR-agonist causing modest CPP, which attenuated a high-dose KOR-agonist CPA (reducing KOR-agonist aversion), and combined medium doses of MOR and KOR agonists that resulted in mutually abolished CPP and CPA, respectively (n.s. compared to saline [106]);

(d) three prolonged inflammatory pain states, two with cFA [67] and [78], that abolished KOR-agonist CPA only, and the other with formalin [96], that suppressed both MOR CPP and KOR CPA.

It is tempting to speculate on the driving force for development and persistence of opposing neural MOR and KOR systems in mammalian speciation. MOR- and KOR-agonist combinations, both agents producing analgesia while provoking opposite-type side effects, may have survival value in controlling severe pain. Opposing endogenous MOR and KOR motivational/mood states in healthy subjects appear to modulate an effective balance of responses to environmental challenges [100, 103, 106, 109]. Impairments in these balances may be effectively treated by adding a low-dose antagonist (NLX, NTX) to modulate activation or suppression of inhibitory or excitatory opioid receptors.

Abbreviations

b-FNA:	Beta-funaltrexamine
CPA:	Conditioned place aversion
CPP:	Conditioned place preference
CRD:	Colorectal distension
CWTF:	Cold-water tail-flick
DAMGO:	MOR agonist peptide
EKC:	Ethylketocyclazocine

i.v.:	Intravenous
i.c.v.:	Intracerebroventricular
KOR:	Kappa opioid receptor
MOR:	mu opioid receptor
NAcc:	Nucleus accumbens, midbrain nucleus
NLX:	Naloxone
NTX:	Naltrexone
PC:	Place conditioning
s.c.:	Subcutaneous injection
s.s.:	Self-stimulation via brain electrodes
U69593:	Selective KOR-1 agonist
cFA:	Complete Freund's adjuvant
VTA:	Ventral tegmental area
CTOP:	MOR selective antagonist
DA:	Dopamine
Dynorphin A:	MOR agonist peptide
5-HT:	5-Hydroxytryptamine
i.p.:	Intraperitoneal
i.t.:	Intrathecal
KOR-1:	Subtype of KOR-receptor
MR2033:	KOR agonist
n-BNI:	Norbinaltorphimine
n.s.:	Nonsignificant
OIH:	Opioid-induced hyperalgesia
RVM:	Rostral-ventromedial medulla
U-50,488H:	Selective KOR-1 agonist
SNL:	Sciatic nerve ligation
VTA:	Ventral tegmental nucleus.

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Review Article

Combination Drug Therapy for Pain following Chronic Spinal Cord Injury

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A number of mechanisms have been elucidated that maintain neuropathic pain due to spinal cord injury (SCI). While target-based therapeutics are being developed based on elucidation of these mechanisms, treatment for neuropathic SCI pain has not been entirely satisfactory due in part to the significant convergence of neurological and inflammatory processes that maintain the neuropathic pain state. Thus, a combination drug treatment strategy, wherein several pain-related mechanisms are simultaneously engaged, could be more efficacious than treatment against individual mechanisms alone. Also, by engaging several targets at once, it may be possible to reduce the doses of the individual drugs, thereby minimizing the potential for adverse side effects. Positive preclinical and clinical studies have demonstrated improved efficacy of combination drug treatment over single drug treatment in neuropathic pain of peripheral origin, and perhaps such combinations could be utilized for neuropathic SCI pain. At the same time, there are mechanisms that distinguish SCI from peripheral neuropathic pain, so novel combination therapies will be needed.

1. Introduction

Tissue injury or disease may lead to a persistent pain state. Chronic pain is maintained through a combination of neural and nonneural mechanisms operating simultaneously at peripheral and central nervous systems [1, 2]. At the site of peripheral tissue damage, a number of inflammatory mediators are released that activate and recruit immune cells, initiating the process of tissue repair. Also, many of these mediators released from recruited immune cells, including excitatory amino acids, neuropeptides, and cytokines, sensitize primary afferent nociceptors [3–5]. The persistent pain state could be maintained, in part, by the overproduction of these mediators and by the overexpression by genes of cell membrane-bound proteins, such as receptors and ion channels, and intracellular signaling complexes in peripheral nerves [6–8]. Furthermore, the physiological responses of spinal dorsal horn neurons and primary afferent neurons are permanently altered, such that their responses to peripheral, cutaneous stimulation are exaggerated and persist long after the application of stimulation. These physiological changes

are believed to be maintained by long-lasting changes in genes and, akin to the process observed in peripheral nociceptors, overexpression of membrane-bound proteins and overactivation of intracellular signaling [9, 10].

Spontaneous activity has been demonstrated from injured peripheral sensory neurons and from CNS neurons, proximally and distally to the site of injury [11–13]. The abnormal neurophysiological responses to peripheral injury, sensitization, and spontaneous activity are believed to be the neural basis of tissue injury-induced chronic pain, which is characterized by cutaneous hypersensitivity and spontaneous pain.

In spinal cord injury (SCI), spontaneously active CNS neurons, found spinally and supraspinally, have been documented in both clinical and experimental settings [14–20]. Experimental evidence suggests that reducing the activity of these neurons leads to a decrease in chronic pain symptoms. Drugs that have demonstrated to decrease CNS neural activity, including opioids, γ -aminobutyric acid (GABA) receptor agonists, and sodium channel blocking drugs, are antinociceptive in animal pain models and these drugs are

also analgesic in clinical pain states [21]. The data suggest that robust pain relief can be obtained though suppressing abnormal neural activity. Obtaining direct evidence, such as electrophysiological and neurochemical, of drug effects from patients as performed in animals, is a tremendous technical hurdle. However, noninvasive imaging may be an alternate method to quantify drug effects on brain neurochemistry and activity and these data could correlate with pain relief [22, 23]. Furthermore, such data could be used to guide drug discovery.

The existence of multiple, often overlapping mechanisms that have been identified so far not only underscores the biological complexity of chronic pain, but the difficulty in providing significant pain relief with currently available analgesic pharmacotherapies. The vast array of pain-related mechanisms, however, invites development of a nearly endless list of potential treatments, especially treatments that target more than one mechanism.

2. Combination Therapy: Synergism

One could take advantage of the parallel activities of molecular targets by engaging several of these targets at once, wherein the goal is a combination treatment that is superior to that of individual target-specific treatments [24, 25]. The concept of synergism has been demonstrated in the clinical setting for a variety of indications such as the treatment of cancer and infections [26]. Combining drugs may lead to either additive or non-additive effects. If the effect of a combination of two or more drugs does not significantly deviate from the theoretical or expected effect based on their individual dose-response curves, then the effect of the combination is said to be additive. There are two types of nonadditive effects that may result with combination treatment. First, if the effect of the combination is greater than expected, then the combination is said to be superadditive, or synergistic. The total dose of the synergistic combination can be lower than what would be expected from the individual dose-response curves, which may also diminish the risk of adverse side effects associated with either drug. Secondly, the total dose needed to induce a certain effect may be higher than what is expected. In this case, the combination is said to be subadditive or antagonistic.

The key is that the experimentally derived result be statistically significant from the expected result. One method to determine this is by isobolographic analysis, wherein the doses of the constituents that give, for example, a 50% antinociceptive effect, are plotted on the x -axis and y -axis [26, 27]. The line connecting these points is said to be the “line of additivity,” a locus of dose pairs of the constituents expected to demonstrate equal antinociception (“zero interaction”). The amount of each constituent in the combination can be based on the relative potency of the constituents. Following construction of a dose-response curve of the combination, the 50% antinociceptive dose is determined and statistical analysis is used to determine whether or not the effect of the combination significantly deviates from zero interaction, whether the effect of the combination is either synergistic, antagonistic, or merely additive. While one ratio of the

constituents may be merely additive, other ratios may lead to either synergistic or antagonistic effects [28]. Thus, the lack of synergy for a given combination should not automatically exclude that combination from further consideration—perhaps other combination ratios could lead to synergy.

There are cases of synergism in which one of the drugs in a two-drug combination demonstrates no efficacy [29]. To demonstrate synergism, a statistically significant increase in the potency of the active drug of the combination compared to the active drug alone is required. Thus, drugs that do not demonstrate efficacy on their own may still be useful when given with drugs that do demonstrate efficacy [30–32]. The advantage of this type of combination is similar to that of a combination in which both drugs demonstrate efficacy—the dose of the efficacious drug in the combination is decreased thereby reducing the potential for adverse side effects.

3. Neuropathic Spinal Cord Injury Pain

In the U.S., there are an estimated 256,000 SCI patients and there are approximately 12,000 new cases of SCI each year, most commonly due to motor vehicle accidents and falls [33]. As medical breakthroughs increase life expectancy in general, there will be a growing population of elderly, and life expectancy of current SCI patients could increase as well. In addition, with increasing numbers of older persons, it is anticipated that they may develop SCI, due to, for example, accidents [34, 35]. Older SCI, as well as non-SCI, patients are more likely than younger people to report chronic pain [36]. Thus, with the significant expansion of the elderly population in the U.S. and other industrialized countries projected by midcentury and the potential for an increased number of elderly SCI patients, there is an urgent need to develop effective pain therapeutics [37, 38].

In addition to significant losses in motor and visceral functionalities, intractable pain may also result following SCI, a majority of SCI patients reporting the severity of pain as either moderate or severe, such that there is greatly diminished mood and motivation to participate in rehabilitation programs and social interaction [39–41]. Pain may be localized in dermatomes either above, at, or below the level of injury [42–44]. Interestingly, below-level pain has been described as “burning” or “shooting” and accompanied by cutaneous hypersensitivity, symptoms that are characteristic of peripheral neuropathic pain [44, 45]. There is also the possibility of “autonomic dysreflexia,” a condition in which noxious somatic or visceral stimulation below the level of SCI could lead to an acute, uncontrolled sympathetic nervous system response, including a life-threatening increase in blood pressure and tachycardia [46]. Thus, treatments that are tolerated in other pain populations may not be suitable for SCI patients. For example, visceral distention by opioids and antidepressants such as amitriptyline could lead to autonomic dysreflexia.

Furthermore, treatments that are efficacious in peripheral neuropathic pain do not appear to demonstrate the same level of efficacy in neuropathic SCI pain. Amitriptyline, for example, in addition to adverse side effects in SCI patients

such as urinary retention, does not appear to be as effective in neuropathic SCI pain as it is in peripheral neuropathic pain [47]. Mexiletine, an orally active analogue of the sodium channel blocking drug lidocaine, also does not show the level of efficacy in SCI patients that has been demonstrated in peripheral neuropathic pain patients [48, 49]. Perhaps the lack of efficacy across patient populations could be due in part to varied testing protocols and outcome measurements in the clinical trials, but some of the differential efficacy could also be due to underlying differences in pain mechanism. If standard pharmacotherapies, when given alone, do not demonstrate efficacy, then combination drug therapy could be a viable option for SCI pain patients.

4. Preclinical Combination Drug Therapy in the SCI Rat

To facilitate the evaluation of novel pharmacotherapies for potential clinical use, a rat model of chronic neuropathic SCI pain was recently developed [50, 51]. Four weeks following a brief midthoracic spinal compression, a massive infiltration of monocytes and a robust gliotic response were observed at the injury site. In addition, syringes were observed extending for several segments from the injury site. The histopathological findings are reminiscent of that reported following an acute spinal contusion, a widely used method of inducing SCI in rats, and in clinical SCI [52, 53]. Despite significant bilateral motor dysfunction below the level of the injury, similar in degree and extent to that following a contusion injury, rats were responsive to cutaneous stimulation. The methods of quantifying below-level cutaneous hypersensitivity used were the same as those commonly used in rat models of peripheral nerve injury [54, 55]. A long-lasting below-level hypersensitivity to cutaneous stimuli, as observed in clinical SCI pain, was obtained beginning one week following spinal compression, which lasted for at least 12 weeks after-injury [56].

A variety of clinically used analgesic drug were assessed in these rats. The anxiolytic drug diazepam did not demonstrate antinociceptive efficacy, indicating that sedative or muscle relaxant property is not sufficient to ameliorate pain-related behaviors [57, 58]. While mexiletine and the anti-convulsant drug carbamazepine were found to be efficacious in other chronic pain models, these did not demonstrate significant effects in SCI rats [59, 60]. The anti-inflammatory cyclooxygenase-2 selective inhibitor rofecoxib and the non-selective cyclooxygenase inhibitor naproxen also did not affect below-level cutaneous hypersensitivity, suggesting that prostaglandins are not a critical factor in maintaining the neuropathic state in these rats [61]. The doses of rofecoxib and naproxen tested in SCI rats were efficacious, however, in rat models of inflammation-induced pain. The lack of efficacy of several analgesics in SCI rats compared to other rat pain models suggests fundamental differences in mechanisms among the chronic pain states.

At the same time, several drugs that demonstrated antinociception in peripheral neuropathic pain models also demonstrated efficacy in the current SCI model, suggesting

that both peripheral and SCI pain share a few common mechanisms. Clinical drugs that are generally characterized as selective for a particular target, including opiates, the GABA_B receptor agonist baclofen, the voltage-gated calcium channel (VGCC) blocker gabapentin, and noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists such as ketamine, showed efficacy in both models of SCI and peripheral nerve injury pain [60, 62–66]. The analgesic tramadol also demonstrated efficacy in both SCI and peripheral neuropathic pain models [66–69]. Interestingly, the efficacy of tramadol is likely to be mediated by a combination of several mechanisms: its metabolite is a μ -opioid receptor agonist and the drug itself increases synaptic levels of analgesic neurotransmitters serotonin and norepinephrine by blocking the reuptake of these neurotransmitters [69]. Both separate and simultaneous activation of rat spinal cord dorsal horn μ -opioid and serotonergic receptors and α -adrenoceptors leads to marked antinociception in acute pain tests [70, 71]. Since drugs are usually dosed systemically, antinociceptive effects could be the result of engaging pain-related targets in several sites within the CNS and those targets may also be found in peripheral nerves [72–74]. Given the presence of a number of pain-related mechanisms involved in modulating pain perception, there are potentially numerous combinations that may lead to synergistic analgesia [75].

Some of the common side effects observed with analgesic drugs with systemic bioavailability include somnolence, sedation, dizziness, and nausea [41]. Adverse side effects are inevitable since most currently available analgesics freely distribute throughout the CNS. With combination drug therapy, it may be possible to significantly reduce the incidence of side effects by reducing the doses of the constituents. Alternatively, there are drug delivery methods which may further minimize the incidence of side effects. One method is to deliver drugs into the intrathecal (i.t.) space of the spinal cord [76]. Some analgesics that demonstrated efficacy when given systemically also demonstrated robust efficacy following i.t. injection in SCI rats, indicating that the spinal dorsal horn is a key site of action of these drugs [77–79]. In rats with a spinal hemisection, blockade of spinal NMDA receptors at the site of spinal injury with i.t. injection of the competitive NMDA receptor antagonist AP-5 ameliorated below-level hypersensitivity to innocuous mechanical stimulation (but not injury-induced heat hypersensitivity) [80]. The effects of clinically used NMDA receptor antagonists, such as ketamine or memantine, were not evaluated in these rats. While block of dorsal horn NMDA receptors in general leads to an antinociceptive effect in chronic pain models, it appears that the degree of efficacy depends on whether the antagonist used is a competitive or noncompetitive antagonist [81, 82]. In-house data indicates that i.t. ketamine, at doses that do not induce hind limb dysfunction, does not ameliorate below-level cutaneous hypersensitivity in rats with a spinal compression injury. In contrast, systemic NMDA receptor antagonist treatment is effective, suggesting that supraspinal NMDA receptors have a prominent role in maintaining below-level cutaneous hypersensitivity in spinal compression-injured rats [50]. The main disadvantage of systemic NMDA receptor antagonists, however, is the overlap

in doses that lead to significant supraspinally mediated psychomimetic effects and those that lead to antinociception [83]. Even though i.t. ketamine alone was not efficacious, as noted earlier, it could be effective if combined with other i.t. delivered analgesic drugs.

A number of combination therapies have been evaluated for efficacy in preclinical models of neuropathic pain but few have been tested specifically in a preclinical model of neuropathic SCI pain and so their potential clinical usefulness is unknown [24]. Because of possible differences in mechanism between peripheral and SCI pain states, combinations that maybe useful in one state might not show efficacy in the other state. Therefore, it will be crucial to test potential combination drug therapies in models of SCI pain.

Recently, a number of drug combination therapies have been evaluated for antinociceptive efficacy in rats with spinal compression injury. While baclofen is approved for use in spasticity, it has been used off label for the treatment of neuropathic pain, including neuropathic SCI pain [84, 85]. Because systemic dosing can lead to side effects such as sedation and muscle weakness, i.t. baclofen has been utilized as a means of long-term pain treatment. However, pharmacological tolerance to the beneficial effect of i.t. baclofen has been reported, and potentially dangerous withdrawal symptoms may occur if i.t. infusion is suddenly interrupted [86–88]. One method of reducing the dose of baclofen and reducing the possibility of tolerance and the severity of withdrawal is to combine it with other drugs. Preliminary data from SCI rats indicates that i.t. injection of a combination of ketamine and a 50% efficacious dose of baclofen leads to a significant enhancement of baclofen antinociception (unpublished data). The combination also underscores a mechanistic hypothesis, that chronic SCI pain is maintained by a simultaneous decrease in GABAergic inhibition and increase in NMDA receptor-mediated excitation [80, 89, 90]. Therefore, considerable pain relief could be obtained by blocking the NMDA receptor and activating GABA receptors. While ketamine was synergistic with baclofen, combination i.t. treatment of GABA_A receptor agonist muscimol and ketamine did not lead to synergism. That there was synergism with GABA_B, but not with GABA_A, receptors is puzzling. The lack of synergism could be explained via a paradoxical in vitro finding that activation of the GABA_A receptor leads to the activation of NMDA receptors [91]. Thus, there is the need for further elaboration of possible interactions between pain-related targets in order to find useful combinations for clinical efficacy. Given all of the potential interactions within the pain transmission system, it appears that synergism is a novel occurrence at best [29].

Ziconotide, a synthetic analogue of a peptide derived from the marine snail *Conus magus*, is prescribed for use as an i.t. monotherapeutic for severe chronic pain [92]. Preclinical and limited clinical studies indicate that ziconotide may be useful in below-level SCI pain [93, 94]. Ziconotide acts via blockade of the N-type VGCC, which are expressed on central terminals of primary afferent nociceptors, which synapse with dorsal horn spinal neurons [95]. Blocking spinal N-type VGCCs prevents an influx of calcium ions and the subsequent increase in intracellular calcium concentration,

thereby preventing the calcium-mediated release of neurotransmitter from central terminals and transmission of nociceptive signaling across the synapse. N-type VGCC found on spinal neurons are also blocked by ziconotide, thereby reducing nociceptive signaling within the CNS. Furthermore, N-type VGCCs in the “neuropathic state” appears to be more sensitive to ziconotide block compared to N-type channels from uninjured animals, since treatment with ziconotide does not affect nociception in uninjured animals [96, 97]. There are reports of psychiatric effects with i.t. ziconotide treatment, which are ameliorated when the dose is lowered, indicating that the side effects are target mediated [98]. Another naturally derived peptide, conantokin-G, blocks NMDA receptors, with an antinociceptive effect similar to that of small molecule NMDA receptor antagonists [99]. Interestingly, i.t. treatment with conantokin-G in rats does not lead to the characteristic side effects typically observed with small molecule NMDA receptor antagonists, so this peptide could find potential use as a monotherapeutic. Nonetheless, the i.t. combination of ziconotide and conantokin-G leads to a synergistic antinociception in SCI rat, whereas the combination of the two leads to additive antinociception in other rat pain models [93]. Why synergism of this combination is observed in SCI compared to other injury states is not entirely clear at this point. There are a number of naturally derived substances, other than peptides, that block ion channels and receptors which may confer significant clinical analgesia alone and which may also significantly enhance the analgesic efficacy of currently available drugs [100, 101].

Cannabinoids have been used for hundreds, if not thousands, of years as a therapeutic for a variety of conditions, including pain [102]. Cannabinoid (CB) receptor agonists have demonstrated robust antinociceptive effects in a variety of preclinical models of chronic pain [103, 104]. Activation of CB receptors alone and in combination with other receptors involved in nociceptive processing leads to synergistic antinociception in rodent pain models [105–107]. There are varying degrees of efficacy of CB receptor ligands in a number of clinical pain states, although they are not as robust as reported in preclinical studies [108]. The mixed levels of clinical efficacy could be due in part to pharmacokinetics. Efficacy has been reported with inhaled CB receptor ligands but not for orally ingested CB receptor ligands in central pain states [49, 109, 110]. A problem that arises from systemic delivery of CB receptor agonists is activation of both spinal and supraspinal receptors, which not only leads to antinociception but also significant psychomotor effects [111]. Given the strong psychomotor side effects observed with therapeutic doses of CB1 receptor agonist, and the sociopolitical controversy surrounding the use of this class of drug for medical use in general, alternative means by which to engage CB receptors for pain relief are needed.

One method to indirectly engage CB receptors would be to increase synaptic concentrations of endogenous cannabinoid receptor ligands (or “endocannabinoids”), such as anandamide (*N*-arachidonylethanolamide), by inhibiting the enzyme which degrades it, fatty acid amide hydrolase (FAAH). Other enzymes involved in metabolizing other

endocannabinoids could also be utilized as pain targets [112]. Antinociceptive effects have been demonstrated by increasing CNS anandamide with FAAH inhibitors in pre-clinical models of neuropathic and inflammatory pain, and the effects were CB receptor dependent [113–115]. Furthermore, the antinociceptive effects were not accompanied by the adverse side effects commonly observed with efficacious doses of small molecule CB receptor agonists. Development of selective and potent FAAH inhibitors for clinical use is currently on-going. However, a metabolite of the over-the-counter drug acetaminophen (acetyl-para-aminophenol), AM404, has been shown to increase synaptic levels of anandamide by blocking the neuronal reuptake of anandamide [116]. Acetaminophen itself acts through various mechanisms and these mechanisms in total, including its effect on synaptic endocannabinoid levels, could explain its analgesic effects [117]. Acetaminophen is safe when taken as directed, and has a long clinical history, either alone or as a combination therapeutic [118, 119]. Until recently, acetaminophen-based combination drug therapy has not been evaluated in SCI pain models [120]. Acetaminophen alone did not alter below-level cutaneous hypersensitivity, even at doses that demonstrated efficacy in other pain models. However, combining acetaminophen with a 50% efficacious dose of gabapentin significantly increased the efficacy of gabapentin. In addition, the antinociception was attenuated with treatment of the CB1 receptor antagonist rimonabant but not the CB2 receptor antagonist AM630, indicating that the combination antinociceptive effect is mediated through endocannabinoids activating CB1 receptors (the antinociceptive effect of gabapentin alone was not blocked with pretreatment of rimonabant). The combination of acetaminophen with morphine was also synergistic and partially mediated by CB1 receptors. Not all acetaminophen combinations demonstrated synergism, however, as combination with either memantine or tramadol were merely additive. These results suggest that acetaminophen combinations could be useful in clinical SCI pain by combining indirect CB receptor activation with modulation of other pain-related targets. In addition, increasing endocannabinoids could be a method to circumvent the use of potent CB receptor agonists which lead to supraspinally mediated side effects. In practice, there may be a period of trial and error in determining an optimal combination that will lead to synergism in humans.

Potentially useful combinations are not always available in convenient oral formulation and this may hamper patient compliance. The possibilities of adverse drug interactions and, for i.t. administration, tissue toxicity need to be carefully considered. As a potential alternative to pharmacotherapeutics, transplantation of cells that release a mixture of analgesic substances could be a long-term means to reduce neuropathic SCI pain. A number of studies have demonstrated that adrenal medullary chromaffin cells, which release catecholamines, opioid peptides, other neuropeptides including the NMDA receptor antagonist histogranin, and neurotrophic factors, implanted in the spinal subarachnoid lumbar space, lead to significant antinociception in various animal models of pain [121, 122]. Because of the difficulty of obtaining cadaver adrenal medullary tissue for human

implantation, cell lines have been engineered to secrete analgesic neurotransmitters, such as GABA and serotonin [123]. The genes for neuroactive peptides, such as ziconotide and histogranin, could be inserted into the genome of these cells [124, 125]. Thus, a mixture of substances would be continuously released into the CSF to ameliorate pain on a long-term basis, without the need for maintenance or refilling the reservoir of an i.t. drug infusion pump. An added advantage is that these mixtures could be designed to reduce the potential for analgesic tolerance. The addition of NMDA receptor antagonists, for example, appears to delay or inhibit the onset of tolerance to the antinociceptive effects of opioids that emerges over time when they are given alone in animals [126].

5. Clinical Use of Combination Drug Therapy in SCI Pain

The preclinical data suggests that clinically used drugs in combination could be useful in ameliorating SCI pain. Even drugs that do not demonstrate efficacy alone could still be useful if combined with a drug that is known to offer pain relief. While the preclinical data are indeed promising, few clinical studies have been performed and fewer still have demonstrated analgesic synergism on the order of magnitude observed in preclinical studies. Ideally, the demonstration of synergism should be carried out with methodological rigor similar to that performed in preclinical studies, such as generation of dose-effect curves of the constituents alone and a dose-effect curve of the combination, the proportion of the constituents of the combination determined by the individual curves. Also, each drug alone and in combination would be compared with a placebo treatment group [25, 127]. The presence of genuine synergism is further complicated by the fact that few, if any, studies suspend analgesic usage prior to the commencement of the study, such that the supra-additive effect of the drugs under investigation could be due to nonstudy medications. Given the limited number of suitable clinical subjects and ethical reasons, analgesic synergism studies are few and far between. Nonetheless, a few carefully designed studies have demonstrated synergism in the clinical setting. One study demonstrated a synergistic interaction between i.t. morphine and clonidine, an α_2 -adrenoceptor agonist, in SCI patients [128]. Doses of i.t. morphine and clonidine were titrated in each patient until either efficacy or side effects was obtained. A 50% efficacious dose of each drug was calculated, then given as a mixture, which yielded analgesia greater than either drug alone and equally analgesic in SCI patients with either at-level or below-level neuropathic pain.

Other studies evaluated the effect of a second therapeutic as an “add-on,” wherein the second drug is added to an already existing drug treatment. For example, i.t. morphine was evaluated as an add-on in SCI patients who were undergoing treatment with a stabilized dose of i.t. baclofen for pain and spasticity [129]. Although most of the patients who received add-on i.t. morphine tolerated the combination, the average reduction in pain was modest, about 35%. A case

report noted improved pain and spasm relief in a SCI patient with clonidine added to i.t. baclofen [130]. Prior to the addition of clonidine, the patient found it necessary to escalate the dose of baclofen needed for pain relief over time. Two years following the initiation of the combination therapy, no further increase in the dose of baclofen was necessary. Ziconotide has also been used as an add-on to i.t. baclofen (and, alternatively, baclofen as an add-on to i.t. ziconotide) and in combination with i.t. hydromorphone, an opioid [94, 131].

One novel combination evaluated in neuropathic SCI pain patients was i.v. ketamine (given once a day for seven days) as an add-on to oral gabapentin, compared to i.v. saline treatment and oral gabapentin (300 mg TID) [132]. During the week of i.v. treatment, the ketamine add-on group showed markedly lower average pain scores compared to the saline-treated group. Furthermore, the group that received i.v. ketamine continued to show reductions in pain scores for at least two weeks after the last infusion of ketamine. After this period, the pain scores of the ketamine-treated group were similar to that of the saline-treated group. This study also demonstrated two interesting effects of oral gabapentin treatment in SCI patients. First, in both groups, pain scores at the end of the study (five weeks in duration) were reduced to about half that of baseline, pretreatment pain scores. Thus, the study confirmed the persistent analgesia obtained with regular gabapentin treatment in SCI patients [41]. Second, in the i.v. saline-treated group, a significant analgesic effect with oral gabapentin treatment can be discerned on the first day of treatment, and analgesia improved over the course of gabapentin treatment. An acute analgesic effect of oral gabapentin has also been reported in clinical peripheral neuropathic pain, reducing both spontaneous pain and cutaneous hypersensitivity within hours of treatment [133]. The mechanism of the two-week analgesic enhancement following ketamine treatment is unknown. It is possible that other combinations with gabapentin may lead to an enhanced and persistent analgesia.

Dose-dependent effects of the add-on drug or the ongoing therapeutic were not established in these studies. Perhaps the effect of the combinations was derived mainly from the add-on drug rather than the combination per se. Clearly, further studies are needed to determine if these combinations fulfill the definition of synergism and what the optimal drug ratio would be, but concurrent activation of spinal GABA_B receptors with either N-type VGCC block, μ -opioid receptor, or α_2 -adrenoceptor activation could be a potentially therapeutic combination. Currently, the only drugs that are approved by the U.S. Food and Drug Administration for i.t. in humans are ziconotide, morphine, and baclofen, and no recommendation has been issued regarding the mixture of these drugs for intrathecal use [134]. Furthermore, the safety of other unapproved drugs for i.t. use has not been extensively determined.

While it may be relatively straightforward for some SCI patients to take medications orally, other patients may have difficulty swallowing. In the case where i.t. drug delivery may not also be an option, topical drug application may be warranted. At-level neuropathic SCI pain is hypothesized to

result from the sensitization of primary afferent nociceptors due to the trauma that led to SCI [42]. Alternatively, centrally mediated processes resulting from SCI feedback onto central terminals of nociceptors, which in turn leads to nociceptor sensitization [135]. Activation of nociceptors in some SCI patients with topical capsaicin leads to increased hypersensitivity to cutaneous stimulation and a burning painful sensation [136]. This indicates that not only are at-level nociceptors intact, but normal functionality has been significantly altered. Application of lidocaine patches in the painful dermatome reduces spontaneous and evoked pain. Other topical treatments have been tested in peripheral neuropathic pain and perhaps these could be used, either alone or in combination, for at-level neuropathic SCI pain [137–139]. It is not known if topical treatment would be effective on below-level SCI pain. A significant peripheral contribution to SCI pain suggests that targeting nociceptors could be an effective treatment and that the drug (or combination of drugs) does not have to enter the CNS, thereby circumventing the problem of CNS-mediated adverse side effects [74, 140, 141].

The contribution of peripheral nociceptors in neuropathic SCI pain, however, could vary between patients. A clinical report was unable to demonstrate a change of peripheral nociceptor responsiveness to capsaicin treatment, either at, below, or above the lesion, in SCI patients [142]. This result suggests that in some patients, central processes, rather than functional changes in peripheral nociceptors, maintain neuropathic SCI pain. Thus, treatments that focus on attenuating the abnormal neural activity at spinal and supraspinal levels would benefit these patients. Perhaps a topical capsaicin test could be used to assess peripheral nociceptor functionality and based on the result, tailor treatment for that patient. A pressing challenge for health care providers will be to identify the relevant mechanism in each SCI patient such that therapeutic intervention will address those particular mechanism and yield pain relief.

6. Other Possible Combination Treatments

One other instance of synergy demonstrated in animals, which may not have immediate clinical applicability, is injection of a drug at different sites of the neuraxis [143, 144]. Such “autosynergy” suggests an interaction of two or more neural sites are required for the analgesic effect of a given drug and that loss or dysfunction of one site will result in decreased efficacy of that drug. This concept could be applied to the use of electrical stimulators implanted in CNS regions involved in nociceptive processing [145]. Neither deep brain stimulation nor spinal cord (dorsal column) stimulation in SCI patients have demonstrated efficacy on pain, but perhaps the combination of the two, spinal and supraspinal or into distinct but complementary brain nuclei, could lead to robust analgesia [146, 147]. Furthermore, drugs, either systemic or i.t., could also be combined with stimulators, to enhance the effect of the stimulator or vice versa [148, 149]. Thus, the application of synergism may not be limited to pharmacotherapeutics.

7. Conclusion

Combination drug therapy could fulfill current needs in at least two areas. It is foreseen that noteworthy new treatments will emerge in the near future with the increased elucidation of the mechanism that underlies neuropathic SCI pain. However, the discovery process involving novel therapeutic targets is both expensive and highly time consuming, and that the safety of treatments based on those targets will not be clear until the completion of extensive human trials [150]. Until the day when novel treatments are ready for widespread use, patients could be treated with currently available pharmacotherapies with known biological and safety profiles in novel combinations. Soon-to-be-initiated clinical studies will evaluate the suitability of cellular transplants to repair SCI and to promote functional recovery. However, there is the possibility that transplantation will induce sprouting of afferent central terminals, such that SCI patients who have not experienced pain may begin to experience it or that ongoing pain in other patients will worsen [151–154]. Again, combination drug therapy could be used as these patients undergo transplantation treatment.

There are challenges that will need to be addressed with combination drug therapy for SCI pain, similar to the challenges noted for other patient populations, including timing of drug dosing and dose ratio [25]. Currently, the emergence and submergence of particular pain-related mechanisms over time are not well delineated, and it is assumed that many of the processes occur all at once. Aging may alter the temporal aspect of pain mechanisms which could in turn alter responsiveness to therapeutics [155–158]. Perhaps greater efficacy and safety could be obtained if drugs are combined at defined times during the course of treatment. With greater understanding of the temporal aspects of pain mechanisms, irrelevant drugs can be excluded depending on the duration of the pain symptoms. As mentioned earlier, the ratio of constituents in a combination could also change, depending on the prevalence or robustness of a particular mechanism [65, 68]. Thus, greater understanding of post-injury mechanism timing will be needed.

Finally, although animal models have been useful in elaborating the in vivo neurological and biochemical mechanisms of pain, one limitation is the difficulty of obtaining spontaneous or unevoked measures of pain. As pain involves an affective as well as sensory component, the effect of novel analgesics on this component is currently unknown, and may be as therapeutically important as reducing the somatosensory component of pain. It is clear that below-level cutaneous stimulation in rats leads to pain-related behaviors such as vocalization and licking and biting of the stimulated area, indicating a supraspinally mediated component [159, 160]. In fact, such an overlap, between cutaneous hypersensitivity and below-level pain in SCI patients is clinically observed [56]. Given the significant contribution of supraspinal structures, including cortical structures, in pain, models of integrated, “purposeful” behaviors in animal pain models have been proposed [161–163]. There are neuroanatomical and cognitive issues that will need to be addressed in tying complex behavior in nonhuman species to human behavior,

which should be made clear when drawing conclusions from such behavioral models [164–166]. As with other preclinical models, the responses to pharmacological manipulation, to both analgesics and nonanalgesics (as “active placebos”), will need to be elaborated. Testing in alternate species and evaluating spontaneous behavior could also prove highly useful in closing the gap between laboratory proof-of-concept and utilizing discoveries in the clinic [167].

8. Summary

The benefits of combination drug therapy for the treatment of neuropathic SCI pain include potential analgesic synergism, wherein the efficacy of the combination is significantly greater than that of the constituents alone and decreased potential for adverse side effects. Recent advances in the neurosciences have uncovered numerous pain-related molecular targets. However, neuropathic SCI pain remains difficult to treat with available pharmacotherapeutics since they do not specifically address neuropathic SCI pain. Engaging more than one relevant target via combination drug therapy may significantly improve pain management in SCI patients. Further clinical studies will be needed to address key issues such as identifying which target combinations could yield the most robust efficacy and the optimal dose ratio of a given combination drug therapy.

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Research Article

Efficacy and Safety of Duloxetine in Patients with Chronic Low Back Pain Who Used versus Did Not Use Concomitant Nonsteroidal Anti-Inflammatory Drugs or Acetaminophen: A Post Hoc Pooled Analysis of 2 Randomized, Placebo-Controlled Trials

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This subgroup analysis assessed the efficacy of duloxetine in patients with chronic low back pain (CLBP) who did or did not use concomitant nonsteroidal anti-inflammatory drugs (NSAIDs) or acetaminophen (APAP). Data were pooled from two 13-week randomized trials in patients with CLBP who were stratified according to NSAID/APAP use at baseline: duloxetine NSAID/APAP user ($n = 137$), placebo NSAID/APAP user ($n = 82$), duloxetine NSAID/APAP nonuser ($n = 206$), and placebo NSAID/APAP nonuser ($n = 156$). NSAID/APAP users were those patients who took NSAID/APAP for at least 14 days per month during 3 months prior to study entry. An analysis of covariance model that included therapy, study, baseline NSAID/APAP use (yes/no), and therapy-by-NSAID/APAP subgroup interaction was used to assess the efficacy. The treatment-by-NSAID/APAP use interaction was not statistically significant ($P = 0.31$) suggesting no substantial evidence of differential efficacy for duloxetine over placebo on pain reduction or improvement in physical function between concomitant NSAID/APAP users and non-users.

1. Introduction

Low back pain has a lifetime prevalence rate of 80% in the United States and is one of the primary causes of disability in individuals younger than 45 years of age [1, 2]. Low back pain usually resolves spontaneously within a few days or weeks, but for some individuals, this pain becomes chronic [1]. Commonly prescribed medications for chronic low back pain (CLBP) include nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, muscle relaxants, anticonvulsants, and tricyclic antidepressants (TCAs) [3]. Over-the-counter medications that are frequently used include acetaminophen (APAP), aspirin, and certain NSAIDs [4]. However, there is no clinical evidence to support the efficacy of any of these agents in CLBP [4, 5]. Furthermore, a number of these treatments pose safety risks that include sedation, respiratory depression and addiction (opioids), gastrointestinal bleeding

and ulcers, and cardiovascular events (NSAIDs) [6]. In addition, antidepressants with serotonin reuptake inhibition properties may increase the risk of bleeding events [7, 8], either when taken alone or in combination with other drugs that affect coagulation, such as NSAIDs [9].

Duloxetine hydrochloride (hereafter referred to as duloxetine) is a potent serotonin and norepinephrine reuptake inhibitor (SNRI) that has been approved by the United States Food and Drug Administration for the management of diabetic peripheral neuropathic pain, fibromyalgia, and chronic musculoskeletal pain (as established in studies in CLBP and chronic pain due to osteoarthritis). It has also been approved for the treatment of major depressive disorder (MDD) and generalized anxiety disorder (GAD) [10].

In two 13-week trials of duloxetine versus placebo in patients with CLBP, one trial [11] reported significantly greater pain reduction with duloxetine treatment at endpoint;

whereas the other reported significant separation from placebo at weeks 3–11, but superiority was not demonstrated at endpoint [12]. Because these trials allowed concomitant use of NSAIDs or APAP if patients used these analgesics regularly prior to study entry, subgroup analyses were conducted to assess whether or not concomitant use of the allowed analgesics had an effect on the efficacy of duloxetine. The results of the subgroup analyses were not significant for either trial, but were limited by sample size. To increase the statistical power and to better understand the advantage of duloxetine over placebo between the groups of patients who concomitantly used these analgesics and those who did not, we conducted a post hoc analysis of data pooled from these two studies. The safety of duloxetine with concomitant use of these analgesics was also evaluated.

2. Materials and Methods

This was a post hoc analysis of data pooled from two 13-week, multicenter, randomized, double-blind trials of the efficacy of duloxetine (doses of 60 QD and 120 QD were pooled for this analysis) compared with placebo on the reduction of average pain severity, improvement in physical function, and in patient global impression of improvement [11, 12]. Both studies were compliant with International Conference on Harmonization guidelines on good clinical practices, and each protocol was approved by the Ethical Review Board for each site. All patients provided written informed consent before beginning any study procedures.

Patients included in these studies were outpatients who were at least 18 years of age with a clinical diagnosis of CLBP; with pain restricted to the lower back (Class 1) or associated with radiation to the proximal portion of the lower limb only (Class 2) according to the Quebec Task Force (QTF) on Spinal Disorders [13]; with pain present on most days for ≥ 6 months, and weekly average pain severity ratings ≥ 4 (on a 0–10 numerical scale) during the week prior to randomization. Exclusion criteria included clinical or radiographic evidence of radicular compression or spinal stenosis, presence of spondylolisthesis grade 3–4, history of ≥ 1 low-back surgery, any low-back surgery within 12 months, or invasive procedures to reduce low-back pain within 1 month. Patients with MDD, body mass index >40 , or seeking disability compensation related to back pain were excluded.

At baseline, patients were stratified according to concomitant NSAID and/or APAP use status prior to study entry and were randomized to duloxetine and placebo within each stratum. Users were defined as those patients answered “yes” to a question soliciting whether or not they were taking a therapeutic dose of NSAID and/or APAP for ≥ 14 days per month for 3 months immediately preceding the study. Because the use of these analgesics was recorded as a global “yes” response, this stratum was referred to as the NSAID/APAP user group. Patients who were NSAID/APAP users were allowed to continue with their stable regimen of NSAID/APAP throughout the entire study.

For this analysis, efficacy measures included the Brief Pain Inventory (BPI) [14] 24-hour average pain severity item

(referred to hereafter as BPI average pain severity) (range, 0 = no pain to 10 = pain as bad as you can imagine); the Roland-Morris Disability Questionnaire (RMDQ-24) [15] (scale range, 0 = no disability to 24 = severe disability); the Patient Global Impression of Improvement (PGI-I) [16]. The PGI-I is a scale on which patients provide ratings of their overall impression of how they are feeling since treatment began with the following range of choices from 1 = very much better to 4 = no change to 7 = very much worse. Response to treatment was defined as at least a 50% decrease from baseline in BPI average pain severity.

To assess and compare the efficacy of duloxetine over placebo between NSAID/APAP use subgroups, we utilized an analysis of covariance (ANCOVA) model to estimate least-squares mean changes from baseline to endpoint in BPI average pain severity ratings and RMDQ-24 scores. The ANCOVA model included a fixed continuous covariate of baseline value, fixed categorical effects of therapy (duloxetine or placebo), study, NSAID/APAP use (yes/no), and therapy-by-NSAID/APAP subgroup interaction. Response at endpoint was also analyzed using a logistic regression model with terms for therapy, study, NSAID/APAP use (yes/no), and therapy-by-NSAID/APAP subgroup interaction. Statistically significant difference in duloxetine efficacy between subgroups for reduction in BPI average pain severity, improvement in RMDQ-24 scores, and BPI pain response was determined by a therapy-by-NSAID/APAP subgroup interaction that was $P < .1$. The number and proportion of patients who reached a PGI-I rating of 1 or 2 at endpoint were summarized by treatment and by NSAID/APAP use subgroup. Subsequent treatment odds ratios were calculated for each subgroup, then compared between subgroups with the Breslow-Day test, and statistical significance was noted at $P < .1$. For patients with missing outcomes (due to early dropout), the last nonmissing observation was treated as their endpoint value in the analyses.

Safety assessments included discontinuation due to adverse events (AEs), the most common treatment-emergent adverse events (TEAEs), and those possibly related to NSAID/APAP use (bleeding and cardiovascular events). Incidence rates were compared between treatment groups using Cochran-Mantel-Haenszel test controlling for study, and statistical significance was noted at $P < .05$.

3. Results

3.1. Patient Disposition. There was no significant between-treatment difference in rates of study completion in either NSAID/APAP use subgroup (Table 1). There was a higher percentage of duloxetine-treated patients versus placebo, who discontinued due to AEs in the NSAID/APAP nonuser subgroup ($P = .002$). For any of the other reasons leading to discontinuation, there were no significant between-treatment differences in either NSAID/APAP use subgroup.

3.2. Demographics and Clinical Characteristics. Patients were stratified according to NSAID/APAP use at baseline: duloxetine NSAID/APAP user ($n = 137$), placebo NSAID/APAP

TABLE 1: Patient disposition.

	NSAID/APAP user			NSAID/APAP nonuser		
	Duloxetine N = 137 n (%)	Placebo N = 82 n (%)	P value	Duloxetine N = 206 n (%)	Placebo N = 156 n (%)	P value
Completed study	90 (65.7)	63 (76.8)	.10	136 (66.0)	117 (75.0)	.08
Reason for discontinuation:						
Adverse event	21 (15.3)	5 (6.1)	.051	39 (18.9)	12 (7.7)	.002
Lack of efficacy	7 (5.1)	4 (4.9)	1.00	5 (2.4)	7 (4.5)	.38
Lost to follow up	5 (3.6)	0	.16	7 (3.4)	3 (1.9)	.53
Protocol violation	5 (3.6)	4 (4.9)	.73	5 (2.4)	2 (1.3)	.70

Abbreviation: APAP, acetaminophen; NSAID, nonsteroidal anti-inflammatory drug.

TABLE 2: Baseline characteristics.

	NSAID/APAP user		NSAID/APAP nonuser	
	Duloxetine N = 137	Placebo N = 82	Duloxetine N = 206	Placebo N = 156
Age in years, mean (SD)	53.1 (14.7)	51.4 (13.3)	53.5 (14.9)	53.1 (13.6)
Female, n (%)	89 (65.0)	52 (63.4)	114 (55.3)	85 (54.5)
Ethnicity, n (%)				
African American	3 (2.2)	3 (3.7)	19 (9.2)	13 (8.3)
Caucasian	108 (78.8)	61 (74.4)	160 (77.7)	123 (78.9)
East Asian	0	0	2 (1.0)	3 (1.9)
Hispanic	25 (18.3)	17 (20.7)	22 (10.7)	15 (9.6)
Native American	0	0	1 (0.5)	2 (1.3)
West Asian	1 (0.7)	1 (1.2)	2 (1.0)	0
CLBP duration since onset, years, mean (SD)	11.4 (11.5)	9.2 (9.1)	10.8 (11.1)	10.3 (9.0)
QT F class 1, n (%)	94 (72.9)	63 (79.8)	150 (75.4)	99 (68.3)
BPI average pain, mean (SD)	6.1 (1.6)	6.0 (1.6)	5.9 (1.6)	6.1 (1.7)
RMDQ-24, mean (SD)	9.6 (5.0)	8.6 (4.8)	9.1 (4.5)	9.8 (5.3)

There was a statistically significant difference in RMDQ-24 scores between treatments in the nonuser subgroup, but this difference was not considered clinically significant.

Abbreviations: APAP, acetaminophen; BPI, Brief Pain Inventory; CLBP, chronic low back pain; NSAID, nonsteroidal anti-inflammatory drugs; QTF, Quebec Task Force; RMDQ-24, Roland-Morris Disability Questionnaire, SD, standard deviation.

user ($n = 82$), duloxetine NSAID/APAP nonuser ($n = 206$), and placebo NSAID/APAP nonuser ($n = 156$). Patient demographics and clinical characteristics are summarized in Table 2. Most of the patients were female, Caucasian, and in their early fifties. Most had pain restricted to lower back (Class 1 per QFT on spinal disorders), with an average duration of CLBP since onset of at least 9 years. At baseline, the mean BPI average pain severity rating was 6, and the mean RMDQ-24 rating for physical function was about 9.5.

4. Efficacy

Mean changes from baseline in efficacy measures are shown in Figures 1 and 2. Treatment-by-NSAID/APAP use subgroup interactions were not significant for reduction in BPI average pain severity ($P = .31$, Figure 1), or for improvement in physical function assessed by the RMDQ-24 ($P = .35$,

Figure 2). These results suggest that there was no substantial evidence of differential duloxetine efficacy on pain reduction or improvement in physical function between concomitant NSAID/APAP users and nonusers. The frequency of PGI-I responses that were “much better” or “very much better” (PGI-I endpoint score ≤ 2) is presented in Figure 3. In both NSAID/APAP use subgroups, a higher percentage of duloxetine-treated patients achieved PGI-I ≤ 2 at endpoint, but the treatment odds ratios were not significantly different between the two subgroups ($P = 0.32$). Therefore, significant differential treatment effects of duloxetine on PGI-I were not observed between concomitant NSAID/APAP users and nonusers.

The criterion for achieving a pain response was met by a higher percentage of duloxetine-treated patients in both NSAID/APAP use subgroups (46.2% of users, and 43.6% of nonusers) than patients treated with placebo (38.0% of users,

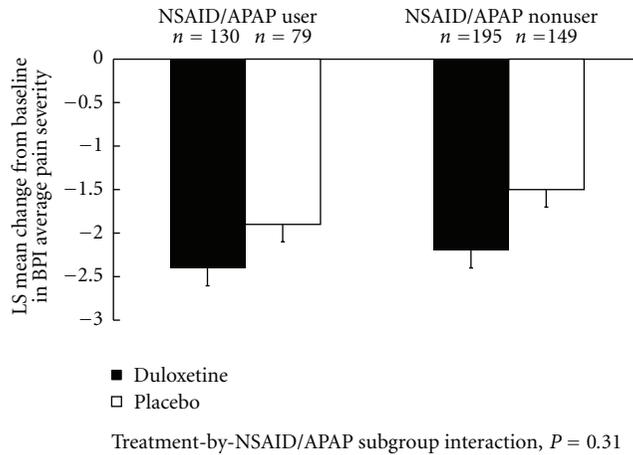


FIGURE 1: Estimated least-squares (LS) mean changes from baseline and standard errors in BPI average pain severity in patients who concomitantly used or did not use NSAID or APAP.

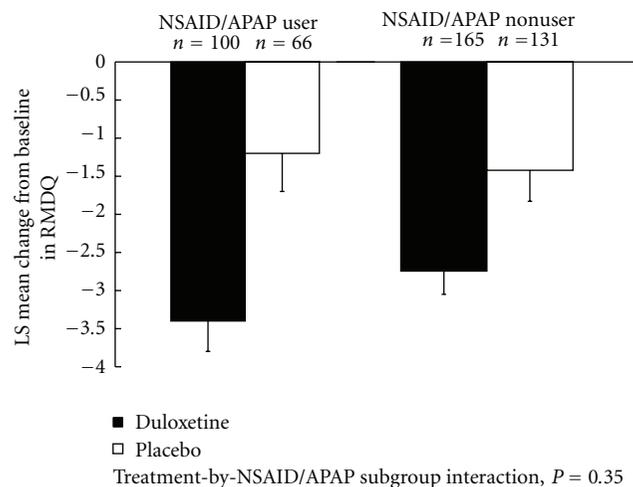


FIGURE 2: Estimated least-squares (LS) mean changes from baseline and standard errors in RMDQ rating in patients who concomitantly used or did not use NSAID or APAP.

and 27.5% of nonusers). The treatment-by-NSAID/APAP use subgroup interaction was not significant ($P = 0.28$), which suggests that the duloxetine treatment effects on achieving a pain response were not statistically significantly different between concomitant NSAID/APAP users and nonusers.

5. Safety

The most common AEs that lead to discontinuation in the NSAID/APAP use subgroup in duloxetine-treated patients were erectile dysfunction and nausea (both events, $n = 2$, 1.5%); events in the nonuser subgroup included nausea, insomnia and somnolence (each event, $n = 3$, 1.5%).

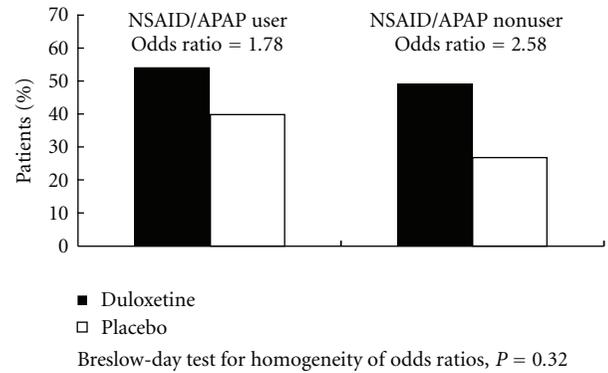


FIGURE 3: Percentage of patients who felt "much better" or "very much better" at endpoint.

TEAEs within NSAID/APAP use subgroups that occurred at a rate of at least 5% with duloxetine treatment and were significantly more frequent than with placebo are summarized in Table 3. Among NSAID/APAP users, the frequency of nausea, dry mouth, constipation, somnolence, and fatigue were significantly greater in patients who received duloxetine versus placebo. In addition to these TEAEs, insomnia, and dizziness were significantly more frequent in patients who received duloxetine versus placebo among the NSAID/APAP nonusers. Cardiovascular and bleeding-related TEAEs are summarized in Table 4. In either NSAID/APAP use subgroup, between-treatment differences in the frequency of these events were not significant.

6. Discussion

There are few published CLBP studies with nonopioid analgesics that allowed concomitant NSAID/APAP use. Two studies investigated the efficacy of TCAs for pain reduction in patients who were allowed to continue taking NSAIDs. One of those two evaluated nortriptyline against placebo [17] and the other compared maprotiline with paroxetine [18], but neither study reported efficacy outcome comparisons between NSAID/APAP users and nonusers. Another CLBP study examined the efficacy of pregabalin combined with celecoxib, and the results suggested that combination treatment was more efficacious than treatment with either medication alone [19].

The post hoc analysis reported here included two clinical trials of duloxetine that allowed concomitant NSAID/APAP for those patients who regularly used them prior to study entry. The use of additional analgesics in a pain trial is associated with the risk of reduced assay sensitivity, and possibly a high placebo response [17]. This was observed in one of the two duloxetine CLBP trials [12] and in the NSAID/APAP use subgroup in this analysis. However, the treatment-by-NSAID/APAP use subgroup interaction was not significant in the analyses of various efficacy measures, which suggests that the advantages of duloxetine over placebo in pain reduction and improvement in function were not significantly different between subgroups.

TABLE 3: Treatment-emergent adverse events within either NSAID/APAP use subgroups that occurred at a rate of at least 5% with duloxetine treatment and were significantly more frequent than with placebo.

	NSAID/APAP User		P value	NSAID/APAP Nonuser		P value
	Duloxetine N = 137 n (%)	Placebo N = 82 n (%)		Duloxetine N = 206 n (%)	Placebo N = 156 n (%)	
At least 1 adverse event	92 (67.2)	51 (62.2)	.850	141 (68.5)	77 (49.4)	<.001
Nausea	25 (18.3)	3 (3.7)	.006	26 (12.6)	4 (2.6)	<.001
Dry mouth	14 (10.2)	1 (1.2)	.018	21 (10.2)	4 (2.6)	.004
Constipation	13 (9.5)	1 (1.2)	.041	17 (8.3)	1 (0.6)	.001
Somnolence	13 (9.5)	0	.006	12 (5.8)	1 (0.6)	.012
Fatigue	10 (7.3)	0	.012	15 (7.3)	1 (0.6)	.003
Insomnia	13 (9.5)	4 (4.9)	.346	23 (11.2)	4 (2.6)	.004
Dizziness	10 (7.3)	2 (2.4)	.197	15 (7.3)	3 (1.9)	.023

P values from Cochran-Mantel-Haenszel test.

Abbreviation: APAP, acetaminophen; NSAID, nonsteroidal anti-inflammatory drugs.

TABLE 4: Bleeding-related or cardiac-related treatment-emergent adverse events.

	NSAID/APAP user		P value	NSAID/APAP nonuser		P value
	Duloxetine N = 137 n (%)	Placebo N = 82 n (%)		Duloxetine N = 206 n (%)	Placebo N = 156 n (%)	
<i>Bleeding-related</i>						
Tendency to bruise	1 (0.7)	0	.52	0	0	—
Eye hemorrhage	0	0	—	1 (0.5)	1 (0.6)	.70
Hemorrhagic cyst	0	0	—	1 (0.5)	0	.31
Rectal hemorrhage	0	0	—	1 (0.5)	0	.44
<i>Cardiac-related</i>						
Palpitations	5 (3.7)	0	.14	1 (0.5)	1 (0.6)	.86
Hypertension	3 (2.2)	1 (1.2)	.84	4 (1.9)	2 (1.3)	.76
Myocardial infarction	1 (0.7)	0	.52	0	1 (0.6)	.32
Tachycardia	1 (0.7)	0	.29	2 (1.0)	0	.15
Transient ischemic attack	1 (0.7)	0	.52	1 (0.5)	0	.31
Heart rate increased	0	0	—	1 (0.5)	0	.44
Hypertensive crisis	0	0	—	1 (0.5)	0	.44
Carotid artery stenosis	1 (0.7)	0	.52	0	0	—

Abbreviation: APAP, acetaminophen; NSAID, nonsteroidal anti-inflammatory drugs.

The safety profile with regards to TEAEs in either NSAID/APAP use subgroup did not differ from those reported previously in duloxetine trials. Although the occurrence of bleeding-related and cardiac-related events noted in this post hoc analysis was low in both NSAID/APAP use group, caution is warranted for concomitant use of NSAID/APAP with duloxetine. This precautionary statement is based upon observations that medications that act to inhibit serotonin reuptake may be associated with an increased risk of bleeding events [9], and the use of these drugs

in combination with medications that affect coagulation, including NSAIDs, may increase this risk.

This study is limited by the lack of complete information regarding dosing and frequency of concomitant NSAID or APAP use. In addition, any NSAID or APAP use less than 14 days/month would have classified patients as nonusers, which also included patients that did not use these analgesics at all, and patients who used them sporadically. In addition, users were identified at baseline by responding “yes” to a questionnaire regarding the use of either NSAIDs or APAP,

instead of regarding the use of one or the other or both, so this lack of information limited further analysis. Also, the studies included in these analyses were not powered to detect differential treatment effect between NSAID/APAP use subgroups. Therefore, the comparisons between NSAID/APAP use subgroups in this study should be viewed in that light. In addition, the sample size and the short duration of the studies also limited the occurrence and detection of rare bleeding events. Finally, these studies excluded individuals with certain comorbidities, so these results may not extend to all individuals in the general population who present with CLBP.

7. Conclusions

In this post hoc analysis of data pooled from two studies in patients with CLBP, there were no statistically significant differences in the treatment advantage of duloxetine over placebo on measures of pain reduction, improved physical function, or patient global impression of improvement observed between concomitant NSAID/APAP users and nonusers. In other words, concomitant use of an NSAID or APAP did not significantly enhance or interfere with the efficacy of duloxetine. The safety of duloxetine with concomitant NSAID/APAP use was consistent with the known duloxetine safety and tolerability profile.

Conflict of Interests

All authors are employees of Eli Lilly and Company.

Acknowledgments

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Clinical Study

Effect of Diclofenac with B Vitamins on the Treatment of Acute Pain Originated by Lower-Limb Fracture and Surgery

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The aim of this study was to compare the efficacy of diclofenac, for the treatment of acute pain originated by lower-limb fracture and surgery, with that of diclofenac plus B vitamins. This was a single-center, prospective, randomized, and double-blinded clinical trial. Patients with lower-limb closed fractures rated their pain on a 10 cm visual analog scale (VAS). Patients were then randomized to receive diclofenac or diclofenac plus B vitamins (thiamine, pyridoxine, and cyanocobalamin) intramuscularly twice daily. Patient evaluations of pain intensity were recorded throughout two periods: twenty-four hours presurgery and twenty-four hours postsurgical. One hundred twenty-two patients completed the study. The subjects' assessments of limb pain on the VAS showed a significant reduction from baseline values regardless of the treatment group. Diclofenac plus B vitamins combination was more effective to reduce the pain than diclofenac alone. The results showed that the addition of B vitamins to diclofenac increased its analgesic effect. The novelty of this paper consists in that diclofenac and diclofenac plus B vitamins were useful for treatment of acute pain originated by lower-limb fracture and surgery.

1. Introduction

A number of situations are prone to develop pain symptomatology, such as tissue degeneration, infection, inflammation, cancer, trauma, surgery, and limb fractures. Each of these physiological abnormalities requires a therapeutic approach different from the last. In acute pain, caused by fracture and/or surgery, several classes of analgesics have been utilized. These basic remedies for analgesia, however, are still confined to a small number of medications, including nonsteroidal anti-inflammatory drugs (NSAIDs), local anesthetics, and opioids. In addition, most of these drugs have side effects, limiting their use in clinical practice [1, 2].

The clinical use of combinations of analgesic agents has increased significantly in the last few years. The purpose is

to associate two or more drugs with different mechanisms of action, in hopes of achieving a synergistic interaction that yields a sufficient analgesic effect with low doses of each agent, therefore, reducing the intensity and incidence of untoward effects [3].

B vitamins are a water-soluble group of vitamins including thiamine, riboflavin, niacin and niacinamide, pyridoxine, cobalamin, folic acid, pantothenic acid, biotin, choline, inositol, and para-aminobenzoic acid (PABA). In particular, some of these B vitamins (thiamine, pyridoxine and cyanocobalamin) have been used, not only in the treatment of pain and inflammation resulting from vitamin deficiency but also alone or in combination with diclofenac or other NSAIDs for various painful diseases such as polyneuropathies, degenerative diseases of the spinal column,

rheumatic diseases lumbago and pain originated from tonsillectomy [4–8]. However, most clinical studies have evaluated this combination in neuropathic pain [4–6, 8]. Recently, a study demonstrated the utility of the diclofenac-B vitamins combination in the pain originating from tonsillectomy surgery [7]. Nevertheless, the sample size of this last study was too small to be representative and the administration route was the intravenous via. On the other hand, this diclofenac-B vitamins combination has never been tested in the acute pain produced by fracture or other kind of surgical procedures. Therefore, the main objective of the present clinical study was to compare the efficacy and tolerability of diclofenac for treatment of acute pain following lower-limb fracture and surgery, with that of diclofenac in combination with B vitamins (thiamine, pyridoxine, and cyanocobalamin). Indeed, we previously conducted a pilot study with 14 patients, with the aim of establishing the adequate experimental conditions as well as to calculate the appropriate sample size, wherein both treatments were equally effective in reducing pain [9].

2. Materials and Methods

This was a single-center, prospective, randomized and double-blinded clinical trial. The study was carried out at the Hospital General SSH Pachuca, Hidalgo, Mexico from January 2008 to February 2010. The study protocol was approved by the Ethic and Investigation Committees from the Hospital General SSH Pachuca, Hidalgo, Mexico as well as this was conducted according to the Declaration of Helsinki.

The participants of the study were patients with lower-limb closed fractures, ranging in age from 18 to 55 years, with acute pain ≥ 5 cm according to a 10 cm visual analog scale (VAS; 0 = no pain and 10 = the worst pain), good health determined by clinical history and laboratory studies, without sanguineous dyscrasias or hypersensitivity to drugs to be employed, and who consented to participate voluntarily.

After giving their consent, patients rated their pain on a VAS, and they were then randomized into one of two groups receiving 75 mg diclofenac or 75 mg diclofenac plus B vitamins (thiamine: 100 mg, pyridoxine: 100 mg, and cyanocobalamin: 1 mg) twice daily (all intramuscularly). Patient evaluations of pain intensity were recorded throughout two periods: twenty-four hours presurgical and twenty-four hours postsurgical. Twenty-four hours after the first drug administration, patients underwent elective lower-limb surgery. Standardized general anesthetic techniques were used for all patients. Patients received 50 mg ranitidine intravenously twice a day throughout the study. If the pain was not controlled after two hours, patients received rescue treatment with morphine. At the end of the study, the improvement in pain levels was evaluated by a Likert scale. The categorical Likert response alternatives consisted of four descriptions. Responses were rated 0–3: 0 = complete relief, 1 = moderate relief, 2 = slight relief, 3 = without relief. Gastrointestinal side effects, rash, or spontaneous complaints of other adverse effects such as postsurgical bleeding problems during the postoperative phase were registered.

Data are shown as the mean \pm SEM. Data were evaluated using *t* student and a nonparametric statistical analysis, the Mann-Whitney *U* test. $P < 0.05$ was required for significance.

3. Results

One hundred twenty-two patients completed the study, sixty-two in the diclofenac group (forty-two male and twenty female) and sixty in the diclofenac with B vitamins group (twenty-seven male and thirty-three female). The mean \pm standard deviation age in the diclofenac group was 37.9 ± 10.5 years and 35.0 ± 8.8 years in the diclofenac plus B vitamins group, which does not represent a significant difference between the groups ($P > 0.05$).

In the study presented here, all patients received medication for forty-eight hours and the acute pain induced by lower-limb fracture and surgery was monitored and recorded. The lower-limb fractures that the patients presented with were 8 fractures of the patella, 47 ankle fractures, 24 tibia shaft fractures, 14 tibial plateau fractures, 20 diaphyseal fractures of the femur, 6 subtrochanteric femoral fractures, 2 fractures of the calcaneus, and 1 fracture of the talus. There was no statistically significant difference in the type of fracture and gender between the two treatment groups ($P > 0.05$).

The subjects' assessment of limb pain on the VAS in both treatments showed a significant reduction from baseline at 4, 8, 12, 24, 36, and 48 hours after treatment. Diclofenac plus B vitamins was more effective to reduce the pain than diclofenac alone at 8, 12, 24, 36, and 48 hours after treatment ($P < 0.05$) (Figure 1). However, diclofenac was more successful to decrease the pain than diclofenac plus B vitamins at 4 hours after treatment ($P < 0.05$) (Figure 1). The value in the Likert scale of the diclofenac plus B vitamins group was 1.37 ± 0.5 with this value being statistically different at the value of 1.56 ± 0.5 for the diclofenac group. No statistical difference was found when comparing the groups according to gender and type of fracture ($P > 0.05$).

Rescue treatments were not applied. All the patients reported pain in the administration site, but generally speaking, all the regimens were well tolerated. None of the patients had any bleeding complication or gastrointestinal complain before or after surgery.

4. Discussion

Fractures of the lower limb are common, especially in the elderly, and are often associated with considerable morbidity and lengthy hospitalization. The immediate goal of treating acute lower-limb fractures is to decrease pain and swelling as well as to protect adjacent structures from further injury. For this reason, after immobilization of the lower limb, NSAIDs are invaluable in treating these musculoskeletal conditions, primarily due to their analgesic and anti-inflammatory effects. Unfortunately, NSAIDs propensity to cause gastrointestinal damage and patient discomfort limits their use. It is known that as many as two to four percent of patients who take NSAIDs during long-term therapy may have serious

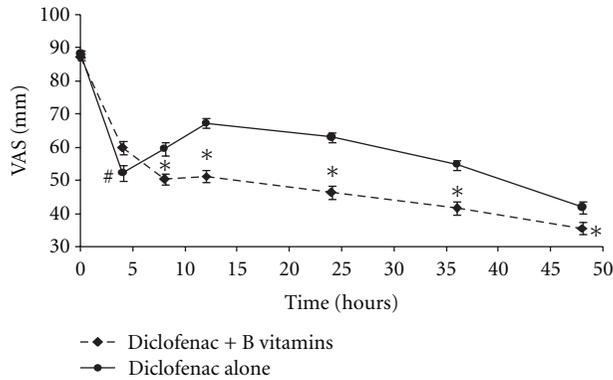


FIGURE 1: Time course of the effect of diclofenac alone or diclofenac plus B vitamins for the treatment of pain produced by lower-limb fracture and surgery. The points represent the average \pm standard error of the media values of the visual analogous scale (VAS) evaluated at different hours. *Significantly different from diclofenac group ($P < 0.05$). #Significantly different from diclofenac + B vitamins group ($P < 0.05$).

gastrointestinal side effects such as perforation, ulceration, or bleeding [10, 11]. An effective strategy to decrease the occurrence of these adverse effects is to combine an NSAID with two or more analgesics, each one with different mechanisms of action. The synergistic outcome achieved yields a sufficient analgesic effect with relatively low doses, reducing the intensity and incidence of untoward effects accordingly [3]. In this regard, it has been demonstrated that the combination of diclofenac and B vitamins is effective in relieving neuropathic pain [4, 5, 8]. Likewise, it is possible to reduce the diclofenac dosage and/or the duration of the treatment [4–6, 8]. A recent study showed that diclofenac plus B vitamins were not superior to diclofenac alone in the treatment of pain originated from tonsillectomy [7]. However, in this same study, the total dose of diclofenac was 45% less in the diclofenac plus B vitamins group suggesting a potential benefit to this approach to analgesic therapy. In a more recent report, it was demonstrated that after 3 days of treatment, a statistically significant higher proportion of subjects with lumbago who received diclofenac plus B vitamins completed the study due to the treatment success compared with patients that received diclofenac alone [8]. Furthermore, the combination therapy yielded superior results in pain reduction, improvement of mobility and functionality [8].

In the present study, both diclofenac and diclofenac plus B vitamins were able to produce an analgesic effect in patients with acute pain originating from lower-limb fracture and surgery. Likewise, at certain points of time the diclofenac-B vitamins combination provided a significantly greater analgesia than the single-agent diclofenac. In this last case, B vitamins potentiated the analgesia produced by diclofenac. There is evidences that B vitamins in their separate forms (B1, B6, and B12) have antinociceptive or analgesic effects. In this regard, in animal experiments, thiamine (B1 vitamin) was able to produce antinociception in the model of pain induced by acetic acid in mice, in the second phase of the formalin test in mice and in the model of pain induced by

electrical stimulation of afferent fibers [12, 13]. Clinical studies showed that administration of a derivative of thiamine, benfotiamine, caused a significant improvement in patients with alcoholic polyneuropathy and in patients with diabetic neuropathy [14, 15]. On the other hand, pyridoxine (B6 vitamin) produced antinociception during the formalin test in mice and in the model of acetic acid in mice [12]. Clinical assays revealed that administration of pyridoxine improves symptoms that occur in the carpal tunnel syndrome and peripheral polyneuropathy [16, 17]. Finally, a clinical study demonstrated that systemic administration of cyanocobalamin (vitamin B12) to patients with low back pain was able to produce a significant decrease in pain and also decreased the consumption of acetaminophen as adjuvant therapy [18]. However, with the present experimental design it is impossible to know which B vitamin is the responsible of the better analgesia.

The increased analgesia with the diclofenac-B vitamins combination differs from the similar analgesic effects observed with diclofenac alone and diclofenac plus B vitamins in the treatment of acute postoperative pain after tonsillectomy [7]; probably, such a difference was due to the different kind of acute pain and the dissimilar administration pathway, since we used the intramuscular administration, while in that study the authors used intravenous infusion over a 12 h period [7]. To our knowledge, in our country there is no the pharmaceutical form of B vitamins for intravenous administration; there are only presentations for oral and intramuscular administration. A dosage form for intravenous administration would be very useful in cases where it cannot be used orally (e.g., in unconscious patients) or intramuscularly.

Diclofenac is an NSAID that exhibits potent analgesic and anti-inflammatory properties. It is known that diclofenac as well as other nonselective NSAIDs are able to impair prostaglandin synthesis by inhibiting the cyclooxygenase isozymes COX-1 and COX-2 in injured tissues and in the central nervous system. However, there is also evidence that diclofenac exhibits additional prostaglandin-independent properties that mediate its antinociceptive effects. For instance, diclofenac is able to inhibit H^+ -gated channels in sensory neurons, increase the concentration of kynurenate (an endogenous antagonist of NMDA receptors), stimulate the nitric oxide-cGMP- K^+ channels pathway, and activate metformin- and phenformin-dependent mechanisms to induce antinociception [19–23]. On the other hand, several studies demonstrated antinociceptive and antihyperalgesic effects with the mixture of thiamine, pyridoxine, and cyanocobalamin in the models of hyperalgesia induced by carrageenan, in the pressure testing of the tail, and in the formalin model [24, 25]. Regarding the action mechanisms by which B vitamins produce their effects, it has been suggested that these result from the activation of several systems. For example, pyridoxine alone or in combination with thiamine and cyanocobalamin was able to increase the synthesis and secretion of serotonin in various brain regions [26, 27]. Furthermore, the analgesic effects of B vitamins have been associated with an increase in inhibitory control of afferent nociceptive neurons in the spinal cord [28] and reduced the response of thalamic neurons to nociceptive stimulation [29]. More recently,

it was confirmed that the analgesic effects induced by B vitamins were partially blocked by naloxone, suggesting that B vitamins could release endogenous opioids that could activate opioid receptors [25]. Besides, there is experimental evidence suggesting that the effects induced by the combination of B vitamins involve the nitric oxide-cGMP system [30, 31]. However, other mechanisms have been proposed, for example, it has been shown that pyridoxine is capable of blocking the synthesis of prostaglandin E2 in humans [32]. In light of this evidence, it is possible to suggest that several mechanisms could be implicated in the diclofenac-B vitamins combination to obtain a major analgesia in comparison with the analgesia by diclofenac alone. The real mechanisms involved in the potentiation for the combination await future elucidation.

Patients often experience unpredictable therapeutic and diagnostic procedural-related pain in emergency rooms that can be associated with considerable stress and anxiety [33]. Although procedural pain may be reduced by a variety of psychological and pharmacological interventions [33], most of these are not given in all the nonelective settings. For example, in our study, after the intramuscular injection all the patients reported pain at the administration site; however, the characteristics of this pain were not evaluated or recorded.

One weakness of our study was that participants had different types of lower-limb fractures and surgeries, and this diversity could have affected the results in favor of providing a better analgesic effect with the combination of diclofenac with B vitamins. However, it was noted that although the patients had different types of fractures, there was no statistically significant difference in the level of pain that both groups of patients presented with on their admission. Therefore, it is necessary to evaluate the effectiveness of this combination in a particular type of fracture or surgery, either member.

In conclusion, the present study gives evidence that the combination of diclofenac plus B vitamins could be a safe and inexpensive postsurgical analgesic strategy. Likewise, it is necessary to undertake controlled studies using this combination in different states of acute postsurgical pain to demonstrate its security and efficacy.

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