Controlled Animal Study

The Effects of Dexmedetomidine Alone and in Combination with Tramadol or Amitriptyline in a Neuropathic Pain Model

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Background: Interactions between the sympathetic and somatic nervous system play an essential role in the pathophysiologic mechanisms of neuropathic pain. The α2-adrenoceptor agonists produce effective antinociception, but sedation is an important adverse effect. Multidrug therapy is potentially valuable to decrease side effects.

Objective: The aim of the present study was to investigate the possible antinociceptive effect of dexmedetomidine, an α2-adrenoceptor agonist, and its combination with front-line treatment of neuropathic pain, i.e., amitriptyline or tramadol, in a chronic constriction injury (CCI) model of the sciatic nerve in rats.

Study Design: Controlled animal study.

Methods: Following unilateral ligation of the left sciatic nerve, the effect of intraperitoneal (i.p.) dexmedetomidine (5µg/kg), tramadol (5 mg/kg), and amitriptyline (30 mg/kg) on mechanical allodynia (measured by electrical von Frey apparatus) and hyperalgesia (measured by Randall and Selitto test) was studied.

Results: The sham-operated rats and un-operated hind paw (right paw) press normally on the floor reproduced by a weighted pain score of 0. Behavioral and mechanical tests confirmed the development of neuropathic pain after CCI. All individual drugs and dexmedetomidine combination with either tramadol or amitriptyline were effective in reducing mechanical allodynia and hyperalgesia. Dexmedetomidine, amitriptyline, tramadol, amitriptyline+dexmedetomidine, and tramadol+dexmedetomidine combination did not produce any sedation/motor impairment ($P > 0.05$).

Limitations: Although the combination of these drugs improved the CCI model of neuropathic pain in this study, an additional interpretation of the underlying mechanism(s) will be needed to confirm these findings.

Conclusion: The combination of these drugs appears to be more effective in increasing the pain threshold after peripheral nerve injury, when compared with the administration of either of amitriptyline or tramadol alone and should be considered as a possible alternative to decrease side effects of individual drug therapy.

Key words: Allodynia, amitriptyline, chronic constriction injury, dexmedetomidine, hyperalgesia, neuropathic pain, rat, tramadol

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Neuropathic pain, "recently defined as pain arises from a direct consequence of a lesion or disease affecting the somatosensory system" (1), is responsible for chronic pain in up to 8% of the general population (2). Many disorders including diabetic polyneuropathy, radicular back pain, postherpetic neuralgia, stroke, and spinal cord injury cause neuropathic pain by producing lesions in somatosensory pathways in the peripheral or central nervous system (3-5). There are several important differences between neuropathic pain and nonpathological pain as reviewed in Woolf et al (6). First, neuropathic pain is persistent. Second, neuropathic pain results in enhanced response to noxious stimuli, thermal and mechanical hyperalgesia, and a pain response to non-noxious stimuli, known as allodynia. Finally, neuropathic pain is characterized by spontaneous pain which is the phenomenon of stimulus-independent pain which takes place in the apparent absence of any stimuli.

Experimentally, various in vivo models of peripheral neuropathic pain have been developed: chronic constriction injury (CCI) of the sciatic nerve with loose ligatures (7-9), partial sciatic nerve injury with tight ligatures (10), total sciatic nerve ligation (11-13), sciatic nerve transaction (14), axotomy of lumbar roots entering the sciatic nerve (15), and Chung's model in which the left L5 and L6 spinal nerves were ligated (16). The CCI of the sciatic nerve is a widely used model of neuropathic pain which evokes a series of molecular, biochemical, and cytoarchitectural changes in primary sensory neurons and produces neuropathic pain sensations similar to those observed in humans. Ligation of the sciatic nerve also leads to the cardinal symptoms of neuropathic pain, namely apparent spontaneous pain, allodynia, and hyperalgesia (7).

Neuropathic pain remains difficult to manage with conventional analgesics such as non-steroidal anti-inflammatory drugs and opiates. The European Federation of Neurological Societies (EFNS) described guidelines and recommendations on classification of evidence for drug treatments in various studied neuropathic pain conditions as reviewed in Attal et al (17). Moreover, the use of first line drugs, i.e. tricyclic antidepressants (TCAs) and anticonvulsants, are limited by a wide spectrum of adverse effects (18,19).

Interactions between the sympathetic and somatic nervous system play an essential role in the pathophysiologic mechanisms of neuropathic pain (20,21). Furthermore, several animal studies have implicated the α2-adrenoceptors in experimental neuropathic models of pain (22,23). Presynaptic activation of the α2-adrenoceptor inhibits norepinephrine release and thus can stop the propagation of pain signals (24,25). In addition, postsynaptic activation of α2-adrenoceptors in the central nervous system inhibits sympathetic activity and can decrease blood pressure and heart rate. Dexmedetomidine (Dex), the pharmacologically active dextroisomer of medetomidine, is a highly selective α2-adrenoceptors agonist. Dex was studied to have an antinociceptive effect both in humans (26) and animals (27). However, the sedative adverse effect limits its application as a potent analgesic and encourages the potential use of combination pharmacotherapy to treat neuropathic pain.

Tramadol (Tram) is a centrally acting opioid analgesic drug. Animal studies suggest that Tram may exert part of its analgesic action through weak opioid (μ-opioid receptor agonistic activity) and non-opioid (norepinephrine and serotonin reuptake inhibition) mechanisms (28,29). Many studies have shown that compounds known to block monoamine uptake, such as TCAs, potentiate the antinociceptive effects of opioids (30,31). Amitriptyline (Ami), a serotonin and noradrenaline re-uptake inhibitor, has been extensively used in neuropathic pain (32).

Therefore, the authors thought that a combination of Dex with serotonin and noradrenaline re-uptake inhibitors may be useful to enhanced neuropathic pain relief. Thus, the aim of our study was to determine the antinociceptive effect of Dex when administered alone or in combination with Ami or Tram in neuropathic pain model in rats.

Methods

Experimental Animals

Adult male Wistar rats, 150 – 200 g at the time of surgery, were kept under standard conditions of light and temperature and allowed *ad libitum* access to food and water. The animals were obtained from the animal's house facility, Faculty of Medicine, Assuit University. The experiments were carried out according to the protocol approved by our local ethics committee in accordance with the ethical guidelines for pain research in conscious animals. We were considerate about the involved suffering and stress of the rats. Thus, we found at least 6 rats/group was statistically and economically appropriate (33).

One week before experimentation the animals were kept in the laboratory for acclimation to minimize

animal stress. The animals were subjected to a neuropathic pain model in the form of a unilateral CCI of sciatic nerve. Administration of drugs and decapitation of the animals were carried out during the interval from between 9:00 a.m. and 5:00 p.m. For all experimental groups, group size was 6 – 8 rats. Before and after drug administration, the animals were subjected to behavioral pain score recording (resting paw posture), mechanical allodynia, and mechanical hyperalgesia tests. Sedation/motor incoordination was assessed on rotarod (Fig. 1).

Surgical Procedure

The CCI procedure was performed as previously described (7). Briefly, rats were anesthetized with 50 mg/kg i.p. sodium pentobarbital. The common left sciatic nerve was exposed and a 7 – 10 mm length of the sciatic nerve, proximal to the sciatic trifurcation was carefully cleared from underlying tissue using blunt dissection. Four ligatures (4.0 braided silk) were tied loosely around it at one mm intervals. The muscle groups approximated and the skin incision was closed with silk suture and was then covered with iodine. In sham-operated animals, an identical dissection was performed in the left paw, except that the sciatic nerve was not ligated. All surgical procedures were carried under strict sterile conditions.

Drugs and Chemicals

Amitriptyline hydrochloride (30 mg/kg, a gift from Kahira Pharmaceuticals and Chemical Industries Company, Egypt), dexmedetomidine hydrochloride (5 µg/kg, Precedex®, Hospira, Inc. Lake Forest, IL, USA), and tramadol hydrochloride (5 mg/kg, Tramal®, 100 mg/2ml, Minapharm, Hilupollis, Cairo, Egypt under licence of German Grunenthal) were used. All drugs were dissolved in 0.9% (w/v) sodium chloride solution (saline) and were administered intraperitoneal (i.p.) in weight-related doses. The doses were chosen according to previous studies in which no toxicity or motor impairment was identified (34,35).

Assessments of Motor Coordination (Rotarod Test)

Preliminary experiments were performed on a rotarod apparatus at a rotating speed of 16 r.p.m. Rats that remained on the rotarod bar for 2 consecutive periods of 45 s each were included in the study. The test was then repeated at 15, 30, 45, 60, 75, 90, 105, and 120 min after injection of saline, Tram, Dex, Ami Ami+Dex, and Tram+Dex. The results are expressed as percentage of animals that succeeded in remaining on the rod with a cut-off time of 45 s (36).

Assessments of Resting Paw Posture

Animals were observed for the resting paw posture of each hind paw without intervention on eighth day after operation prior to and at 15, 30, 45, 60, 75, 90, 105, and 120 min after i.p. drug administration. Each animal was placed in clear rectangular Plexiglas cage and allowed to habituate for 15 min. Different positions of the operated or sham-operated hind paw were rated using a time sampling technique, paw position was recorded for 2 min (120 s) every 15 min according to the following scale: 0 = paw pressed normally on

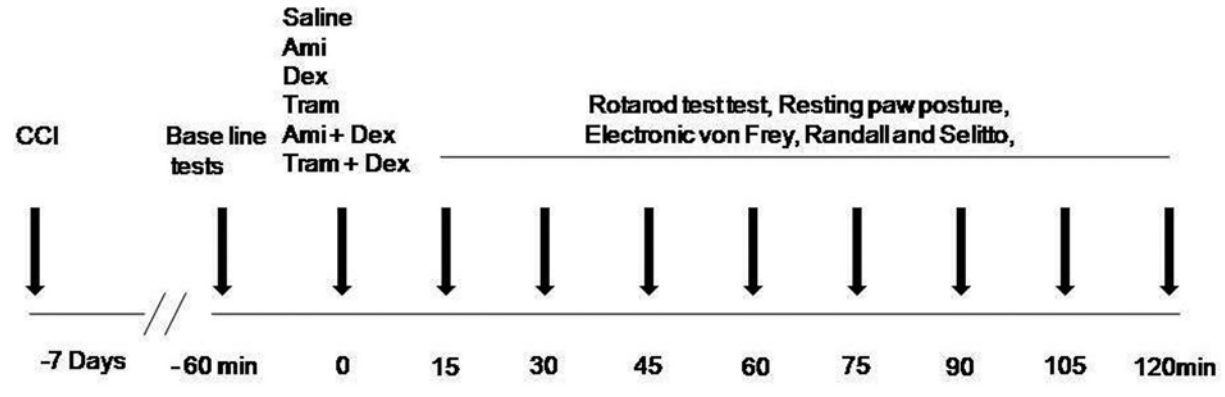

Fig. 1. *Experimental design. Ami: amitriptyline; CCI: chronic constriction injury; Dex: dexmedtomidine; Tram: tramadol. All drugs and saline (vehicle) were injected intraperitoneally.*

floor; 1 = paw rests lightly on floor, toes ventroflexed; 2 = only internal edge of paw pressed to floor; 3 = only heel pressed to floor, hind paw in inverted position; 4 = whole paw is elevated; 5 = animal licks paw. Therefore, the possible scores range from 0 to 5, representing a continuum in pain scores of the paw from normal to abnormal (37).

Assessments of Tactile Allodynia (Electronic von Frey Test)

Allodynia was measured in CCI rats by the use of an electronic von Frey apparatus (Model EVF3, Bioseb, France). Animals were placed in a quiet room in plastic cages with a wire grid floor 30 min before paw stimulation. The electronic von Frey polypropylene tip was applied perpendicularly to the midplantar surface of the hind paw and the intensity of the stimulus was automatically recorded when the paw was reflexly flexed followed by a clear flinch response after paw withdrawal (38).

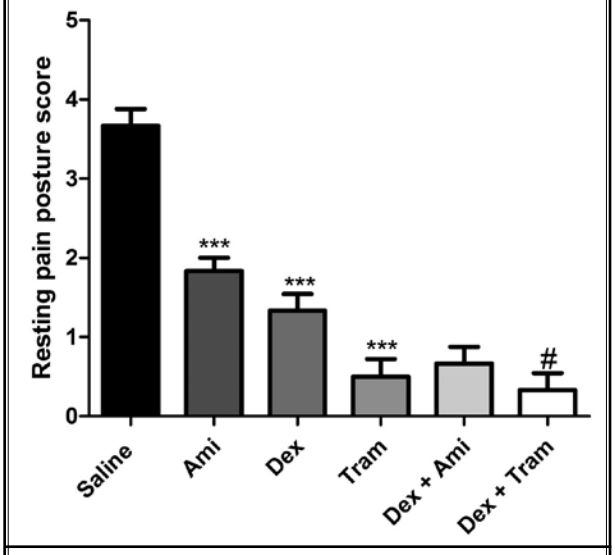

Fig. 2. *Resting paw posture for the left hind paw of the saline-treated group, amitriptyline (Ami)-treated group, dexmedtomidine (Dex), tramadol (Tram), Dex + Ami and Dex + Tram group of the CCI animals at 8th day after surgery. Each column represents the mean ± S.E.M measurements of various time points for n = 8 in each group (except saline-treated group where n = 6). * and # indicate a significant difference compared to saline-treated and Dex-treated group respectively (P < 0.05, P < 0.001 ANOVA followed by Dunnett's test).*

Assessments of Mechanical Hyperalgesia (Randall and Selitto Test)

Based on the Randall and Selitto test (39), the nociceptive threshold after induction of neuropathic pain was measured with an analgesymeter (Ugo Basile, Italy). Measurements were performed on the eighth day after operation prior to and at 15, 30, 45, 60, 75, 90, 105, and 120 min after i.p. drug administration. The stimulus was applied between the third and fourth metatarsals with a cutoff pressure of 200 g. The force needed to induce paw withdrawal was recorded as the pain threshold (expressed in grams). In order to avoid tissue damage, only a trial was performed at each time.

Statistical Analysis

Data are represented as the group means ± S.E.M. The significance of differences between groups was analyzed using a Student's t-test or one-way analysis of variance (ANOVA) followed by the post hoc Dunnett's test for multiple comparisons. All statistical analyses were calculated with Prism software (Graph-Pad Software, version 5, La Jolla, CA, USA).

Results

Rotarod Test Assay

We performed rotarod to exclude the possible influence of motor deficits on the pain behavioral tests. Administration of Tram (5 mg/kg), Dex (5 µg/kg), Ami (10mg/kg), and the combination of Dex with either Tram or Ami had no significant effect on motor coordination in rotarod tests at 15 min intervals and up to 120 min after i.p. administration in rats (P >0.05).

Resting Paw Posture

The sham-operated rats and un-operated hind paw (right paw) pressed normally on the floor reproduced a weighted pain score of 0. CCI produced a significant (P < 0.05) increase in pain scores in all pretreated groups compared with sham-operated groups. Treatment with i.p. Ami, Dex, and Tram produced significant improvement in pain scores compared with saline-treated animals. Combined administration of Dex with Tram showed a significant decrease in pain scores in the left hind paw compared with Dex-treated animals. However, the combination of Dex with Ami produced a non-significant change in pain scores as compared with Dex-treated animals (Fig. 2).

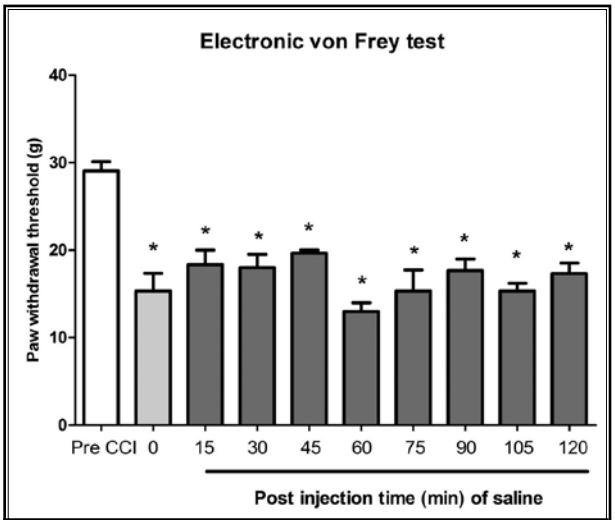

Fig. 3. *Time course effect of saline on paw withdrawal threshold in rats with CCI. Significant differences evoked by electronic von Frey test every 15 minutes up to 120 minutes (n = 6–8) between saline-treated group compared with Pre CCI value; *, P < 0.05.*

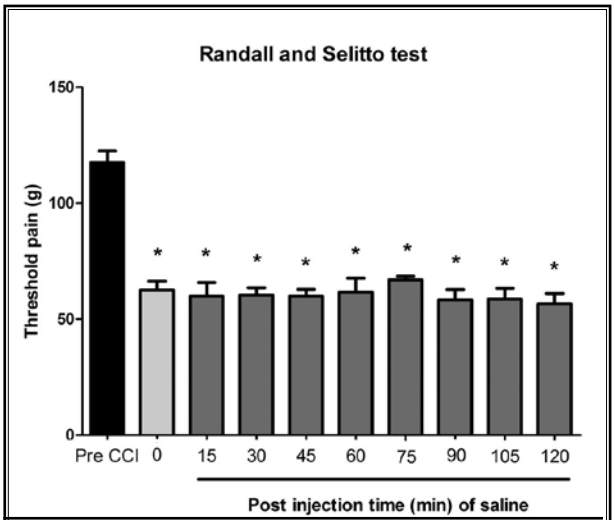

Fig. 4. *Time course effect of saline on the pain threshold in rats with CCI. Significant differences evoked by Randal and Sellito test every 15 minutes up to 120 minutes (n = 6) between saline-treated group compared with Pre CCI value; *, P < 0.05.*

Effect of Saline on Mechanical Hyperalgesia and Allodynia

One week after ligation of the left sciatic nerve, all studied groups developed allodynia and mechanical hyperalgesia. The allodynia paw withdrawal threshold in these animals decreased significantly from 29.07 ± 1.053 to15.33 ± 2.03 prior to drug(s) administration with no significant difference in pain threshold for the selected time points after saline administration (Fig. 3). Similarly, the mean hyperalgesia pain threshold decreased significantly from 117.50 ± 4.96 to 56.67 ± 4.41 prior to drug(s) administration (Fig. 4). Sham-operated rats did not show mechanical hypersensitivity (data not shown).

Action of Individual Drugs on Allodynia and Mechanical Hyperalgesia

The animals were then divided into the assigned treatment groups. All rats displayed a predrug decrease in pain threshold (at 0 time). Initially, Ami, Dex, Tram, and the assigned combination did not produce any motor impairment in 100% of the injected animals at 15 min intervals up to 120 min after administration. Afterward, Ami, Dex, or Tram were found to produce a time dependent reversal of allodynia and mechanical hyperalgesia over 120 minutes with a maximum effect nearly at 45 minutes and continued up to 120 minutes after administration of the individual drugs compared with saline-treated rats (Fig. 5 and Fig. 6 respectively). Combined administration of Dex and Ami was as effective in reducing mechanical allodynia as combined administration of Dex and Tram, but with decreased duration. Furthermore, the Dex–Tram combination group had withdrawal thresholds of mechanical hyperalgesia that were of the same time points (same duration) to those of the Dex-Ami group.

Discussion

Treatment of neuropathic pain remains a clinical challenge. The $\alpha 2$ adrenoceptors are distributed in the primary afferents, spinal cord, and brain (40). Recently, great concern has been shown for the use of Dex, a highly selective $\alpha 2$ agonist, particularly for acute postoperative and chronic pain conditions (41,42). The most common side effect of Dex is sedation, which limits the use of higher doses (43).

First, to exclude any interference of saline on the mechanical allodynia and hyperalgesia (44,45), we carried out the analgesic tests at the same time points of our present experimental paradigm after saline administration. Secondly, a rotarod test was conducted to exclude rats in this study that were sedated by Ami, Dex, and Tram because the analgesic effect of Dex would have been exaggerated. In the present study, no positive results were observed by individual drugs or combination of Dex with Ami or Tram. It was studied that the anti-allodynic effect of intraperitoneal injected Dex at 12.5, 25, and 50 µg/kg is not exaggerated by its sedative

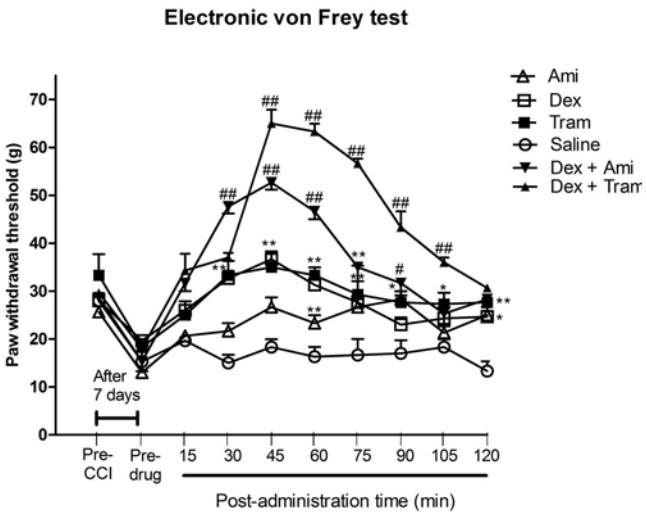

Fig. 5. *The effects of amitriptyline (Ami), dexmedetomidine (Dex), and tramadol (Tram) in electronic von Frey test. Points indicate mean ± S.E.M. for n = 8 in each group (except saline-treated group where n = 6). * and # indicate a significant difference compared to saline-treated and Dex-treated groups, respectively (P < 0.05, P < 0.01 ANOVA followed by Dunnett's test) at the assigned time. The drugs were administered just after the predrug baseline (at 0 time) values were determined.*

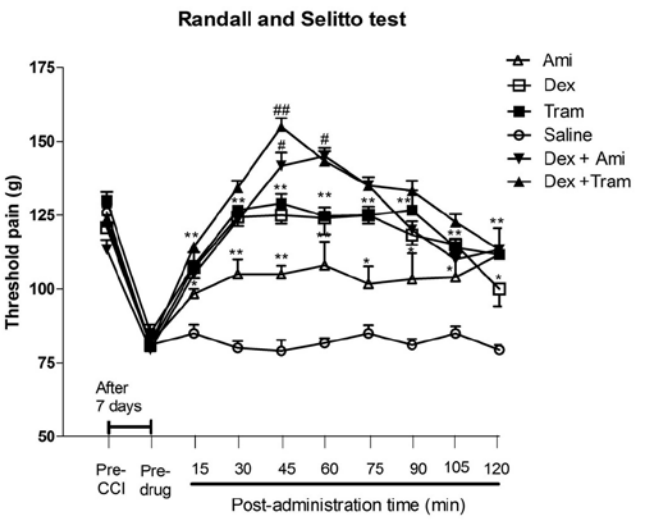

Fig. 6. *The effects of amitriptyline (Ami), dexmedetomidine (Dex), and tramadol (Tram) in Randall and Selitto test. Points indicate mean ± S.E.M. for n = 8 in each group (except saline-treated group where n = 6). * and # indicate a significant difference compared to saline-treated and Dex-treated groups, respectively (P < 0.05, P < 0.01 ANOVA followed by Dunnett's test) at the assigned time. The drugs were administered just after the predrug baseline (at 0 time) values were determined.*

effect in vincristine-induced model of neuropathic pain (46). Therefore, the non-sedating dose which matched the dose that produced a 50% antinociceptive response (ED50) for mechanical allodynia (von Frey test) in rats was chosen (34,47). Indeed, sedation should be considered when using large doses of these drugs.

This study demonstrates antiallodynic and antihyperalgesic actions of Dex in an experimental neuropathic pain model. The effect was time dependant starting its maximum response after 45 min and continued up to 120 min post administration without a significant sedative effect or motor impairment. We performed similar pain tests to that of a previous work from our group (48) and we again elicited a comparable resting pain posture and pain threshold for the Ami-treated group. Preliminary data from our laboratory showed that Ami alone is an effective analgesic in the CCI model of neuropathic pain. However, our data in the present study demonstrated that the maximal antinociceptive effects of Dex or Tram were observed earlier after drug administration for both mechanical allodynia and hyperalgesia and again seem to be more effective than Ami. Nerve injury increased the binding affinity of the μ-opioid receptor 2 – 5 days post injury and returned to control levels within 10 days (49). Guneli et al (34) have reported considerable disagreement to our study. While they studied the antinociceptive effect of either i.p. Dex or Tram alone in the same doses used in our study, they found no statistical difference from control in thermal hyperalgesia induced by peripheral sciatic nerve mononeuropathy in rats, while their combination showed significant difference without sedative side effects.

Data about the role of α2-adrenoceptors after nerve injury are contradictory (50). A previous study has shown that infusion of high dose Dex (30 – 60 pg/d) for 7 days had no effect on von Frey or cold allodynia after a spinal nerve ligation model of neuropathic pain (51). Furthermore, spinal nerve injury itself did not alter either the total number or affinity of α2-adrenoceptors but activated downstream effector molecules (52); other studies have demonstrated damage of noradrenergic fibers after nerve injury (53,54). Yet another study has shown that depletion of spinal noradrenaline by intrathecal 6-hydroxydopamine

increases the α2 adrenoceptor number in the spinal cord or affinity to clonidine in normal animals (55). Likewise, clonidine's behavioral effect was enhanced after nerve injury in animals consistent with denervation supersensitivity or increased receptor expression (54). We believe that a likely interpretation of these conflicting results is differences in research methods or variation of species.

It is noteworthy to consider that the pharmacology of analgesia achieved in neuropathic pain involves many neurotransmitters (56). Opioidergic and noradrenergic pathways can interact synergistically to modulate pain perception, thus combination of drugs with Level A ratings for treatment of neuropathic pain is expected to have high efficacy and low dose-related side effects (17,57).

To our knowledge, limited studies have been conducted for the combined use of Dex-Tram (34,58), and there have been no studies that examined the pharmacologic combination of Dex with Ami in the CCI model of neuropathic pain. Our obtained data suggest that the combination of Dex with either Tram or Ami was more effective in increasing the pain threshold in an experimental neuropathic pain model when compared with administration of Dex alone. In accordance with our findings, at a clinical level, Belgrade and Hall (59) found that infusion of Dex increased the morphine antinociceptive effect. Those results were supported by a recent subsequent study by Gursoy et al (60), who showed that administration of Dex with morphine increased morphine analgesia while MK-467 (an α2-adrenoceptor antagonist) decreased morphine analgesia in rats.

The observed increase in pain threshold in our study for both combinations to detect mechanical hyperalgesia lasted for a short period which may denote development of acute tolerance for hyperalgesia. In contrast, we could not observe development of acute tolerance for allodynia when a Dex-Tram combination was used. Also, we did not find significant difference of baseline pain threshold in Randall and Selitto tests and von Frey tests which might support the use of this combination to ease allodynia rather than hyperalgesia. The difference might be explained in terms of a rapid return to low pain threshold and that the changes of sensory information during hyperagesia occur mainly in the central nervous system (61). Although tolerance usually occurs with chronic administration, Horvath et al (62) observed that morphine pretreatment significantly decreased the antinociceptive effect of morphine in the acute heat-pain test 7 days later and suggested a delayed tolerance mechanism. In contrast, Dex did not cause cross-tolerance with morphine. Not only do the mechanism(s) mediating the action of Ami or Tram and Dex carry some similarity explaining the analgesic action of these drugs, the antinociceptive actions can also coexist on the same cells. Also, the addition may be at the postreceptor signalling transduction pathways. Dex induced antinociception was attenuated by naloxone (63) and morphine-induced antinociception was reduced by yohimbine, an α2-adrenoceptor antagonist (64). Moreover, analgesic synergism of opioids with clonidine and Dex was lost in the α2 adrenoceptors-knockout mice (65). Malmberg et al (50) found that the α2A, α2B, and α2C adrenoceptors mutant mice developed a thermal and mechanical allodynia after sciatic nerve injury that did not differ from those observed in wild-type mice and concluded that α2 adrenoceptors subtypes are not required for the development of these cardinal signs of neuropathic pain. However, they demonstrated that the observed antiallodenic effect of Dex at a dose less than that used in our study (3 μg/kg, s.c.) is mediated via the α2A adrenoceptors.

A clear-cut analysis of our results is limited by only single-dose responses rather than defining a full-dose response curve for the tested behavioral responses at each of allocated time points. Neuropathic hyperalgesia is associated with an increase in glutamate content, activation of N-methyl-D-aspartate (NMDA) receptors, and subsequent intracellular cascades including nitric oxide (NO) production (66). Our future studies will be conducted to demonstrate whether the NMDA receptors would be involved in the antinociceptive effect of Dex-Ami or Dex-Tram combinations in neuropathic pain models and will be extended to the use of clinically available NMDA receptor antagonists, for example ketamine and memantine.

Conclusion

In conclusion, on the basis of our present data, systemically administered low doses of a Dex and Tram combination appears to be effective in increasing the pain threshold in rat models of CCI and we are inclined to consider Dex-Ami a good alternative combination to Dex-Tram to increase antinociceptive effect.

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