ORIGINAL ARTICLE

The synergistic antinociceptive interactions of morphine and dexmedetomidine in rats with nerve-ligation injury

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Abstract

Background: Peripheral nerve injury may result in chronic neuropathic pain, which is characterized by spontaneous pain, hyperalgesia, and allodynia. Intrathecal administration of opioids and α_2-adrenoceptor agonists produces spinal analgesia by activation of opioidergic and noradrenergic systems. In our study, we have compared the synergistic antiallodynic interaction and side-effects of intrathecal morphine and dexmedetomidine in a rat model of neuropathic pain.

Methods: Male Wistar rats, weighing 380-440 g, were treated with tight ligation of left L5-6 spinal nerves and a chronic catheter was implanted intrathecally. Morphine and dexmedetomidine were administered intrathecally to obtain the dose-response curves and the 50 % effective doses (ED_{50}) for each drug and fractional analysis of the ED_{50} of each drug administered concurrently was performed to examine the interaction. Mechanical allodynia was measured by using application of von Frey filaments to the hindpaw.

Results: Intrathecal administration of morphine and dexmedetomidine alone and in combination resulted in a dose-dependent antiallodynic effect, and the combination produced a synergistic effect-state magnitude. Moreover, the incidence of side-effects was higher when morphine or dexmedetomidine were administered in high doses alone and extremely low when these two drugs were used in combination. These results are suggestive of a synergistic effect at lower doses of both drugs.

Conclusion: These findings may provide a rationale for combining such drugs for the improvement of human postoperative or neuropathic pain treatment in the future. Hippokratia 2013; 17 (4): 326-331.

Keywords: Analgesia, neuropathic pain, intrathecal administration, morphine, dexmedetomidine

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Introduction

Injury of a peripheral nerve can lead to a pain syndrome that is characterized by spontaneous pain, hyperalgesia, and allodynia[1].

The spinal cord is an important neuronal structure for pain transmission, and nociception is attenuated by local opioids, norepinephrine and serotonin[2,3]. Therefore, an interaction of these systems at the spinal cord level may affect antinociception. In addition, the spinal cord is one of the pharmacological sites of action for the antinociceptive effects of different drugs. However, the efficacy of morphine in a state of neuropathic pain is still controversial[4,5].

Opioids, administered intrathecally or epidurally, are widely used for postoperative, post-labor and chronic nociceptive pain secondary to cancers[6,7]. Intrathecal administration of narcotics has not been used widely in patients with chronic neuropathic pain. This lack of experience stems firstly from empiric studies which have demonstrated poor outcomes of parenteral or oral opioids in the treatment of neuropathic pain syndromes. Secondly, opioid therapy is limited because of the side effects (hypotension, pruritus, nausea, urinary retention, respiratory depression) and intolerance[7-9]. Spinal α_2-adrenoceptor activation enhances antinociceptive effect of intrathecal morphine[4]. α_2-adrenoceptor-mediated spinal analgesia has been investigated extensively in animal and human studies[10,11]. Dexmedetomidine is a highly selective and potent α_2-adrenoceptor agonist. Its intrathecal administration leads to antinociceptive effects in animals, although it does have some undesired side effects (sedation, mydriasis, diuresis)[11-13].

Combination of several agents that produce similar therapeutic effects or synergistic interactions may also decrease the side-effects by permitting dose reduction dose of individual agents[14-16].

In this study, we have compared the synergistic interaction and side-effects of intrathecal morphine and dexmedetomidine in the rat model of neuropathic pain.

Material and Method

The experimental protocol of this study was approved by

the Animal Investigation Committee of Ministry of Health. Male Wistar rats weighing 380-440 g were studied. Rats were housed individually in a temperature controlled vivarium and were allowed to acclimate in a 12/12-h light/dark cycle.

Rats were prepared surgically under anesthesia with ketamine and xylazine (72 and 8 mg/kg intraperitoneally, respectively). A dorsal midline incision was performed from L_3 to S_2 dermatomal areas. Left L_6/S_1 posterior interarticular processes were resected and left L_5-L_6 spinal nerves were isolated and ligated tightly with 6/0 black silk distal to the dorsal root ganglion. After a 7-day postoperative period, an intrathecal catheter was implanted if the rat showed a withdrawal threshold of 4.0 g or less. Under ketamine and xylazine anesthesia, the heads of rats were fixed on a stereotaxic head device. The intrathecal space was punctured through the atlantooccipital membrane and a polyethylenecatheter (PE-10) was inserted and passed to the T_{12}-L_1 spinal cord level of the lumbar enlargement. The catheter was tunneled subcutaneously and externalized through the skin[17,18]. Rats exhibiting postoperative neurologic deficits (non-withdrawal reflex after mechanical stimulation in paws) were excluded. The animals were randomly assigned to treatment groups (n=6, total 24 rats). Each animal was studied three times in an experimental series; with 6 to 8 days intervals between studies to minimize the possibility of tolerance developing. After experimental usage, rats were sacrificed with pentobarbital overdose (100 mg/kg, intraperitoneally).

All pharmacological experiments were conducted 2 weeks after catheterization because tactile allodynia develops within 1 week after spinal nerve ligation. Behavioral testings were performed during the same time period within the day (between 11.00 am and 5.00 pm) to exclude diurnal variations in pharmacological effects. All drugs were dissolved in 5 μl physiologic saline. Drugs were given intrathecally via a microinjection syringe over a 30 seconds interval in a volume of 5 μl, followed by a 10 μl flush of physiologic saline. Intrathecal injections were performed concurrently because of the coincidence of the effects of both drugs.

The first series of experiments defined the dose-response curves of intrathecally administered morphine and dexmedetomidine to determine for each drug. For the evaluation of the reversal of mechanical allodynia and the 50% effective dose (ED_{50}) estimated to produce 50% maximal possible effect (%MPE) of each drug, dexmedetomidine and morphine sulphate were administered intrathecally. The doses of 1, 3, 10, 30 μg (n=6, total 24) and 0.3, 3, 6, 10 μg (n=6, total 24) were injected for morphine and dexmedetomidine, respectively. Animals were given a 5-day resting period between drug injections to minimize any possibility of tolerance developing.

In the second series of experiments, fractions (1/2, 1/4, and 1/8) of ED_{50} of each drug (n=6, total 18 rats), were administered concurrently in an equal dose ratio to establish the ED_{50} of morphine-dexmedetomidine combinations (n=18). Antinociceptive effects were determined as the percentage of the maximal possible effect (%MPE) according to the formula: %MPE= (test latecy-predrug latency)/(cut-off time-predrug latency) x 100 %.

Rats were placed in individual plastic covered compartments. Mechanical threshold was measured by applying a von Frey filament to the midplantar surface of the lesioned hindpaw until a positive sign for pain behavior was elicited[19]. Von Frey filaments of sizes 0.70, 2.00, 5.50, 8.50 and 15.1 g were applied and cut-off in the absence of a response was 15.1 g. Measurements were taken before and 15, 30, 45, 60, 90 and 120 min after the intrathecal administration of the drug.

Analysis of dose-response curves and statistics were obtained via ANOVA test program and included calculation of the ED_{50} values and 95% confidence intervals of them (CI). The difference between the theoretical additive point and the experimentally derived ED_{50} was compared by using the Student's test. For experimental values lower than theoretical additive values, a p value < 0.05 for the differences in both X and Y directions were considered as a significant synergistic interaction.The SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

Results

All rats displayed a significant decrease in the mechanical threshold after spinal nerve ligation. In all groups, mean withdrawal thresholds were in the range of 2.24 ± 0.68 g and 3.28 ± 0.52 g at the baseline measurement.

A dose-dependent increase in withdrawal latency after intrathecal administration of morphine (1, 3, 10, 30 μg) and dexmedetomidine (0.3, 3, 6,10 μg) alone were seen in the hindpaws. Maximal effects were observed at 15 min from injection of morphine. Its effect started to decrease after 60 minutes. ED_{50} value was found 12.1 μg for morphine. Morphine at smaller doses (1-3 μg) produced a moderate increase in %MPE (29%-48%); larger dose (10 μg) caused very effective (65%), long lasting antinociception. The largest dose (30 μg) caused close to

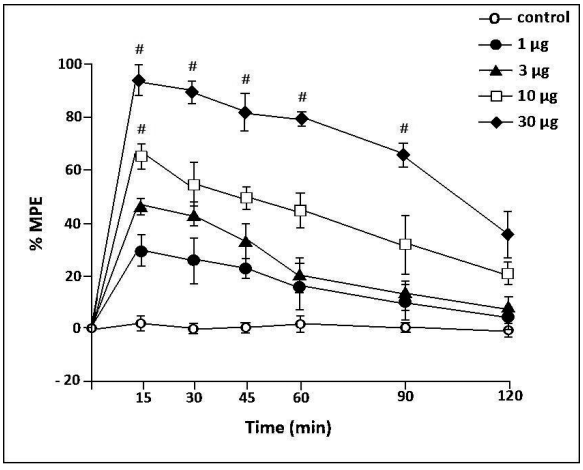

Figure 1: Time-course curves plotting %MPE for the antiallodynia of morphine. Antinociceptive effect (%MPE) of various doses of intrathecal administered morphine

The points are expressed as mean ± SEM of six rats. Times (min) are represented logarithmically on the x axis, and %MPE is represented on the y axis, †: $p < 0.05$, #: $p < 0.05$.

%100 MPE (Figure 1). Maximal effects were observed within 15-30 min from injection of dexmedetomidine. Its effect started to decrease after 45 min. ED_{50} value was found 1.12 µg for dexmedetomidine. Dexmedetomidine at smaller doses (0.3- 3 µg) produced a slight increase in %MPE (22%-27%); two larger doses (6-10 µg) caused very effective (60%-90%), long lasting antinociception (Figure 2).

Injection of morphine prolonged the latency of withdrawal starting at the dose of 3 µg, injection of dexmedetomidine prolonged the latency of withdrawal starting at the dose of 6 µg (Table 1).

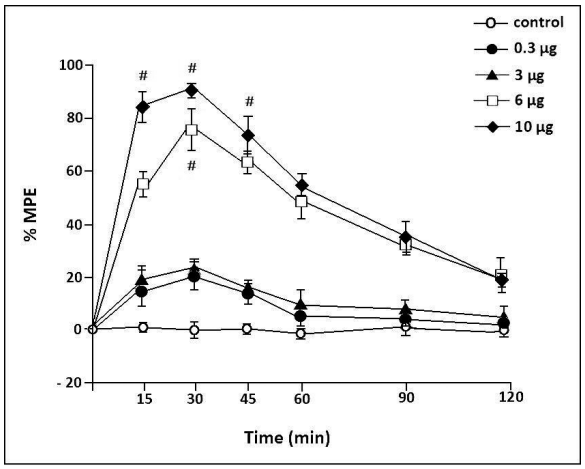

Figure 2: Time-course curves plotting %MPE for the antiallodynia of dexmedetomidine. Antinociceptive effect (%MPE) of various doses of intrathecal administered dexmedetomidine.
The points are expressed as mean ± SEM of six rats. Times (min) are represented logarithmically on the *x* axis, and %MPE is represented on the *y* axis, †: $p < 0.05$, #: $p < 0.05$.

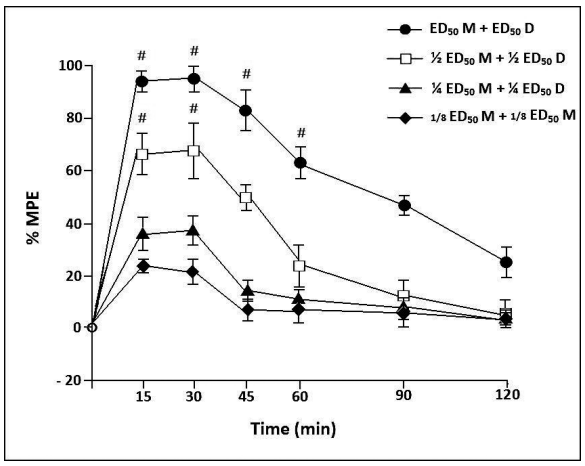

Figure 3: Time-course curves plotting %MPE for the antiallodynia of morphine and dexmedetomidine in combination. Antinociceptive effect (%MPE) of various mixtures of intrathecal administered morphine and dexmedetomidine. Analysis of ED_{50} values and fractions (1/2, 1/4, and 1/8) of dexmedetomidine and morphine combinations.
The points are expressed as mean ± SEM of six rats. Doses (µg) are represented logarithmically on the *x* axis, and %MPE is represented on the *y* axis, †: $p < 0.05$, #: $p < 0.05$.

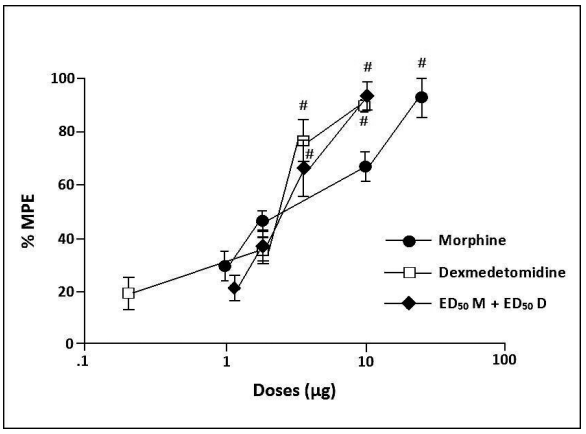

Figure 4: Dose-response curves plotting %MPE for the antiallodynia of morphine and dexmedetomidine, and in combination of ED_{50} of each drugs. Dose-response curves for the antinociceptive effect of the single-drug treatment and double combination at %MPE was seen.
The points are expressed as mean ± SEM of six rats. Doses (µg) are represented logarithmically on the *x* axis, and %MPE is represented on the *y* axis, †: $p < 0.05$, #: $p < 0.05$.

ED_{50} values and fractions (1/2, 1/4, and 1/8) of ED_{50} of each drug (Table 2), were administered concurrently in an equal dose ratio to establish the ED_{50} of morphine-dexmedetomidine combination. %MPE of combination was analyzed[20] (Figure 3). Intrathecal co-administration of 1/2 of ED_{50} of each drug resulted in a significant increase in the withdrawal latency following a dose-dependent fashion. Isobolografic analysis demonstrated that this interaction was synergistic, because the doses of morphine and dexmedetomidine necessary to produce 50 %MPE were significantly less than those calculated to be necessary for an additive interaction. Therefore, the dose-effect curve of morphine shifted to the left when it was combined with dexmedetomidine in combination of 1/2-1/4 doses (Figure 4). The statistical significance for the differences in the degree of synergism reached the level of $p < 0.05$ between the binary combinations.

Similar patterns of time course were shown in the combination groups. Intrathecal administration of morphine and dexmedetomidine alone and in combination resulted in a dose-dependent increase in antiallodynic effect. Experimentally determined mixture ED_{50} were 0.14 (0.12-0.46) µg for dexmedetomidine and 1.51 (0.87-2.36) µg for morphine ($p < 0.05$). The theoretical additive ED_{50} (± SEM) was 0.56 (± 0.09) µg for dexmedetomidine and 6.1 (± 1.11) µg for morphine. According to the fractional method used here, fractions of each drug are both 0.1, and total fractional value less than 1 indicates a synergistic interaction.

A dose related pattern of side effects was shown in the morphine and dexmedetomidine groups. The large dose (10 µg) of morphine caused mild to moderate motor weakness and sedation. The largest dose (30 µg) caused temporary rigidity. Spinal morphine also caused inability

Table 1: ED_{50} (μg) and slope with 95% CI for dose-response curves of intrathecal drugs.

	n	ED_{50} (%95 CI)	Slope (%95 CI)
Morphine	6	12.1 (5.98-23.79)	36.1 (17.2-45.1)
Dexmedetomidine	6	1.12 (0.91-1.87)	41.34 (38.0-57.3)

ED_{50}: 50% effective dose, CI: confidence intervals, n: number of rats used in constructing the dose-response curve.

Table 2: ED_{50} (μg) and fractions (1/2, 1/4, and 1/8) of ED_{50} of each drug in combinations.

	n	combinations
Morphine ED_{50} + Dexmedetomidine ED_{50}	6	12.1 + 1.12
½ of Morphine ED_{50} + ½ of Dexmedetomidine + ED_{50}	6	6 + 0.56
¼ of Morphine ED_{50} + ¼ of Dexmedetomidine ED_{50}	6	3 + 0.28
1/8 of Morphine ED_{50} + 1/8 of Dexmedetomidine ED_{50}	6	1.5 + 0.14

ED_{50}: 50% effective dose, n: number of rats.

Table 3: Incidence of side effects after intrathecal morphine, dexmedetomidine, and combination of both.

Dose (μg)	n	Sedation	Motor weakness	Urination
Morphine				
1	6	—	—	—
3	6	1 (17%)	—	—
10	6	2 (33%)	1 (17%)	—
30	6	3 (50%)*	2 (33%)	—
Dexmedetomidine				
0.3	6	—	—	—
3	6	2 (33%)	1 (17%)	2 (33%))
6	6	3 (50%)*	2 (33%)	3 (50%)*
10	6	4 (67%)*	2 (33%)	6 (100%)*
Morphine + Dexmedetomidine				
1.5 + 0.14	6	—	—	—
3 + 0.28	6	—	—	—
6 + 0.56	6	1 (17%)	—	1 (17%)
12 + 1.12	6	2 (33%)	1 (17%)	2 (33%)

n: number of rats, *: $p< 0.05$.

to diuresis. Dexmedetomidine in larger doses was associated with substantial diuresis and sedation (decreased spontaneous exploring activity, but the animals were still responsive to acoustic or tactile stimuli), (Table 3). The incidence of side-effects generally reduced when combined. At antiallodynic doses, none of the drugs obtunded the brisk hind paw withdrawal induced by the higher von Frey stimuli.

Discussion

In the experiments described here, an intrathecal injection of morphine produced a mild to moderate reduction of mechanical allodynia at the analgesic dose range. These data suggest that mechanical allodynia may be mediated by the spinal opioid system[7]. Intrathecal injection of dexmedetomidine resulted in a mild reduction in mechanical allodynia at lower doses and an antinociceptive effect at higher doses. Furthermore, intrathecal administration of morphine and dexmedetomidine produced a significant synergistic effect with regard to a spinally-mediated mechanical allodynia. These results clearly confirmed the interactive role between both drugs.

Some investigators have suggested that morphine was ineffective against neuropathic pain in animal studies[21,22], whereas others, in clinical studies[7,14,23], have reported that opioids may alleviate neuropathic pain. They, however, measured only analgesic effects, not the mechanical allodynic component. Nicholas et al[22], reported that intrathecal morphine did not alter allodynia at doses up to 100 μg and a high-efficacy μ-opioid agonist exhibited a significant, dose-related antiallodynic action. Furthermore, this antiallodynic efficacy of morphine was regulated by cholecystokinin in the same neuropathic rat model. In contrast, morphine sulphate in doses of 90-150 μg evoked a prominent behavioral syndrome suggestive of allodynia[24]. Clinical reports have suggested this phenomenon may also occur in humans after large doses of intrathecal morphine[25].

Intrathecal injection of dexmedetomidine led to antinociception via decreasing the release of glutamate from primary afferent nerve terminals and suppressing the noxiously evoked activity of wide dynamic range neurons[12,13]. Another study is shown; brain derived nerve growth factor in the spinal cord is needed for normal synthesis of acetylcholine by cholinergic neurons and excitation of these neurons by α_2-adrenoceptor agonists after nerve injury[26]. The antinociceptive action of systemically administered α_2-adrenergic agonists, such as clonidine, has been documented, but is frequently associated with adverse effects on blood pressure[10].

There are several possibilities for synergistic interaction between morphine and dexmedetomidine. Since the opioid, glutamate, and α_2-adrenoceptors are abundant in the spinal cord[27], coactivation and antagonism of these receptors could have a beneficial effect on the inhibition of pain sensation at low dosages, which cause minimal side-effects. There are differences in mechanisms of action between morphine and dexmedetomidine. One of those is the inhibition of the release of transmitters from the C-fiber primary afferent terminal, although they have inhibitory effects on both interneurons and projecting neurons[28,29]. The augmented activity may also attribute to a decreased clearance of drugs from the intrathecal space, since the duration of action of the combination was longer than that of morphine or dexmedetomidine alone. In another study, the combination of tramadol and dexmedetomidine was more effective in increasing the pain threshold in acute and neuropathic pain when compared with the administration of either of these drugs alone[30].

Naguib and Yaksh[31] reported that antinociceptive effects of co-administrated drugs at the spinal level were mediated by independent receptor systems and there was a reduction in doses for either drug, suggesting synergistic interaction. It is possible, however, that the enhanced effectiveness resulted from a decreased clearance, changes in agonistic affinities or functional interactions. Although the redistribution of the present drugs was not examined, we do not believe that these results reflect an altered clearance of either drug. In a similar study conducted with α_2 and μ agonists, clearance was not altered[32]. Stevens et al[33] used a different pain test, (hot-plate withdrawal latency), and examined the effects of these drugs in a reflex nociceptive model opposite to our neuropathic pain model. Regardless, the significance of the potential cross tolerance between α_2-adrenoceptor agonists and morphine is felt to be minimal, as Yaksh[24] has demonstrated only a minimal loss of response between such agents which interact with distinct receptors. Our results supported this conclusion.

Intrathecal morphine and dexmedetomidine appeared to be effective analgesics in this experimental model of neuropathic pain. In the future, synergistic interaction between these drugs may be of therapeutic significance via allowing reducing of the dose of each drug required to achieve an acceptable level of analgesia. Also, the side-effects were observed often in response to high doses of morphine and dexmedetomidine alone, but the incidence was very low when combined. These findings may provide a rationale for combining such drugs for improved human postoperative or neuropathic pain management in the future. In order to determine the most appropriate α_2-adrenoceptor agonists to be used for potentialization of opioid analgesia and its possible mechanism of action, further experimental studies are needed.

Conflict of interest

Authors report no conflict of interest.

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