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Analysis of the antinociceptive effect of systemic administration of tramadol and dexmedetomidine combination on rat models of acute and neuropathic pain

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Abstract

The aim of the present study was to investigate the possible antinociceptive effect of systemic administration of tramadol and dexmedetomidine either alone or in combination on acute and neuropathic pain models in rats. The antinociceptive effects of intraperitoneal (i.p.) tramadol (5–20 mg/kg) and dexmedetomidine (5–20 μg/kg) and three different combinations of tramadol+dexmedetomidine (5+5, 5+10 and 10+5, mg/kg+μg/kg, respectively) were measured by tail-flick and hot-plate methods in acute pain. The effects on the sciatic nerve ligation-induced neuropathic pain was tested by i.p. administration of tramadol (5 mg/kg), dexmedetomidine (5 μg/kg) and tramadol+dexmedetomidine combination (5+5) using a thermal plantar test. Sedation/motor-incoordination was assessed on rotarod. Tramadol and dexmedetomidine produced dose-related antinociception in tail-flick and hot-plate tests. In both tests, combination of these drugs produced an antinociceptive effect that is greater than that produced by tramadol or dexmedetomidine alone at several time points. In hot-plate test, tramadol+dexmedetomidine combination (5+10) exerted the strongest antinociceptive effect, while tramadol+dexmedetomidine combination (10+5) was significantly most effective in tail-flick test. In the neuropathic pain, the antinociceptive effect exerted by tramadol+dexmedetomidine combination (5+5) was also significantly greater than their applications alone. In rotarod test, tramadol (30 and 40 mg/kg), dexmedetomidine (30 and 40 μg/kg), tramadol+dexmedetomidine combination (10+10, 20+20) produced sedation/motor-incoordination, whereas tramadol (5–20 mg/kg), dexmedetomidine (5–20 μg/kg) and tramadol+dexmedetomidine combination (5+5, 5+10 and 10+5) did not produce any effect on sedation/motor-incoordination. 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.
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Keywords: Tramadol; Dexmedetomidine; Combination; Antinociception; Sedation; Acute pain; Neuropathic pain; Rat

1. Introduction

Although, several drugs are available for acute pain therapy, research is continuing in a search for the most appropriate drugs. The opioid analgesics remain the major drugs for the treatment of moderate-to-severe pain. However, the side effect profile of opioids, which includes nausea/vomiting, sedation, constipation, and respiratory depression, should be considered when using large doses of these drugs. Therefore, drug combination therapy is recommended for management of pain. Thus, multimodal analgesia combinations often achieve analgesia at lower doses than required for either compound alone, leading to enhanced pain relief and a reduction of side effects (Jin and Chung, 2001).

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Nerve damage that affects peripheral or central nerves leads to abnormal pain states referred to as neuropathic pain. Although this pain syndrome is usually poorly controlled by currently available medications, pharmacotherapy remains the mainstay of neuropathic pain management. The most widely utilized pharmacotherapy options include anticonvulsants, antidepressants, topical treatments (*e.g.*, 5% lidocaine patch, capsaicin), and opioids (Stacey, 2005). The available drugs to treat neuropathic pain have incomplete efficacy and dose-limiting adverse effects. Recent experimental and clinical data support the potential of combination pharmacotherapy for neuropathic pain (Uyar et al., 2003; Gilron et al., 2005; Jackson, 2006).

Alpha (2)-adrenoceptors (alpha(2)AR) play an important role in the control of pain. Both systemically and intrathecally administered alpha(2)AR agonists produce antinociceptive effect in humans (Aho et al., 1991; Filos et al., 1994) and animals (Kalso et al., 1991; Pertovaara et al., 1991; Takano and Yaksh, 1992; Eisenach et al., 1993, Graham et al., 1997; Kingery et al., 2000). Dexmedetomidine, a highly selective alpha (2)AR agonist, has been shown to produce antinociception at the spinal (Kalso et al., 1991) and supraspinal level (Guo et al., 1996). One of the major side-effects caused by the currently available alpha(2)AR agonists has been sedation (Hall et al., 2000). The sedative and the other side-effects of these agonists limit their application as potent analgesics in clinical practice.

Tramadol is a centrally acting weak opioid analgesic agent that is used in the management of pain (Raffa and Friderichs, 1996). Experimental data suggest that tramadol exerts part of its analgesic effect through the activation of the central inhibitory monoaminergic pathway because its effect has been partially blocked by alpha(2)AR antagonists such as yohimbine (Raffa et al., 1992). Both these opioid and non-opioid mechanisms independently contribute to the analgesic effect of tramadol. Thus, its mode of action and also safety profile distinguishes it from other opioids. With its dual mechanism of action (μ-opioid receptor agonist and a monoaminergic mode of action) tramadol provides a kind of combined/adjuvant pain therapy (Raffa et al., 1993; Uyar et al., 2003; Negro et al., 2005).

As mentioned above, activation of μ-opioid receptor and alpha(2)AR inhibits the transmission of pain sensation. However, the μ-opioid receptors and alpha(2)ARs are thought to interact in modulation of nociceptive processing. Previous animal studies have shown synergistic antinociceptive interaction between μ-opioid receptor agonist and alpha(2)AR agonists in animal models (Ossipov et al., 1989; Sullivan et al., 1992; Przesmycki et al., 1997). From this point of view, these drugs show similarities in their mode of antinociceptive action. However, no study was found determining the interaction between dexmedetomidine and tramadol in acute pain and neuropathic pain. Therefore, we thought that a combination of two different analgesic drugs may be useful for pain therapy if the drugs enhance each others antinociceptive effects, because sedation can be reduced by lowering the doses. Thus, the aim of the present study was to determine the antinociceptive effect of systemic dexmedetomidine and tramadol on acute and neuropathic pain methods in rats when administered separately and in combination.

2. Materials and methods

2.1. Animals

The experiments were performed on adult male Wistar rats weighing 200–250 g obtained from Breeding Center of Experimental Animals in Dokuz Eylul University, Faculty of Medicine, Izmir, Turkey. Animals were housed four per cage (Tecniplast, Italy) in a room maintained at 22±1 °C with an alternating 12 h dark/12 h light cycles. Standard laboratory chow and tap water were available *ad libitum* and the operated neuropathic animals were able to eat and drink unaided. Each animal in each group was used for maximum of 2–4 experiments, with each experiment using a different dosing regimen. A recovery period of at least 7 days was allowed between experiments. All experiments were conformed to ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983). The protocol was approved by the Animal Ethics Committee of Faculty of Medicine, Dokuz Eylul University (12.11.2004, nr. 04/14/66).

2.2. Drugs

Tramadol hydrochloride (Contramal®, Abdi Ibrahim, Turkey) and dexmedetomidine hydrochloride injection (Precedex®, Abbott Laboratories, Chicago) were diluted in physiological saline (0.9% NaCl). Both drug-treated and control animals were administered i.p. in a volume of 10 ml/kg body weight. Control animals received equivalent volume of physiological saline.

2.3. Experimental protocol and behavioral tests

Nociceptive testing was carried out between 09.00 and 12.00 in normal light and temperature (22±1 °C) in a quiet room, away from colony room. Rats were randomly assigned for a series of tests. The animals were allowed to adapt to laboratory for at least 2 h before the experiment and their tails were marked with marker in order to apply the thermal stimulus from the same point and to discriminate the treatment groups. The observers were blind to the experimental and treatment conditions. To minimize experimental variability, all behavioral and neuropathic operations were done by the same person. The number of animals and the intensity of noxious stimuli were the minimum possible with which to demonstrate reliable effects of the agents tested. Dose–response curves for antinociception were constructed using only nonsedative doses of these drugs. Sedation was evaluated by a rotarod test as mentioned below.

2.3.1. Acute nociception assay

The effect of drugs on acute nociception was investigated using the tail-flick and hot-plate tests:

2.3.1.1. Tail-flick test. The tail-flick test was evoked by a source of radiant heat, which was focused on the dorsal surface of the tail. Rats were examined for latency (seconds) to withdraw their tails from a noxious thermal stimulus using a tail-flick meter (MAY-TF 0703, Ankara, Turkey). Withdrawal of the tail exposed to the light, which turned off the thermal stimulus and automatically stopped

the clock. Each rat was tested twice before the administration of the drug and the reaction times were averaged to obtain a baseline. The intensity of heat stimulus was adjusted to achieve a mean tail-flick latency of 3–4 s. The heat source was not adjusted for individual animals. Each rat was then tested before and 30, 45, 60, 75, 90, 105 and 120 min after the i.p. administration of tramadol, dexmedetomidine and tramadol plus dexmedetomidine combinations. Control rats received saline instead of the drugs. Treatments were terminated if the animals did not respond within 15 s in order to avoid tissue damage and the test was stopped. The animals which did not respond within 15 s were used for other experiments with a different treatment (D'Amour and Smith, 1941).

2.3.1.2. Hot-plate test. Rats were placed on aluminum hot-plate (MAY-AHP 0603, Ankara, Turkey) kept at a temperature of 55±0.5 °C for a maximum time of 30 s. Reaction time was recorded (when the animals licked their fore and hind paws and jumped) before and 15, 30, 45, 60, 90 and 120 min after i.p. administration of tramadol, dexmedetomidine and tramadol plus dexmedetomidine combinations. The animals whose basal responses were >6 s were discarded (Franzotti et al., 2000).

In both tests, a time and dose–related curves were constructed to assess antinociceptive activity of dexmedetomidine, tramadol and combination of both agents. The rats were assigned to 10 groups for tail-flick and hot-plate tests. Each group received saline, three different doses of tramadol (5, 10, 20 mg/kg), three different doses of dexmedetomidine. (5, 10, 20 μg/kg), and three different combination of tramadol+dexmedetomidine [5 mg/kg+5 μg/kg (5+5), 5 mg/kg+10 μg/kg (5+10) and 5 mg/kg+5 μg/kg (10+5), respectively], i.p. It has been used the same animals for hot-plate and tail-flick tests. However, at least 7 day intervals were given between the tests.

2.3.2. Neuropathic pain assay

2.3.2.1. Chronic construction injury (CCI) of the sciatic nerve. CCI surgery was produced under ether anesthesia. The common sciatic nerve was exposed at the middle level of right thigh by blunt dissection through the biceps femoris. Proximal to the sciatic's trifurcation, about 7 mm of nerve was freed of adhering tissue and 4 ligatures (chromic catgut 4.0) were tied loosely around it with approximately 1 mm spacing. To observe the effect of CCI-induced pain on immune reaction, all rats received sham surgery consisting of exposure of the sciatic nerve in the same way but without ligation in the left limb. After surgery, the rats were returned to their cages and maintained for 15–21 postoperative days under the same conditions mentioned above (Bennett and Xie, 1988).

2.3.2.2. Thermal plantar test. The Hargreaves method (Hargreaves et al., 1988) was used to assess paw withdrawal latency (PWL) to a thermal nociceptive stimulus. First, the preoperative pain thresholds of the animals were recorded, and then the surgery was performed. The Paw Withdrawal Analgesimeter (MAY PWAM 0903 Plantar Test, Ankara, Turkey) was used to measure thermal nociceptive thresholds. Behavioral responses to thermal stimuli radiant heat was applied from below to the plantar surface of each hind paw and the withdrawal latency was measured with an electronic timer. Three measurements were performed on each hind paw with at least 1 min intervals to determine mean PWL. A preliminary or control threshold was determined for each rat before drug injection. The cut-off value was determined as 30 s in order to avoid tissue damage.

The preoperative and postoperative predrug thresholds of each rat were defined as the mean of the values from the last 3 stable thresholds. Each rat was tested after the i.p. administration of saline (control group), 5 mg/kg tramadol, 5 μg/kg dexmedetomidine and 5 mg/kg tramadol plus 5 μg/kg dexmedetomidine combination (5+5) groups. Nociceptive thresholds were then measured at every 10 min intervals for 2 h after drug administration until they returned to baseline.

2.3.3. Rotarod test

Sedation/motor incoordination was assessed with a rotarod apparatus, at a rotating speed of 16 r.p.m. A preliminary selection of rats was made on the day of experiment to exclude those that did not remain on the rotarod bar for two consecutive periods of 45 s each. Performance was measured before and 30 min and 120 min after the injection of saline, tramadol, dexmedetomidine and tramadol plus dexmedetomidine combinations. The rats were assigned to 15 groups. Each group received i.p. saline, five different doses of tramadol i.p. (5, 10, 20, 30, 40 mg/kg), dexmedetomidine (5, 10, 20, 30, 40 μg/kg) and tramadol+dexmedetomidine (5+5, 5+10, 10+5, 10+10 and 20+20, respectively). Results are expressed as percentage of animals that succeeded in remaining on the rod for 45 s, which was the cut-off time (Pieretti et al., 1999).

2.4. Statistical analysis

Data are given as the mean±standard error of the mean (S.E.M.), with N indicating the number of animals. Pre-drug and post-drug *N* values were equal in each group. Since a significant difference was detected between pain thresholds from the injured and uninjured paws, all the statistical analyses were performed using values expressed in seconds. Peak maximum percent effect (MPE) values of individual animals from the time–response data were pooled for each dose to construct the dose–response curves. MPE was obtained from the raw nociceptive threshold values by the formula: [MPE=(postdrug latency−predrug latency)/(cutoff latency−predrug latency)×100]. MPE data were entered into the GraphPad Prism (San Diego, CA) program to calculate the dose producing a 50% effect (ED_{50}) and associated 95% confidence intervals. Repeated measures ANOVA was used to evaluate both the effect of time for each administered dose and the effect of dose–time interaction. Because the sphericity assumption was not valid, Greenhouse–Geiser adjustment was used for correction of correlation. When the effect of dose–time interaction was statistically significant, Tukey's test was applied following the separate one way analyses for each time point to evaluate the multiple comparisons. The results of sedation/motor incoordination tested with the rotarod were statistically analyzed using two-tailed Fisher's Exact tests. SPSS statistical package was used for

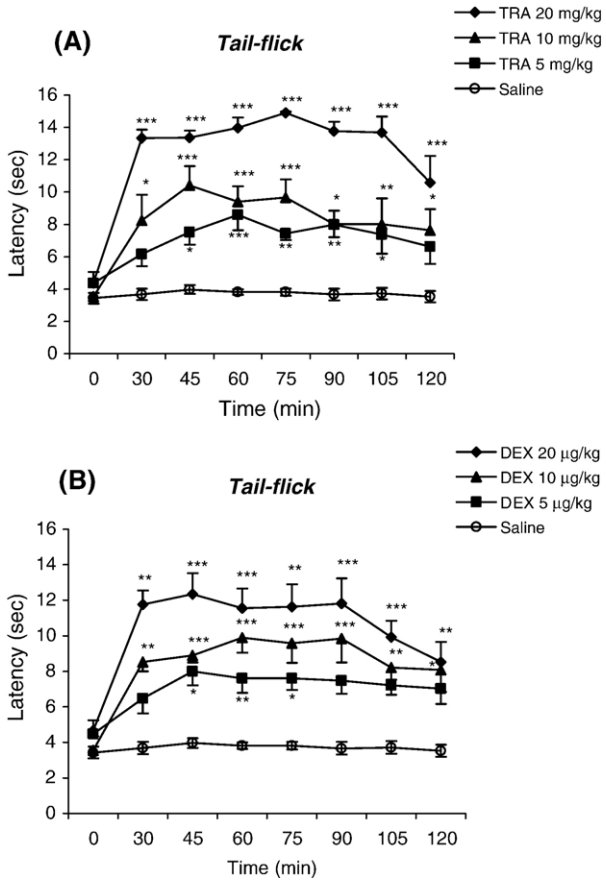

Fig. 1. Time course of antinociceptive effects to tramadol and dexmedetomidine in tail-flick test. (A) Tramadol (TRA), (B) dexmedetomidine (DEX). Data are presented as means±S.E.M. (n=7 in each group). The drug was administered just after the predrug baseline value was determined. *p<0.05 **p<0.01 ***p<0.001 *versus* saline (control).

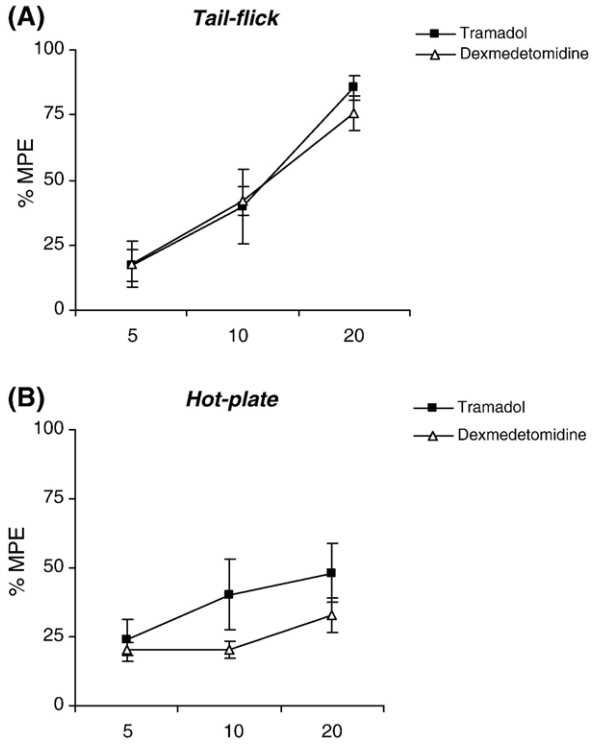

Fig. 2. Dose-antinociceptive response curves to tramadol and dexmedetomidine in the (A) tail-flick test and (B) hot-plate test in rats. Responses are expressed as % MPE (maximum percent effect). %MPE values at the time point at which peak antinociceptive responses were observed for each drugs (see Figs. 1 and 3) were used to plot the curves. Data are presented as means±S.E.M.

statistical analysis (ver. 11.0, Chicago, Illinois, USA). A value of p<0.05 was considered to be statistically significant.

3. Results

3.1. Acute nociception assay

The predrug response latencies in the tail-flick test and the hot-plate test were (4.09±0.12 s) and (4.77±0.16 s), respectively.

3.1.1. Effects of i.p. administered tramadol and dexmedetomidine alone in tail-flick test

Single drug administration of tramadol and dexmedetomidine produced a clear time and dose-related antinociception in tail-flick test (Fig. 1). The drug dose–response curves are displayed in Fig. 2A. The corresponding ED_{50} values from the dose–response curves are shown in Table 1. Effect of tramadol (5 mg/kg, i.p.) was apparent within 45 min, lasting until 105 min. 10 mg/kg and 20 mg/kg of tramadol was apparent within 30 min and lasting until 120 min. Peak effect of 5, 10 and 20 mg/kg of tramadol was achieved by 60, 45 and 30 min, respectively (Fig. 1A). Effect of dexmedetomidine (5 µg/kg, i.p.) was apparent within 45 min, lasting until 75 min. 10 µg/kg and 20 µg/kg of dexmedetomidine was apparent within 30 min and lasting until 120 min. Peak effect of 5, 10 and 20 µg/kg of dexmedetomidine was achieved by 45, 60–90 and 45 min, respectively (Fig. 1B).

3.1.2. Effects of i.p. administered tramadol and dexmedetomidine alone in hot-plate test

Single drug administration of tramadol and dexmedetomidine produced a time and dose-related antinociception in hot-plate test (Fig. 3). The drug dose–response curves are displayed in Fig. 2B. The corresponding ED_{50} values from the dose–response curves are shown in Table 1. Effect of tramadol (20 mg/kg, i.p.) was apparent within 45 min, lasting until 120 min. Tramadol (10 mg/kg) was apparent within 60 min and lasting until 120 min. Tramadol (5 mg/kg) did not produce a significant antinociceptive effect at all time points (p>0.05). Peak effect of 10 and 20 mg/kg tramadol was achieved at 90 and 60 min, respectively (Fig. 3A). Dexmedetomidine (5 µg/kg) did

Table 1
Potency of dexmedetomidine and tramadol as single i.p. drug administration in the tail flick and hot plate tests

	ED_{50}	
	Tail-flick	Hot-plate
Dexmedetomidine (µm/kg)	10.14 (9.6–10.72)	17.87 (very wide)
Tramadol (mg/kg)	10.30 (9.6–11)	9.67 (7.36–11.98)

Values represent ED_{50} (with 95% confidence intervals).

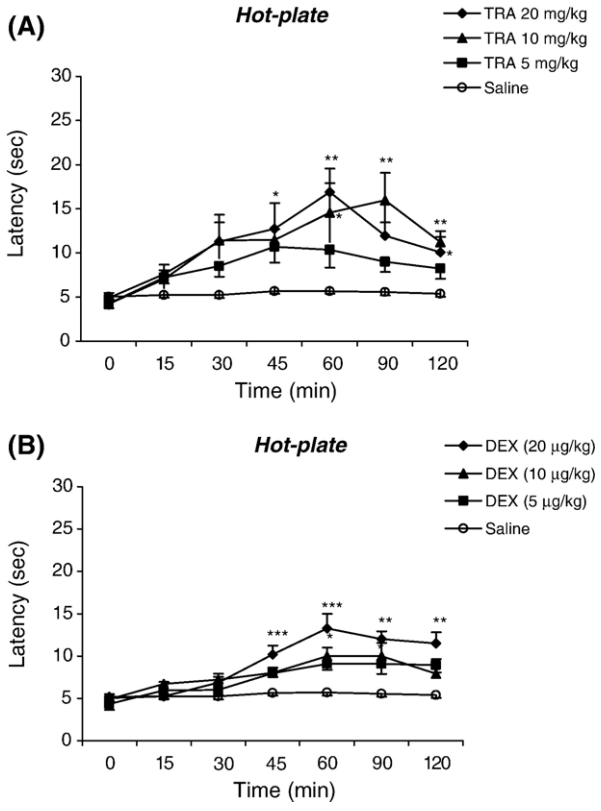

Fig. 3. Time course of antinociceptive effects to tramadol and dexmedetomidine in hot plate test. (A) Tramadol (TRA), (B) dexmedetomidine (DEX). Data are presented as means±S.E.M. ($n=7$ in each group). The drug was administered just after the predrug baseline value was determined. *$p<0.05$ **$p<0.01$ ***$p<0.001$ *versus* saline (control).

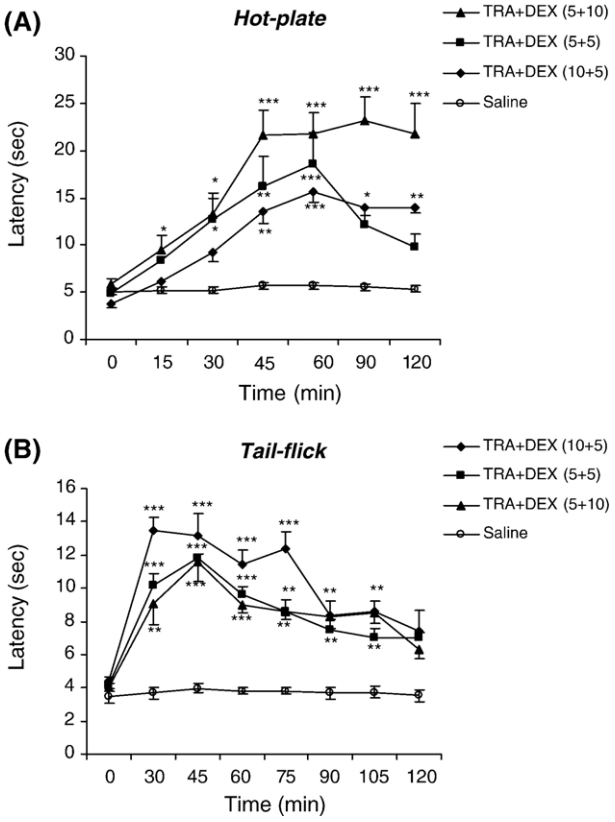

Fig. 4. Time-related response of the combination of the tramadol and dexmedetomidine [5 mg/kg+5 µg/kg (5+5), 5 mg/kg+10 µg/kg (5+10) and 10 mg/kg+5 µg/kg (10+5) respectively]. (A) Hot plate test, (B) tail-flick test. Data are presented as means±S.E.M. ($n=7$ in each group). *$p<0.05$ **$p<0.01$ ***$p<0.001$ *versus* saline (control).

not also produce significantly antinociceptive effect ($p>0.05$). Effect of dexmedetomidine (10 µg/kg) was apparent within 60 min, lasting until 90 min. Effect of dexmedetomidine (20 µg/kg) was apparent within 45 min and lasting until 120 min. Peak effect of 10 and 20 µg /kg dexmedetomidine was achieved at 60 min (Fig. 3B).

3.1.3. Effects of combined i.p. administration of tramadol and dexmedetomidine in tail-flick and hot-plate tests

Dose- and time-dependent antinociceptive effects of the drug combinations in tail-flick and hot-plate tests are shown in Fig. 4. In both tail-flick and hot-plate tests, various combinations of tramadol and dexmedetomidine produced an antinociceptive effect that is greater than that produced by tramadol (5 and 10 mg/kg) or dexmedetomidine (5 and 10 µg/kg) alone at several time points (Fig. 4, $p<0.05$). In hot-plate test, combination of tramadol+dexmedetomidine (5+10) exerted the most significant antinociceptive effect (Fig. 4A), however tramadol+dexmedetomidine (10+5) was significantly most effective in tail-flick test (Fig. 4B). However, combinations of these drugs did not attenuate the antinociceptive effects on each other.

3.2. Neuropathic pain assay

In presurgery, there were no differences between right and left paw withdrawal latencies in each group. CCI surgery (ipsilateral) applied rats showed significant changes in PWL ($p<0.001$) to heat stimuli, at 14–21 days after ligation of sciatic nerve compared to presurgery PWL values (Table 2). There was no obvious difference in the sham surgery (contralateral) applied PWL between groups.

The antinociceptive effects of the investigational drugs are summarized in Fig 5. This figure illustrates time course of the antinociceptive effect of i.p. injection of tramadol (5 mg/kg), dexmedetomidine (5 µg/kg) alone, and combination (5+5) on thermal hyperalgesia induced by CCI in rats. Low doses of tramadol (5 mg/kg) and dexmedetomidine (5 µg/kg) were not statistically effective alone ($p>0.05$), except dexmedetomidine (5 µg/kg) alone at 80 min ($p<0.05$). However, significant differences were detected in the combination group (5+5) when compared to saline, tramadol and dexmedetomidine groups,

Table 2
Induction of thermal hyperalgesia induced by chronic constriction injury (CCI) in rats

Group	Paw withdrawal latency (s)	
	Pre-surgery	Post-surgery
CCI (ipsilateral)	5.65±0.25	3.45±0.18*
Sham (contralateral)	5.00±0.22	4.59±0.24

Data are means±S.E.M.
* $p<0.001$ when compared to presurgery values.

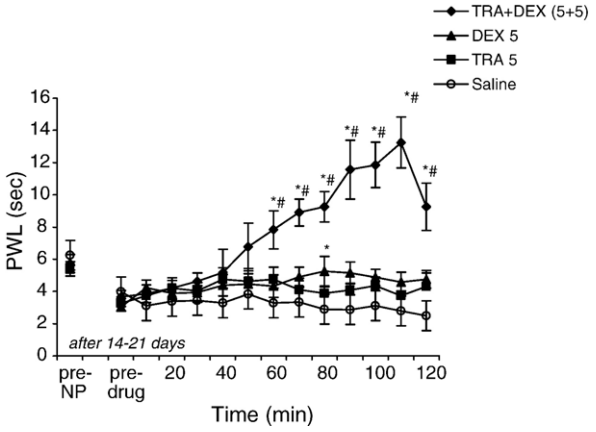

Fig. 5. Time course of the effect of i.p. injection of 5 mg/kg tramadol (TRA 5), 5 μg/kg dexmedetomidine (DEX 5) and 5 mg/kg tramadol+5 μg/kg dexmedetomidine [TRA+DEX (5+5)] on thermal hyperalgesia induced by CCI in rats. Data are presented as means±S.E.M. ($n=7$ in each group). The drug was administered just after the predrug baseline value was determined. PWL: paw withdrawal latency. *$p<0.05$ *versus* saline (control), #$p<0.05$ *versus* TRA and DEX.

between 60 and 120 min ($p<0.05$). On the other hand, no statistically significant differences were detected between the values for PWL before and after i.p. injection of 5 mg tramadol, 5 μg dexmedetomidine and combination dose (5+5) in sham groups ($p>0.05$, data not shown).

3.3. Rotarod test assay

In rotarod performance test; 30 and 40 mg/kg of tramadol, 30 and 40 μg/kg dexmedetomidine, and tramadol+dexmedetomidine combination (10+10 and 20+20) produced sedation/motor impairment at 30 and 120 min after i.p. administration compared to saline (control) in rats (Table 3, $p<0.05$), whereas tramadol (at doses of 5, 10 and 20 mg/kg), dexmedetomidine (at doses of 5, 10 and 20 μg/kg) and tramadol+dexmedetomidine combination (5+5, 5+10 and 10+5) did not produce any sedation/motor impairment ($p>0.05$).

4. Discussion

The main finding of the present study is that systemically administered (i.p.) combination of tramadol and dexmedetomidine provides potent antinociceptive effect at low drug doses that are below the threshold for producing sedation in acute and neuropathic pain.

The μ-opioid receptor and alpha(2)AR are thought to interact in modulation of nociception and analgesia. The interaction between μ-opioid receptor and alpha(2)AR has been reported by several studies; it has been shown that alpha(2)AR agonist-induced antinociception was attenuated by naloxone (Sullivan et al., 1992) and morphine or tramadol-induced antinociception by alpha(2)AR antagonists (Ossipov et al., 1989). Meert and De Kock (1994) found a potentiation in the effect elicited by opioids when alpha(2)AR agonists were concomitantly administered. Tham et al. (2005) found that dexmedetomidine and morphine showed an additive effect in acute pain models using tail-flick and hot-plate tests in mice. We have also used the same tests in our research. Ossipov et al. (1990) suggested that the interaction between the opioid and alpha(2)AR receptors occurred within the spinal cord. Sullivan et al. (1987) investigated the location of alpha(2)ARs and opioid binding sites by using *in vitro* autoradiography with selective ligands, and they demonstrated that both opioid and alpha(2)ARs were present within the same superficial layers of the dorsalhorn (laminae I and II), the site of entry of afferent A-δ and C pain-transmitting fibers into the central nervous system, which provided anatomic evidence for a possible interaction between the two systems. Moreover, analgesic synergism of opioids with alpha(2)AR agonists was lost in the alpha(2)AR-knockout mice, confirming the central role of alpha(2)AR in this effect (Ozdogan et al., 2004). The potent antinociceptive effect observed with systemic co-administrations (dexmedetomidine+tramadol), may be related with a central site of action, since it has been reported that dexmedetomidine crosses the blood–barrier easily (Khan et al., 1999; Smith and Elliott, 2001). It is also well known that tramadol exerts its analgesic effect as a centrally acting weak opioid analgesic agent; binds to μ-opioid receptors, inhibits neuronal reuptake of serotonin and norepinephrine (Raffa et al., 1992; Raffa and Friderichs, 1996).

The μ-opioid receptor and alpha(2)AR agonists have some similar pharmacological effects. It has been known that they have a similar distribution in the brain and that they function through the activation of the same transduction and effector mechanisms, *i.e.* G-proteins and coupling to potassium channels. Therefore, if μ-opioid receptor and alpha(2)AR agonists are administered together they may exhibit a synergistic action (Khan et al., 1999). However, the mechanism of interactions between μ-opioid receptor and alpha(2)AR systems still remains unclear.

Opioids may alleviate neuropathic pain, but at higher than normal doses, thus it can be difficult to treat the patients only with

Table 3
Effects of i.p. tramadol (TRA), dexmedetomidine (DEX) and combination of tramadol+dexmedetomidine (TRA+DEX) on rotarod test in rats

Drug	Dose	Number and (%) of falls in 45 s		
		n	30 min	120 min
Saline	NaCl (%0.9)	5	0 (0)	0 (0)
TRA	5 mg/kg	6	0 (0)	0 (0)
	10 mg/kg	6	0 (0)	0 (0)
	20 mg/kg	6	0 (0)	1 (16.6)
	30 mg/kg	6	5 (83)*	5 (83)*
	40 mg/kg	6	6 (100)**	6 (100)**
DEX	5 μg/kg	6	0 (0)	0 (0)
	10 μg/kg	6	0 (0)	0 (0)
	20 μg/kg	6	1 (16.6)	1 (16.6)
	30 μg/kg	6	6 (100)**	6 (100)**
	40 μg/kg	6	6 (100)**	6 (100)**
TRA+DEX	5 mg/kg+5 μg/kg	6	0 (0)	0 (0)
	10 mg/kg+10 μg/kg	6	5 (83)*	5 (83)*
	10 mg/kg+5 μg/kg	8	2 (25)	2 (25)
	5 mg/kg+10 μg/kg	8	4 (50)	4 (50)
	20 mg/kg+20 μg/kg	6	6 (100)**	6 (100)**

30 min: 30 min after drug administration, 120 min: 120 min after drug administration. *$p<0.05$ and **$p<0.01$ when compared with saline (control).

opioids (Arner and Meyerson, 1988; Jadad et al., 1992; Ossipov et al., 1995). Morphine potency and efficacy decreases in neuropathic pain, but may be restored or enhanced by co-administration of morphine with alpha(2)AR–selective analgesics (Fairbanks et al., 2000). However, the side effect profile of opioids should be considered when using large doses. Tramadol has an FDA-approved use for moderate to moderately severe pain, but as a relatively well-tolerated opioid it has appeal for treatment of neuropathic pain. Tramadol is considered an effective step II agent of the World Health Organization's analgesic ladder for the control of chronic pain conditions, including neuropathic pain, and also exhibits a good safety profile (Negro et al., 2005; Sindrup et al., 1999). Although tramadol is not a first-line drug for monotherapy of neuropathic pain, it has a widespread use for combination with other agents shown to be effective in neuropathic pain (Uyar et al., 2003; Negro et al., 2005). Tramadol may produce side-effects similar to that of other opioids (Raffa and Friderichs, 1996). However, this drug is generally assumed to be associated with fewer side-effects than more potent opioids.

It has been shown that alpha(2)AR agonists have antinociceptive properties under neuropathic pain states in animal (Puke et al., 1994; Ossipov et al., 1997) and human (Carroll et al., 1993; Glynn and Sullivan, 1995) studies. Previous animal studies have also shown synergistic antinociceptive interaction between μ-opioid receptor agonist and alpha(2)AR agonists in neuropathic animal models (Ossipov et al., 1997; Mansikka et al., 2002). Dexmedetomidine, a selective alpha(2)AR agonist, has been approved to be used as a sedative agent in intensive care unit settings (Bradley, 2000). It has been shown that systemically administered dexmedetomidine exerted significant analgesic effect in human studies (Jaakola et al., 1991; Hall et al., 2000). However, there are no studies showing the effects of dexmedetomidine on neuropathic pain behavior in humans. There are limited data about the antinociceptive effect of systemic dexmedetomidine on neuropathic pain in animal models (Kontinen et al., 1998; Poree et al., 1998; Kingery et al., 2000; Malmberg et al., 2001). It has been shown that systemically administered dexmedetomidine exerted dose-related antihyperalgesic effect in neuropathic heat hyperalgesia that is caused by tibial nerve transection in mice (Kingery et al., 2000). 5 μg/kg dexmedetomidine had no antinociceptive effect in this study. Similarly, it has been reported by Poree et al. (1998) that systemically administered dexmedetomidine also had dose-dependent analgesic potential in neuropathic pain in rats caused by spinal ligation and 5 μg/kg dexmedetomidine exerted no effect either. On the other hand, Kontinen et al. (1998) state that even high dose dexmedetomidine infusion was not effective in spinal ligation induced neuropathy in rats. Poree et al. (1998) also stated that the analgesic potency of dexmedetomidine was enhanced after nerve injury. However, these investigators administered dexmedetomidine systemically and suggested that the effect could be peripheral. These controversial results on the antihyperalgesic activity of alpha(2)AR agonists might derive from the differences in the different species from the route of administration and neuropathic models. We have not observed any antinociceptive effect with 5 μg/kg dexmedetomidine doses, however significant antinociceptive effect was seen in the CCI limb in rats with combination therapy. Therefore, dexmedetomidine appears to hold promise as an adjuvant for pain management uses in the future.

Although the primary goal of pain management in clinical practice was to relieve pain using a single agent, the reality is that monotherapy rarely provided adequate relief from chronic neuropathic pain. In these complex and refractory situations, combination therapy was frequently necessary. The combination therapy with two or more agents with different modes of action at suboptimal doses might provide synergistic effects necessary for optimal pain relief without compromising the side-effect profile of each agent (Jackson, 2006). Both dexmedetomidine and tramadol have been shown to exert an antihyperalgesic action in neuropathic pain states (Poree et al., 1998; Apaydin et al., 2000; Tsai et al., 2000). These drugs showed a dose-related reversal of thermal hyperalgesia following ligation of spinal and chronic constriction injury (CCI) of the sciatic nerve in rats (Poree et al., 1998; Tsai et al., 2000). Poree et al. (1998) have shown significant sedation with dexmedetomidine doses below the threshold for effective analgesia in neuropathic pain. This study has indicated that there was no accurate dosage range in this model of neuropathic pain allowing for analgesia without sedative side effects. Authors suggested that one way to overcome this problem might be a combination with other drugs. In the current investigation, using the paw withdrawal threshold to a thermal nociceptive stimulus, we have clearly demonstrated that systemically administered low doses of tramadol and dexmedetomidine alone did not produce significant antinociceptive effect; however their combination attenuated thermal pain-related disorders caused by peripheral sciatic mononeuropathy in Wistar rats. It has been chosen to use 5+5 combination because it was the best combination that the antinociceptive responses were observed and no sedation was determined.

In conclusion, systemically administered low doses of tramadol and dexmedetomidine combination appears to be effective in increasing pain threshold in rat models of acute and neuropathic pain when compared to the administration of these drugs alone. Future research will be needed to interpret the synergistic effects of this combination and required to determine the efficacy of this combination in acute and neuropathic pain in humans.

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