Effects of Perineural Administration of Dexmedetomidine in Combination with Levobupivacaine in a Rat Sciatic Nerve Block[☆]

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ARTICLE INFO

ABSTRACT

Objective: The aim of this study was to assess if perineural administration of dexmedetomidine combined with levobupivacaine increases the duration of the sensory and motor blockade of a sciatic peripheral nerve block in rats.

Methods: Forty male Sprague–Dawley rats were randomly divided into 5 experimental groups: Group 1, sham; Group 2, perineural levobupivacaine (0.2 mL of a 0.5% solution) and subcutaneous saline; Group 3, perineural levobupivacaine (0.2 mL of a 0.5% solution) plus dexmedetomidine (20 µg/kg dexmedetomidine) and subcutaneous saline; Group 4, perineural saline and subcutaneous dexmedetomidine; and Group 5, perineural saline and subcutaneous saline. Pain reflexes in response to a thermal stimulus were measured at 0 and 240 minutes after drug administration by using a hot-plate and tail-flick tests. Neurobehavioral status, including sensory and motor functions, was assessed by an investigator who was blinded to the experimental groups every 30 minutes until normal functioning resumed.

Results: The sensory and motor blockades of the rats did not increase in the treatment with dexmedetomidine plus levobupivacaine when compared with the treatment with levobupivacaine alone at all the time points ($P > 0.05$). Compared with rats in Group 2, those in Group 3 showed significantly higher latency times at 30 and 60 minutes in the hot plate test ($P < 0.01$). At 30 and 60 minutes, the latency times of the rats in Group 3 were longer than those in Group 2 in the tail-flick test ($P < 0.01$). Furthermore, the durations of the complete sensory and motor blockade were similar when treatment with levobupivacaine plus dexmedetomidine was compared with treatment with levobupivacaine alone.

Conclusions: A 20µg/kg dose of dexmedetomidine added to levobupivacaine did not increase the duration of the sensory and motor blockades in rats. However, treatment with dexmedetomidine plus levobupivacaine increased the quality of analgesia in rats.

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Introduction

Single injections of local anesthetics have been used in regional anesthesia and peripheral nerve blocks worldwide for many years. Long-lasting anesthetic solutions such as bupivacaine, levobupivacaine, and ropivacaine provide analgesia for approximately 6 to14 hours.[1] Postoperative pain may continue for several days, disrupting a patient's comfort and increasing the demand for opioids, which have very serious side effects. Because the analgesic effect of local anesthetics is very short, and prolonged block duration is essential for patients who have postoperative pain,[2] local anesthetics may be combined with other drugs to extend the duration of the block, and indwelling catheters may be used for administering them. Generally, the use of indwelling catheters is inconvenient except for administering drugs to the epidural space.[3]

Levobupivacaine is an S(-)-enantiomer of racemic bupivacaine and is a local anesthetic that is currently used in clinical practice. Levobupivacaine exhibits lesser cardiotoxicity and central nervous system (CNS) toxicity than that by R(+)-bupivacaine.[4] Levobupivacaine is widely used in spinal and epidural anesthesia and in supraclavicular nerve blocks.[5] In addition, many clinical studies have shown that clonidine extends the duration of peripheral nerve

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blocks.[6,7] Although its mechanism of action is currently unknown, it is thought the blockade is caused by local vasoconstriction or inhibition of impulse conduction in the peripheral nerves.[8] Additionally, α2-adrenoceptor agonists exhibit antinociceptive properties.[9] Dexmedetomidine exerts specific and selective α2-adrenoceptor effects.[10] Moreover, dexmedetomidine has been shown to be safe and effective in central and peripheral blockades in human beings[11,12] and has been widely used to achieve sedation in intensive care units.[13] Several studies have tested the addition of dexmedetomidine to local anesthetics in sciatic nerve blocks in rats to determine if the addition enhanced the duration of sensory and motor blockades.[1,14–16] For instance, Brummett et al[1] investigated the combined administration of dexmedetomidine and ropivacaine in sciatic nerve block in rats. They reported that dexmedetomidine added to ropivacaine increased the duration of sensory blockade. Another study also by Brummett et al[16] showed that the duration of complete sensory and motor blockades were lengthened in dexmedetomidine and bupivacaine groups. However, in those studies the authors evaluated these effects only through neurobehavioral tests, such as motor functions and sensory response, and observation of histopathology.

In our study we investigated the effects of perineural and intraperitoneal administration of levobupivacaine plus dexmedetomidine in sciatic peripheral nerve block in rats through the use of neurobehavioral tests and analgesia measures such as hot-plate and tail-flick tests.

Methods

Animals

We obtained 40 male Sprague–Dawley rats weighing between 210 g and 240 g from Inonu University Laboratory Animals Research Center, Malatya, Turkey. The rats were maintained in a room at a temperature of 21°C ($\pm$2°C) and relative humidity of 60% ($\pm$5%) under a 12-hour light/dark cycle. The animals were housed in plastic cages (50 $\times$ 35 $\times$ 20 cm, 8 animals per cage). The experiments were performed according to the standards of animal research issued by the National Health Research Institute and with the approval of the Inonu University Ethical Committee.

Drug administration

Levobupivacaine 1% solution was mixed with normal saline or dexmedetomidine to obtain final concentrations of 0.5% levobupivacaine and 0.5% levobupivacaine plus 0.005% dexmedetomidine. In addition, 0.01% dexmedetomidine was mixed with normal saline to obtain 0.005% dexmedetomidine. All dexmedetomidine doses were determined on the basis of the weights of the individual rats (20.0 μg/kg), with a final mean (SD) concentration of 119.3 (4.5) μmol/L.[16]

Experimental design

The rats were randomly divided into 5 groups consisting of 8 animals in each group: Group 1, sham; Group 2, perineural levobupivacaine (0.2 mL of a 0.5% solution) and subcutaneous saline; Group 3, perineural levobupivacaine (0.2 mL of a 0.5% solution) plus dexmedetomidine (20 μg/kg dexmedetomidine) and subcutaneous saline; Group 4, perineural saline and subcutaneous dexmedetomidine; Group 5, perineural saline and subcutaneous saline. The dosage of dexmedetomidine was chosen according to previous dose–response study by Brummett et al.[14]

Perineural sciatic nerve injection

An investigator blinded to the experimental drug conditions performed the sciatic nerve injections. Another investigator performed the subsequent neurobehavioral tests, and the laboratory assistants prepared the drugs. Rats without any signs of preprocedural neurobehavioral impairment were anesthetized using an intraperitoneal administration of 10 mg/kg ketamine (Ketalar; Pfizer, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun; Bayer, Toronto, Canada). The sciatic nerve of the right hind limb was exposed using a lateral incision over the thigh and division of the superficial fascia as previously described.[14,15] After the dissection, the sciatic nerve was identified at the point proximal to its bifurcation. Using a tuberculin syringe, 0.2 mL of the drug was injected into the perineural space. Following the perineural injection, 0.2 mL of the drug was subcutaneously injected into the fold of the skin at the back of the neck. A nonabsorbable muscle fascia suture was performed at the midpoint of the injection site as a marker for subsequent nerve removal. The suturing was performed in the muscle fascia of the biceps femoris below the subcutaneous tissue, and the sutures neither directly touched nor surrounded the nerve. All the incisions were closed. Eight intervals (0–240 minutes) from both the operative and control were obtained every 30 minutes from the time of injection. This interval was chosen according to previously related-studies.[1,14]

Measurement of analgesia

Acute thermal pain was determined using hot-plate and tail-flick tests, which are 2 established methods for measuring thermal analgesia in rodents.

Experimental procedures of the hot-plate test

The surface of the hot plate (Columbus Instruments, Columbus, Ohio) was heated to achieve a constant temperature of 50°C ($\pm$0.5°C), which was confirmed using a built-in digital thermometer. The time (in seconds) between the placement of the rats on the plate and the onset of shaking, paw licking, and jumping off the plate was recorded as the response latency. To avoid tissue damage, 60 seconds was set as the cut-off time after which the rats were returned to their cage, regardless of whether a response was observed.[17] The baseline was considered as the mean response times obtained at 0 and 30 minutes before drug administration and was defined as the normal response of the animal to the temperature stimulus. Latency during the hot plate test was measured at 0, 30, 60, 90, 120, 150, 180, 210, and 240 minutes after drug injection. No further nociceptive thresholds were measured until full recovery from the effects of the general anesthetic was achieved.

Experimental procedures in the tail-flick test

Antinociception and thermal analgesia were assessed using the radiant heat tail-flick test apparatus (Type 812; Columbus Instruments) as previously described.[18] Briefly, the rats were placed in transparent hard plastic tubes, and 3 independent tests were performed (within a 15-second interval) at each time point. The mean tail-flick latency was obtained from the measurements of 3 predrug trials, which represented the individual baseline latency. Animals demonstrating tail-flick latencies ranging from 2 to 5 seconds before the drug treatment were used in the experiments. Immediately after the baseline assessment, either drugs or saline were injected into the rats according to the experimental protocol. The responses were measured at 0, 30, 60, 90, 120, 150, 180, 210, and 240 minutes after treatment by applying pressure using an

analgesiometer on the tail (1 cm from the tail tip). The cut-off time was established at 10 seconds to avoid tissue damage. The timing of the drug injections was adjusted according to that previously described from related studies in rodents.[19,20]

Neurobehavioral examination

Sensory functions were evaluated by the withdrawal response of the paw to a forceps pinch on the lateral foot/toe. The pinch was limited to a maximum of 1 second to avoid direct paw tissue trauma. The rats were able to withdraw the tested paw in response to pain.[21,22] The sensory responses were evaluated on the basis of the withdrawal reflex or vocalization to the pinch and scored as follows: vigorous paw withdrawal response to pinch (normal sensory function) = 0, moderate withdrawal = 1, minimal withdrawal = 2, and full sensory block/no response to pinch = 3.[23–25] Similarly, the motor function was assessed using the 0 to 3 scale as follows: normal motor function = 0, normal dorsiflexion ability and walking with curled toes = 1, moderate dorsiflexion ability and walking with curled toes = 2, and no dorsiflexion ability and walking with curled toes = 3.[21,22] The sensory and motor functions were evaluated every 30 minutes by an investigator blinded to the experimental groups until the blockade was completely recovered.

Statistical analyses

For detecting even minor effects, the required sample sizes used in this experiment were identified using statistical power analysis. The sample sizes necessary for a power of 0.80 were estimated using NCSS software. Data were analysed using the SPSS software program for Windows (version 18.0, 2010; IBM Corp, Armonk, New York). The data were presented as the median (minimum–maximum). The assumption of a normal distribution was confirmed using the Kolmogorov–Smirnov test. The Kruskal–Wallis H-test was used when the assumption of normality was not provided. The Mann–Whitney U test with Bonferroni's correction was used for multiple comparisons. The statistical significance was established at $P < 0.01$.

Results

Measurement of analgesia

Compared with Group 2, rats in Group 3 showed significantly increased latency times at 30 and 60 minutes during the hot plate test (**Table I**) ($P < 0.01$). At 30 and 60 minutes, the latency times

Table II
Tail-flick latency time results, by treatment group.[*]

Time (min)	Group 1 (n = 8)	Group 2 (n = 8)	Group 3 (n = 8)	Group 4 (n = 8)	Group 5 (n = 8)	P Value
Median sec (minimum–maximum)						
Basal	8 (3–11)	9 (5–11)	8 (3–12)	8 (3–11)	8 (6–10)	
30	5 (4–15)	4 (3–7)	8 (5–15)[†]	5 (4–10)	4 (3–11)	0.0019
60	5 (5–12)	5 (4–11)	8 (4–15)[†]	4 (3–12)	5 (4–10)	0.0075
90	5 (3–8)	3 (3–11)	7 (3–14)	4 (2–8)	4 (3–11)	NS
120	5 (3–8)	5 (3–11)	3 (3–11)	5 (2–8)	4 (3–11)	NS
150	3 (2–15)	4 (3–10)	4 (3–7)	4 (4–10)	4 (3–11)	NS
180	6 (2–15)	6 (4–15)	5 (4–13)	4 (3–12)	5 (4–10)	NS
210	4 (3–8)	4 (3–9)	4 (3–7)	4 (3–8)	4 (3–7)	NS
240	3 (1–6)	3 (2–6)	4 (12–19)	5 (2–9)	4 (2–6)	NS

NS = Not significant.

* Group 1: sham, Group 2: perineural levobupivacaine (0.2 mL 0.5% solution) and subcutaneous saline, Group 3: perineural levobupivacaine (0.2 mL 0.5% solution) plus dexmedetomidine (0.2 mL 0.5% levobupivacaine + 20 μg/kg dexmedetomidine) and subcutaneous saline, Group 4: perineural saline and subcutaneous dexmedetomidine, Group 5: perineural saline and subcutaneous saline.
† Group 2 versus Group 3 ($P < 0.01$).

of the Group 3 rats were longer than those of the Group 2 rats in the tail-flick test (**Table II**) ($P < 0.01$). The results of the tail-flick and hot plate tests obtained at 30 and 60 minutes showed that the analgesic effect was better in the Group 3 rats (relative to the control group) than in the Group 2 rats.

Neurobehavioral results

No rats in Group 1 and Group 5 showed complete sensory or motor blocks. Dexmedetomidine combined with levobupivacaine did not enhance the sensory blockade when compared with levobupivacaine alone at all the time points (**Table III**). Moreover, dexmedetomidine and levobupivacaine did not enhance motor blockade when compared with levobupivacaine alone at all the time points (**Table IV**). Furthermore, the durations of the complete sensory blockade (sensory score = 3) and motor blockade (motor score = 3) were similar when treatment with levobupivacaine plus dexmedetomidine was compared with treatment with levobupivacaine alone (Table III). In addition, the time required for recovery of normal sensory function (sensory score = 0) and motor function (motor score = 0) were similar in treatment with levobupivacaine plus dexmedetomidine when compared with treatment with levobupivacaine alone.

Table I
Hot-plate test latency time results, by treatment group.[*]

Time (min)	Group 1 (n = 8)	Group 2 (n = 8)	Group 3 (n = 8)	Group 4 (n = 8)	Group 5 (n = 8)	P Value
Median sec (minimum–maximum)						
Basal	13 (7–17)	12 (8–15)	10 (9–13)	13 (8–17)	13 (7–16)	
30	15 (11–22)	20 (12–24)	25 (14–29)[†]	17 (12–25)	14 (7–19)	0.0001
60	15 (7–24)	20 (11–25)	24 (13–28)[†]	16 (8–18)	14 (6–21)	0.0004
90	14 (11–23)	17 (13–24)	17 (12–25)	15 (9–17)	13 (8–18)	NS
120	13 (8–23)	13 (8–23)	15 (10–25)	17 (10–20)	14 (10–19)	NS
150	13 (11–18)	12 (10–24)	15 (12–23)	17 (10–21)	13 (8–16)	NS
180	14 (8–17)	15 (11–20)	16 (12–24)	18 (11–24)	14 (11–22)	NS
210	14 (7–19)	13 (11–19)	14 (10–22)	15 (12–18)	15 (8–22)	NS
240	13 (11–16)	12 (11–16)	14 (12–19)	16 (12–19)	14 (10–19)	NS

NS = Not significant.

* Group 1: sham, Group 2: perineural levobupivacaine (0.2 mL 0.5% solution) and subcutaneous saline, Group 3: perineural levobupivacaine (0.2 mL 0.5% solution) plus dexmedetomidine (0.2 mL 0.5% levobupivacaine + 20 μg/kg dexmedetomidine) and subcutaneous saline, Group 4: perineural saline and subcutaneous dexmedetomidine, Group 5: perineural saline and subcutaneous saline.
† Group 2 versus Group 3 ($P < 0.01$).

Table III
Sensory blockade scores* for drugs, by group.[†]

Time (min)	Group 1 (n = 8)	Group 2 (n = 8)	Group 3 (n = 8)	Group 4 (n = 8)	Group 5 (n = 8)
			Median (minimum–maximum)		
30	0 (0–0)	2 (1–2)	2 (2–2)	1 (0–1)	0 (0–0)
60	0 (0–0)	2 (0–2)	2 (1–2)	1 (0–1)	0 (0–0)
90	0 (0–0)	2 (2–2)	2 (1–2)	1 (0–1)	0 (0–0)
120	0 (0–0)	2 (1–2)	2 (1–2)	0 (0–0)	0 (0–0)
150	0 (0–0)	1 (1–2)	1 (2–1)	0 (0–0)	0 (0–0)
180	0 (0–0)	1 (1–2)	1 (2–1)	0 (0–0)	0 (0–0)
210	0 (0–0)	1 (1–2)	1 (2–1)	0 (0–0)	0 (0–0)
240	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)

* Sensory scores (complete blockade sensory score = 3; normal sensory function = 0).
[†] Group 1: sham, Group 2: perineural levobupivacaine (0.2 mL 0.5% solution) and subcutaneous saline, Group 3: perineural levobupivacaine (0.2 mL 0.5% solution) plus dexmedetomidine (0.2 mL 0.5% levobupivacaine + 20 µg/kg dexmedetomidine) and subcutaneous saline, Group 4: perineural saline and subcutaneous dexmedetomidine, Group 5: perineural saline and subcutaneous

Table IV
Motor blockade scores* for drugs, by group.[†]

Time (min)	Group 1 (n = 8)	Group 2 (n = 8)	Group 3 (n = 8)	Group 4 (n = 8)	Group 5 (n = 8)
			Median (minimum–maximum)		
30	0 (0–0)	2 (1–2)	2 (2–2)	1 (0–1)	0 (0–0)
60	0 (0–0)	2 (0–2)	2 (1–2)	1 (0–1)	0 (0–0)
90	0 (0–0)	2 (2–2)	2 (1–3)	0 (0–1)	0 (0–0)
120	0 (0–0)	1 (1–2)	1 (1–2)	0 (0–0)	0 (0–0)
150	0 (0–0)	1 (1–2)	1 (1–1)	0 (0–0)	0 (0–0)
180	0 (0–0)	0 (0–1)	0 (0–1)	0 (0–0)	0 (0–0)
210	0 (0–0)	0 (0–1)	0 (0–1)	0 (0–0)	0 (0–0)
240	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)

* Sensory scores (complete blockade sensory score = 3; normal sensory function = 0).
[†] Group 1: sham, Group 2: perineural levobupivacaine (0.2 mL 0.5% solution) and subcutaneous saline, Group 3: perineural levobupivacaine (0.2 mL 0.5% solution) plus dexmedetomidine (0.2 mL 0.5% levobupivacaine + 20 µg/kg dexmedetomidine) and subcutaneous saline, Group 4: perineural saline and subcutaneous dexmedetomidine, Group 5: perineural saline and subcutaneous saline.

Discussion

α2-Adrenoceptors are expressed in the CNS and peripheral tissues, including sympathetic nerve endings, neurons, vascular smooth muscles, and platelets;[7] however, the analgesic effect of peripheral receptors have not been studied in the periphery.[8] Agonists for the α2-adrenoceptor exert analgesic effects on the CNS.[26] The mechanism of action of dexmedetomidine in the CNS was reduced, because dexmedetomidine inhibits the activity of rat hypothalamic paraventricular nucleus neurons and paraventricular nucleus parvocellular neurons.[27]

One of the important findings of our study is that the dexmedetomidine and levobupivacaine group showed significantly more analgesic status than the levobupivacaine alone group, according to both hot-plate and tail-flick test results. This beneficial effect was dominant especially at 30 and 60 minutes parallel to the drug's pharmacokinetics. Although there is no difference between the control and local anesthesia group according to the hot-plate and tail-flick test results at >90 minutes, in fact this is an unexpected result according to the drug's half-time. However, as shown in **Table III** and **Table IV** sensory and motor blockade

continue until 240 and 180 minutes, respectively. There is no literature to explain this circumstance. One possible explanation is the well-known phenomenon that the nerves related with pain—such as A_δ and C fibers—is blocked earlier than motor functions.[28]

Another important finding of our study is that the dose of dexmedetomidine used did not increase the duration of the sensory and motor blockades when added to levobupivacaine. Nor did subcutaneous dexmedetomidine increase the duration of sensory analgesia. Our administered dose of subcutaneous dexmedetomidine was too low to induce a central analgesic effect; previous studies have shown that dexmedetomidine at a dose of 144 µg/kg exerts a central analgesic effect.[8,29] Previous studies have indicated that high doses of perineural dexmedetomidine (28–40 µg/kg) added to ropivacaine and bupivacaine increases the duration of sensory and motor blockades in rats.[14,16] On the contrary, in our study, the combination of dexmedetomidine (20 µg/kg) and levobupivacaine did not increase the duration of the sensory and motor blockade when compared with the levobupivacaine alone. One of the possible explanations of this result is that the duration of sensory and motor blockade is strongly related to the dosage of dexmedetomidine. It has been reported that using clonidine in peripheral nerve blocks, similar to dexmedetomidine, can cause centrally mediated analgesia, α2-adrenoceptor-mediated vasoconstrictive effects, inflammatory responses, and direct activities on the peripheral nerve. Also, Brummett et al[1] reported that clonidine enhances activity-dependent hyperpolarization by blocking the hyperpolarization-activated current. It is well established that clonidine is a postoperative analgesia with an activity-dependent inhibition of inwardly rectifying potassium currents. Moreover, α2-adrenoceptors have a vasoconstriction effect in the periphery; however, the vasoconstrictive properties of clonidine are weaker than that of adrenaline.[30] Based on this relationship, Brummett et al[1] declared that unlike adrenaline, the enhancement of the sensory blockage by clonidine is not attenuated by the coadministration of α-adrenoceptor antagonists.[31,32]

Limitations

In our study, the evaluation of motor block and the paw thrust measures used were limited to subjective assessments that were adopted based on measures described in previous studies.[14] Systemic administration of dexmedetomidine provided analgesia and sedation because high doses of perineural dexmedetomidine could be absorbed, thereby mediating a central effect that might also cause sensory and motor blockades. However, the doses used in this study were too low for use in human studies. We speculate that high doses of dexmedetomidine combined with levobupivacaine could render an increased duration of sensory and motor blockades. Because of our limited facilities, we used ketamine for anesthesia instead of inhaler anesthesia in this study. This is in accordance with previous studies.

Conclusions

Dexmedetomidine combined with levobupivacaine did not increase the duration of the sensory and motor blockade at a dose of 20 µg/kg. However, dexmedetomidine combined with levobupivacaine increased the quality of analgesia in rats. Thus, we propose that drug combinations be used in clinical practice to achieve stable analgesia. Furthermore, our results can be used as a guide for future studies in human beings.

Acknowledgments

Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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