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SCIENTIFIC ARTICLE

Anti-Nociceptive, Analgesic and Pathohistological Effects of Intrathecal Dexmedetomidine and Bupivacaine in Rats

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Abstract

Background and Objectives: This study investigates analgesic and nociceptive effects of adding dexmedetomidine to bupivacaine neuraxial anesthesia through Tail-flick (TF) and Hot-plate (HP) tests and the pathohistological changes on spinal nerves and nerve roots through light microscopy.

Methods: Forty anesthetized, male Sprague-Dawley rats were intrathecally catheterized. Basal values of TF and HP tests were measured before and after catheterization. Thirty-six successfully catheterized rats were assigned to four groups. Group B received 10 µg bupivacaine, Group BD3 received 10 µg bupivacaine + 3 µg dexmedetomidine, Group BD10 received 10 µg bupivacaine + 10 µg dexmedetomidine and Control group received 10 µL volume of artificial cerebrospinal fluid. TF and HP tests were performed between the 5th and 300th minutes of drug administration. Twenty-four hours after administration of drugs, rats were sacrificed and spinal cord and nerve roots were removed for pathological investigation.

Results: Baseline values of the TF and HP tests were not statistically different among the groups (6.8 ± 0.15 s). TF and HP latencies in the Control group did not change significantly during the study. TF and HP test results showed that adding 3 and 10 µg dexmedetomidine caused a dose-dependent increase in duration and amplitude of analgesic and nociceptive effect of bupivacaine (TF: 37.52 ± 1.08%, 57.86 ± 1.16% respectively, HP: 44.24 ± 1.15%, 68.43 ± 1.24% respectively).

Conclusions: There were no apparent pathohistological changes at least 24 hours after the intrathecal administration of a single dose of dexmedetomidine 3 µg and 10 µg. Dexmedetomidine added to bupivacaine for spinal block improves analgesia and prolongs block duration.

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Introduction

Spinal anesthesia is the blockage of nerve transmission through injecting a local anesthetic agent into the cerebrospinal fluid [1]. Its short duration of action and concerns of toxicity resulted in the addition of several adjunct drugs [2,3]. Benzodiazepines [4], opioids [5], neostigmine [6], ketamine [7] and α_2-receptor agonists [8,9] were used to overcome these shortages. Many of these adjuncts have side effects like itching, urinary retention, respiratory depression, nystagmus, cardiac depression, nausea and vomiting. Lacking many of the above-mentioned side effects and with its safer cerebral and respiratory profile, α_2-receptor agonists prolong motor and sensory blockade duration, which makes them very popular adjuncts to neuraxial anesthesia.

In this study, we investigated how dexmedetomidine added to bupivacaine neuraxial anesthesia would affect spinal and supraspinal pain pathways through Tail-flick (TF) and Hot-plate (HP) tests and its neurotoxic effect on spinal nerves and nerve roots through light microscopy.

Materials and Methods

Forty male inbred Sprague Dawley rats (weight, 350-400 g; age, 15-16 weeks) were obtained from Pendik Faculty of Veterinary Science Laboratories (Istanbul, Turkey). The rats were housed in separate cages with unlimited water and food and kept in temperature controlled rooms (20-24°C, relative humidity 50-60%) on a 12-hour light and 12-hour dark cycle (dark cycle beginning at 7:00 p.m.). All tests were performed in the morning. The animals were handled according to the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council. The Ethical Committee of Pendik Faculty of Veterinary Science Laboratories, Istanbul, Turkey (Chairperson Dr. Muhammet AKSIN, Ph.D.) gave ethical approval for this study (Ethical Committee No: 13) on 19 January 2009.

The rats were allowed to acclimatize to the laboratory environment for 3 days. Body weights were measured daily throughout the study. On the fourth day, all rats were implanted with an intrathecal (IT) catheter using a modified method of Yaksh [10]. Anesthesia was provided with 60 $mg.kg^{-1}$ intraperitoneal sodium pentobarbital and periodic supplements of 10 $mg.kg^{-1}$ of pentobarbital. Response to foot stimulation was monitored to ensure adequacy of anesthesia. In a prone position, the cervical region of rats was shaved and sterilized with povidone-iodine. A longitudinal 2 cm incision was made and the neck musculature was gently retracted to expose the atlanto-occipital membrane. After we pierced the membrane using a sterile 18-gauge disposable needle, there was an abundant outflow of clear cerebrospinal fluid. A stainless steel wire guide of a spinal needle fitting into the 32-gauge polyurethane (PE32) catheter was used to ease the insertion and prevent the catheter from bending and the traumatization of the spinal cord. The catheter was carefully pushed until vertebral body T11 (7.4-7.7 cm) into the spinal space from the incision whilst applying slight tension to the tail in order to place the tip at the T_{11-12} vertebral level, estimated to be the L_{3-4} level of the spinal cord. After insertion, we aspirated and flushed it with artificial cerebrospinal fluid (ACSF) prepared using sterile water and adding commercial electrolyte solutions (Merck) to achieve a final concentration of ($mEq.L^{-1}$) Na: 150, K: 3, Ca: 1.4, Mg: 0.8, P: 1 and Cl: 155 with a pH of 7.4 [11]. A bacterial filter was clamped and sealed on the outside end of the catheter. The incision was sutured and the rat returned to a separate cage for 3 days to recover. Rats were checked for motor or sensory deficits after recovery and evaluated daily by using a 4-point scale. Animals with any sign of neurologic deficit, infection, catheter displacement or clogging were discarded. A total of 4 animals were excluded from the study (2 because of death during catheterization and 2 because of infection).

Thirty-six rats were randomly assigned into four groups. Randomization procedure was done through computerized equalization of mean body weights of each group of rats. Drugs for IT injection were dissolved in a 10 µL volume of ACSF. All drugs were administered over a 1-minute time period. Group B received 10 µg bupivacaine, Group BD3 received 10 µg bupivacaine + 3 µg dexmedetomidine, Group BD10 received 10 µg bupivacaine + 10 µg dexmedetomidine and Control group received 10 µL volume of ACSF on the fourth day after catheterization.

The TF test was structured with a heat source (100 Watt light bulb) focused on the dorsal surface of the tail approximately 4 cm from the tip. We measured latency in withdrawal from heat source. Lack of occurrence of withdrawal in 14 seconds resulted in termination of the stimulus to avoid damage to the tail.

HP test was structured with a Hot Plate Analgesia Meter from Columbus Instruments (Columbus, OH). The surface of the hot plate was heated to a constant temperature of 55°C. We measured latency to move the hind-paws or jump. The cutoff time was 50 seconds to avoid tissue damage. Baseline values of the TF and HP tests were measured on the first, fourth and seventh days. Post-injection values of the tests at 5^{th}, 10^{th}, 15^{th}, 20^{th}, 30^{th}, 45^{th} and 60^{th} minutes and every 30 minutes until the 300^{th} minute were recorded. Results were expressed as percentage of maximum possible effect (%MPE) according to the following formula: %MPE = (post-injection latency - pre-injection latency) x 100 / (cut-off time - pre-injection latency). The study was performed double-blindly at the stages of catheterization, drug injection and measurements.

After the tests were completed, animals were put into separate cages and euthanized the next day (approximately 24 hours after injection) with a 1 mL intra-cardiac dose of 26% sodium thiopental and the spinal cord removed *en bloc* via a modified technique as described elsewhere [12]. Briefly, after excising the surrounding skin and muscle tissue, the spinal column was removed and whole lumbar section was separated. The vertebral column was carefully cut. The dorsal and ventral cords were spared and cut from outside the vertebral foramen. During tissue removal, we verified the catheter placement. After tissue fixation in 10% neutral buffered formalin (Fisher, Pittsburgh, PA) for 48 hours, sections were embedded in paraffin, sliced in 5-10 µm thickness, and stained with hematoxylin-eosin, luxol fast blue, trichrome [13] and Weil's myeline sheath [14]. Longitudinal and parallel sections were obtained at the tip of the catheter, 1 mm on either side of the tip, and at 2 mm intervals on either side until a distance of 10 mm was reached. A neuropathologist blinded to the injections evaluated sections.

Statistical Analysis

We used SPSS 17 for Windows to evaluate data. We analyzed starting weights and weight gains of rats throughout the study period using Student's *t*-test. Distributions of TF and HP test results were tested using the Shapiro-Wilk test. Results were analyzed using variance analysis with repeated measures and expressed as mean ± SEM. We used Tukey's procedure for post hoc comparison. We evaluated motor function, sensorial function and pathohistological results using Fisher's exact test. Differences were considered to be significant at $p < 0.05$.

Results

We prepared 40 animals and analyzed the results from 36, as four were excluded from the study. Statistical analysis revealed a normal distribution of starting weights, no difference in trends of weight gain and rats' food and water consumption throughout the study.

Evaluations using the 4-point scale showed no sign of neurologic deficit, infection, catheter displacement or clogging. Baseline values of the %MPE values in the TF and HP tests were not statistically different among the groups (using Student's *t*-test) before drug administration (4 ± 1 s for all groups). TF and HP latencies in the Control group did not change significantly during the study. The measured pH values were 6.36 ± 0.05 for bupivacaine, 6.02 ± 0.04 for dexmedetomidine and 6.08 ± 0.04 for mixtures of dexmedetomidine and bupivacaine.

During the TF test, 10 µg bupivacaine caused a moderate antinociception, which peaked at the 20th min (mean: 48.3%, SEM: 0.9%) and was sustained until the 240th min. Addition of 3 µg dexmedetomidine to 10 µg Bupivacaine caused a high level of antinociception, which peaked at the 30th min (mean: 74.4%, SEM: 1.6%) and was sustained until the 300th min. Addition of 10 µg dexmedetomidine to 10 µg Bupivacaine caused a high level of antinociception, which peaked at the 30th min (mean: 97.9%, SEM: 1.1%) and surpassed the 300th min (Figure 1).

A repeated measures ANOVA test with a Greenhouse-Geisser correction determined that mean %MPE levels differed statistically significantly between time points [F (8.631, 45) = 12460.737, $p < 0.0005$] for the TF test. Post hoc tests using the Tukey's procedure revealed that 10 µg bupivacaine caused an increase in %MPE levels (22.07 ± 0.98% vs. 0.1 ± 0.1%, respectively) which was statistically significant ($p < 0.001$), addition of 3 µg dexmedetomidine to bupivacaine caused an extra increase in %MPE levels (37.52 ± 1.08%) which was statistically significant ($p < 0.001$), addition of 10 µg dexmedetomidine to bupivacaine caused a further increase in %MPE levels (57.86 ± 1.16%) which was statistically significant ($p < 0.001$).

During HP test, 10 µg bupivacaine caused a moderate antinociception, which peaked at the 30th min (mean: 54%, SEM: 1%) and was sustained until the 300th min. Addition of 3 µg dexmedetomidine to 10 µg Bupivacaine caused a high level of antinociception, which peaked at the 30th min (mean: 76.9%, SEM: 1.7%) and was sustained until the 300th min. Addition of 10 µg dexmedetomidine to 10 µg Bupivacaine caused a high level of antinociception, which peaked at the 15th min (mean: 98.7%, SEM: 0.5%) and surpassed the 300th min (Figure 2).

A repeated measures ANOVA test with a Greenhouse-Geisser correction determined that mean %MPE levels differed statistically significantly between time points [F (7.655, 45) = 12612,953, $p < 0.0005$] for the HP test. Post hoc tests using the Tukey's procedure revealed that 10 µg bupivacaine caused an increase in %MPE levels (28.37 ± 0.99% *vs.* 0.1 ± 0.1%, respectively) which was statistically significant ($p < 0.001$), addition of 3 µg dexmedetomidine to bupivacaine caused an extra increase in %MPE levels (44.24 ± 1.15) which was statistically significant ($p < 0.001$), addition of 10 µg

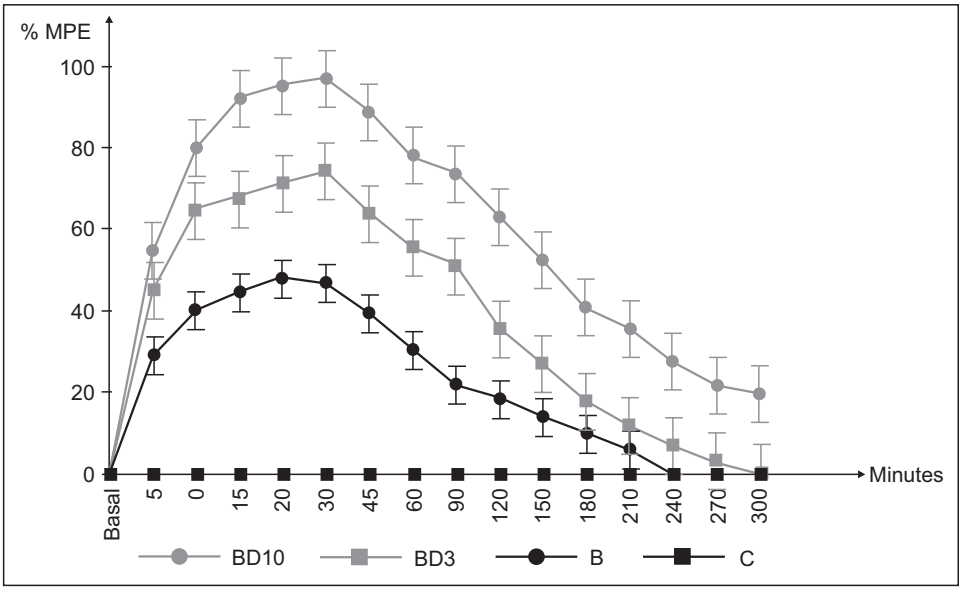

Figure 1 Time course of the antinociceptive effect (%MPE) of intrathecally administered drugs measured by the TF test.

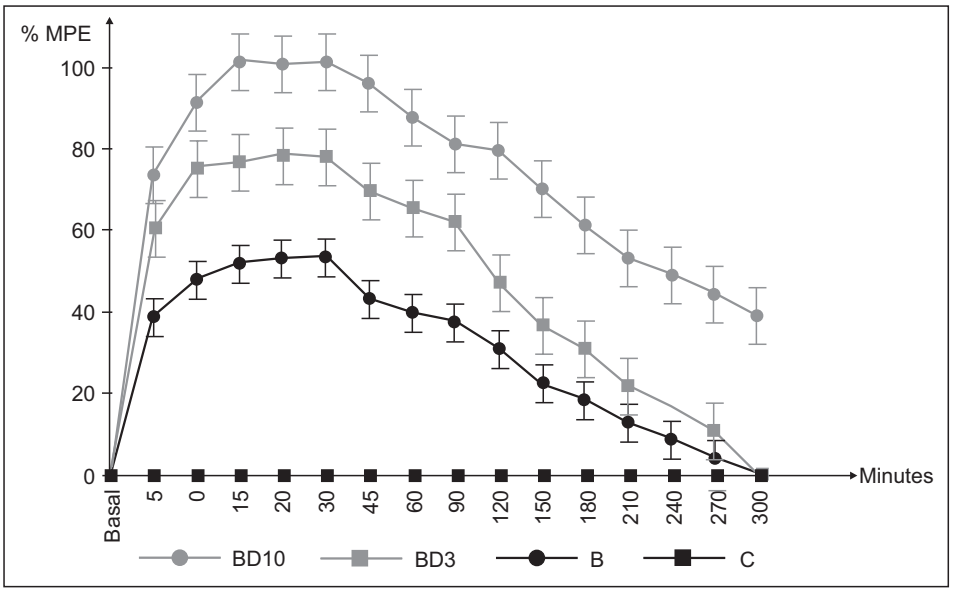

Figure 2 Time course of the antinociceptive effect (%MPE) of intrathecally administered drugs measured by the HP test.

dexmedetomidine to bupivacaine caused a further increase in %MPE levels (68.43 ± 1.24%) which was statistically significant ($p < 0.001$).

At the 24th hour after injections, cellular structure was preserved on all slides. No pathologic change was noted in grey and white matter, dural, pial and arachnoid membranes. Spinal nerve roots and ganglion cells retained their morphology. There was no apparent neurotoxicity at least 24 hours after the administration of single dose of dexmedetomidine 3 µg and 10 µg ($p > 0.05$).

Discussion

This study showed that addition of dexmedetomidine to bupivacaine spinal block enhances the amplitude and duration of nociception to a thermal stimulus. Dexmedetomidine acts on several places to show its antinociceptive action. It binds to presynaptic C-fibers transmitting noxious stimuli from peripheral receptors to spinal cord. It also binds to α_{2A}- and α_{2C}-adrenoceptors of postsynaptic neurons present in superficial layers of the spinal dorsal horn [15]. Its antinociceptive effects result both from inhibition of presynaptic C-fibers and hyperpolarization of postsynaptic dorsal horn neurons [16]. Ishii and colleagues reported that dexmedetomidine induced activation of K^+-channels through α_{2A}- and α_{2C}-adrenoceptors results in hyperpolarization of substantia gelatinosa neurons [15]. Similarly, Brummett and colleagues reported that dexmedetomidine's antinociceptive action depends on inhibiting the build up of excitation via hyperpolarization-activated cation current (I_h) and is reversed by I_h agonist forskolin [17].

Among spinal catheterization methods, sacral approach is mainly suited for epidural drug administration and lumbar approach carries the risks of bone damage and inflammation due to fixatives. Therefore the atlanto-occipital approach has been preferred as it was well described by Yaksh et al. [10]. PE32 catheters were used to minimize damage to spinal cord and nerve roots [2]. We chose the spinal route for drug delivery to eliminate the concerns about insufficient diffusion of drugs through dural membrane and also to observe the effect of drugs, pH and catheters on spinal cord. Several studies verified accurate placement of perineural catheter using lidocaine or any local anesthetic to produce a motor block. In our study, no drug other than the study ones were injected through the catheters to minimize effects, therefore catheter placement was verified by postmortem examination.

Our study showed that dexmedetomidine dose dependently prolonged the duration and augmented the analgesic quality of the spinal block. Our results concord with Calasans and colleagues' study reporting that levobupivacaine-induced spinal block was prolonged by intrathecal dexmedetomidine in doses up to 0.4 µg and intraperitoneal doses up to 40 $\mu g.kg^{-1}$, which was reversed by an α-adrenergic antagonist, yohimbin [18]. These results suggest that dexmedetomidine exerts its effects through α_2-adrenoceptor agonism. Brummett and colleagues' study investigating addition of dexmedetomidine to ropivacaine induced peripheral nerve block [19]. They administered dexmedetomidine in doses up to 20 $\mu g.kg^{-1}$, and the pH value of mixture of ropivacaine and dexmedetomidine was reported to be 5.69 ± 0.05, which is far more acidic than the pH values of drug mixtures measured in our study. This and the fact that a pathohistological examination of nerve tissues were done after 24h of drug administration may explain why our study did not show signs of inflammation or nerve damage.

The TF response is organized at the spinal level, the HP response is mediated by both spinal and supraspinal levels [20]. The increase in withdrawal latency times in our study was similar in both TF and HP test results. This may suggest that intrathecally administered dexmedetomidine did not reach the supraspinal centers or that its effects on supraspinal centers were less prominent than on dorsal horn neurons.

In two different studies investigating dexmedetomidine's effects on nerve blocks, onset time of nerve blocks are reported to be shortened by Gupta and colleagues investigating spinal block in lower abdominal surgery and Esmaoglu and

colleagues investigating axillary nerve blocks [16,21]. In our study, the first measurements in both tests were done at the fifth minute, which prevented the study to show a possible difference between the start times of the anti-nociceptive effect. Since the anesthesia start time is of critical importance in regional anesthesia, frequent measurements starting earlier are needed to clarify any possible difference.

Conclusion

Dexmedetomidine added to bupivacaine for spinal block improves analgesia and prolongs block duration.

Acknowledgments

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