Evaluation of the neurotoxicity of intrathecal dexmedetomidine on rat spinal cord (electromicroscopic observations)

ABSTRACT

Background: Spinal administration of dexmedetomidine has been proposed as an adjuvant in spinal anesthesia. However, there is limited information about its possible neurotoxic effect after its neuraxial administration. Potential spinal neurotoxicity should be investigated in animals before administering drugs through the spinal cord. Our aim was to investigate the neurotoxic effects of intrathecal dexmedetomidine in rats.

Methods: Two groups were performed: the dexmedetomidine (D) group ($n = 10$) received 10 μg (0.5 ml), whereas the control (C) group ($n = 10$) received 0.9% (0.5 ml) sodium chloride through indwelling intrathecal catheter. Seven days after the injection, the medulla spinalis was extracted. Samples were withdrawn from both groups for histologic, electron microscopic examination. The histologic examination was performed separately on each of the four sites. The findings were categorized as follows: 0 - normal neuron; 1 - intermediate neuron damage; and 2 - neurotoxicity.

Results: Intrathecal administration of dexmedetomidine sensorial block was seen in the dexmedetomidine group and significant differences in the dexmedetomidine group than control group in 15th and 30th min ($P < 0.05$). Histological examination did not show evidence suggestive of neuronal body or axonal lesion, gliosis, or myelin sheath damage in any group. In all animals, there were observed changes compatible with unspecific inflammation at the tip of the needle location. On the four-area scoring histologic examination, the scores of both groups were 0–1, and no statistical difference was observed between the groups.

Conclusions: A single dose of intrathecal dexmedetomidine did not produce histologic evidence of neurotoxicity.

Key words: Dexmedetomidine; intrathecal; neurotoxicity

Introduction

Potential spinal neurotoxicity should be investigated in animals before beginning a clinical experiment in which the drugs are administered through the spinal column. Unfortunately, many agents are used by clinical investigators without such preliminary screening.

Neuraxial drug administration describes a technique in which drugs are delivered to the spinal cord in proximity, such as intrathecal administration into the cerebrospinal fluid or epidural administration into the fatty tissues surrounding the dura, by injection or infusion.

Intrathecal α2-agonists are used as adjuvant drugs for local anesthetics.[1-3] Intrathecal α2-agonists potentiate the effect of local anesthetics and decrease the required doses.[2-4]

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Dexmedetomidine, a potent and highly selective α2-adenoreceptor agonist, is currently used in clinical, particularly in the Intensive Care Unit.[5] Dexmedetomidine produces an analgesic effect through pre- and post-synaptic α2-adrenergic receptors in the spinal cord.[6,7] Functional and anatomic studies suggest that this analgesic effect is mediated by cholinergic activation.[5]

Limited electron microscopic, histopathologic investigations exist regarding the intrathecal application of dexmedetomidine.

Some studies have demonstrated an association between parameters, such as analgesic efficacy of the drug and epidural application. The study results regarding the analgesic efficacy and toxicity of clonidine, α2-agonist similar to dexmedetomidine, following epidural and intrathecal application have been reported.[8,9]

In this study, our aim was to investigate the neurotoxic effects of intrathecal dexmedetomidine in rats.

Methods

This study was approved obtained from the Ethical Committee of University Experimental Animal Studies before the study was conducted. The study was performed on twenty adult rats (weighing between 320 and 340 g), which were divided into two groups after randomly assigned (computer generated random numbers). The rats were kept in different cages at a room temperature 21°C ± 2°C in the animal research laboratory for 10 days and fed a standard diet before the intrathecal injection.

Before the procedure, 3 mg/kg of xylazine was administered intramuscularly for sedation. Thereafter, the experimental animals were secured on the study table in the prone position. The lumbosacral area was shaved, and asepsis was achieved with 10% povidone iodine. A 22-gauge nerve stimulator injector (1 Stimuplex® A 50.22-gauge × 2", 0.70 mm × 50 mm, B/BRAUN, Melsungen AG) was placed on nerve stimulator. Both iliac spine of the animal were palpated, and on the line passing both iliac spines, spinous process was labeled. Characteristic bilateral back leg muscle contraction was looked for as a respond to low-density stimulation through nerve stimulator stimulation frequency 1 Hz, 1 ms, and intensity 0.2 mA. After detection of bilateral back leg muscle contraction, the drug was administered into the subarachnoid space of L4–L5 interspace. 10 μg of 0.1 ml dexmedetomidine was injected in dexmedetomidine group and 0.1 ml of 0.9% NaCl was injected in control group.

Following the intrathecal injection, both groups were examined after 5, 15, 30, 45, and 60 min to determine whether or not a motor or sensorial blockade was present. Neurological function was measured by an investigator who was blinded to the solution administered to each animal. Tail-fick (TF) test was performed to examine the response which the tail gives to the noxius heat stimulus. A 100-W projector lamp was focused on a distal segment of the tail approximately 5 cm from the tip. The time at which rats withdrew the tail was defined as the TF latency. A cutoff time of 10 s was used to avoid damage to the tail. To measure the response of the legs to noxious mechanical stimulus, a paw pressure (PP) test was applied to the dorsal surface of both hind paws using a device capable of progressively increasing the pressure at a rate of 15 g. The pressure at which rats withdrew the paw from the device was defined as the PP threshold, and the mean value of both paws was used for analysis. A cutoff pressure of 400 g was used to prevent damage to the paws. Motor function (MF) in lower limbs was also assessed. The grading of the motor block was as follows: 0 = none, 1 = partially blocked, and 2 = completely blocked. The normal baseline score was 0 and the score with bilateral complete block was 2 + 2 = 4. TF, PP, and MF tests were performed sequentially at the same time point with a 15-s interval. Rats were administered 100 IU intraperitoneal heparin 24 h after the injection and before being sacrificed. After waiting for 15 min, 60 mg/kg of intraperitoneal sodium thiopental was injected, and the chest wall was incised, and a catheter was placed in the outlet of the aorta. First, 0.9% of NaCl was passed through the serum set for 10 min, which was placed 120 cm above the catheter; simultaneously, the right atrium was incised, and the efflux of circulating blood was provided. After the fluid emitted from the right atrium became clear at the end of 10 min, fixation was continued by the application of 150–200 ml of fixation solution (2.5% glutaraldehyde solution in phosphate buffer [pH 7.4]) for each rat. After confirming fixation as evidenced by the stiffness of the visceral and peripheral organs of the rats, the medulla spinalis was extracted by laminectomy.

Surgical procedure

The laminectomy procedure was carried out with the aid of an operating microscope. Specimens were collected from both groups for histopathologic and electron microscopic examination. These specimens were stored in 2.5% glutaraldehyde solution after being washed with normal saline. In total, three segments (at the needle tip level and up and down segments) were examined in each rat. After postfixation with 1% osmium tetroxide in the same buffer solution, 0.5 μm sections were taken from the specimens, which were blocked in plastic embedding media following

routine dehydration procedures performed with a graded alcohol series and propylene oxide, and they were contrasted with lead citrate and uranyl acetate solutions. Specimens were examined on a Jeol-1011-B electron microscope. Images were processed by recording on a MegaView III digital imaging system and Analysis (Soft Imaging Systems, GmbH, Germany) software. Histological analysis was performed separately for each of the four areas by blinded histologist, according to the following scoring system. Description of neuronal lesions, gliosis, and damage of the myelin sheath was considered evidence of typical histological changes of neurotoxicity.

The score 0 represents no vascular plethora, cell necrosis, intraparietal hemorrhage, or vacuolization; 1 - vascular plethora, glial cell reaction, or minimal vacuolization; and 2 - intraparietal hemorrhage or disseminated vacuolization. The findings were categorized as follows: 0 - normal neuron; 1 - intermediate neuron damage; and 2 - neurotoxicity.

Electron microscopic assessment was performed by a histologist. The subjects which underwent laminectomy were disposed of by burning in the Research Institute of the Veterinary School.

Statistical analysis was performed using SPSS for Windows, version 10.0 (http://spss-for-windows-10.0.softinfodb.com). The results of the TF and painful stimuli tests were analyzed by ANOVA. A Chi-square test was used for the evaluation of histopathologic sections. $P < 0.05$ was considered statistically significant.

Results

All animals survived the surgical procedure without any complication. Spinal cord extractions were performed without complications, and no apparent lesions were noted by gross examination.

Intrathecal administration of dexmedetomidine did not produce motor block. Sensorial block was seen in the dexmedetomidine group and significant differences in the dexmedetomidine group than control group in 15th and 30th min ($P < 0.05$).

The fixative procedure for microscopy functioned well. Histological examination did not show evidence suggestive of neuronal body or axonal lesion, gliosis, or myelin sheath damage in any group. In all animals, there were observed changes compatible with unspecific inflammation at the tip of the needle location. On the four-area scoring histologic examination, the scores of both groups were 0–1, and no statistical difference was observed between the groups ($P > 0.05$).

Although there was edema in the cytoplasm of the ganglion cells, focal disintegration in the myelin sheath of myelinated axons, pericellular and periaxonal edema, and a minimal decrease in the nonmyelinated axons have been observed in both groups [Figures 1-3].

The relative decrease in the number of neurotubules and neurofilaments as well as notching and invagination on the nuclear sheath of neurons was observed and was more specifically in the dexmedetomidine group [Figure 4]. It was determined that the alterations in the dendritic, axonal, and glial processes were not specifically in the dexmedetomidine group [Figure 5]. No significant findings were observed in the ependymal cells. Edema was observed in the oligodendroglia cells in two groups.

The synaptic structures, containing spherical presynaptic vesicles, were more evident in the dexmedetomidine group. The number of synaptic vesicles in the control group (normal saline) was lower than in the dexmedetomidine group.

Discussion

Adding α_2-adrenoceptor agonist dexmedetomidine to local anesthetics prolongs the duration of both motor and sensory spinal blockade.[1-3] Intrathecal administration of α_2-adrenoceptor agonists produces analgesia by depressing the release of C-fiber transmitters and by hyperpolarization of postsynaptic dorsal horn neurons.[10-14] These responses have been documented animals studies.[15,16] The findings from those studies suggest that this drug might not produce histological changes associated to neurotoxicity.[16-20] After single-dose intrathecal administration of clonidine (3, 12.5, or 25 µg/kg during 14 consecutive days; or 70 µg/kg during 4.5 consecutive days), which is α_2-adrenoceptor

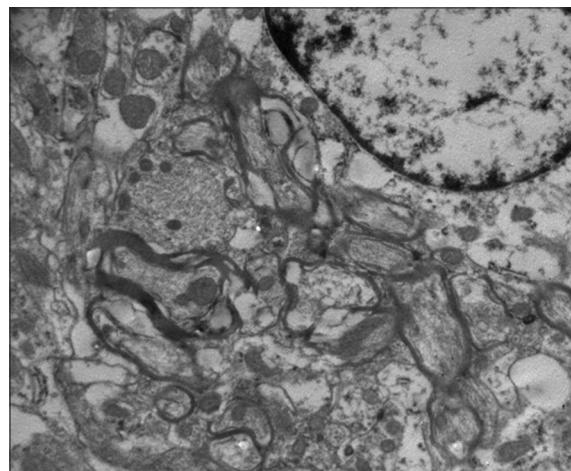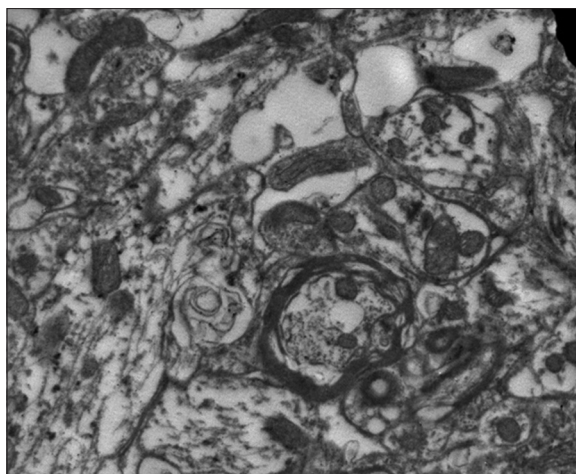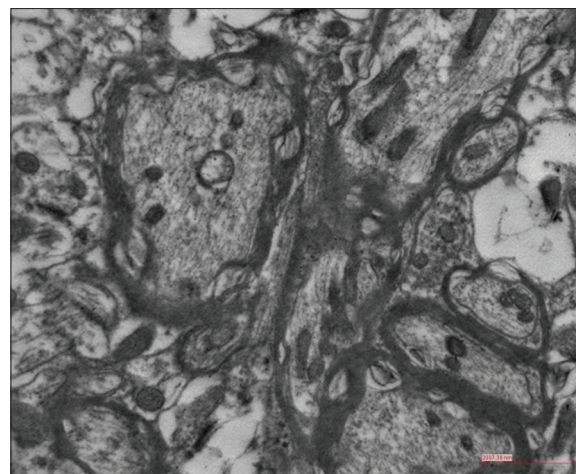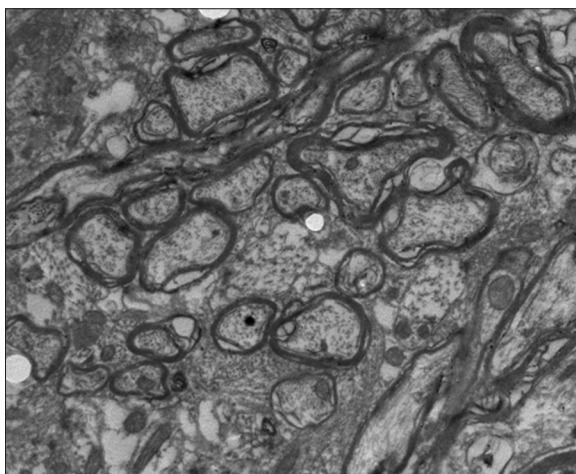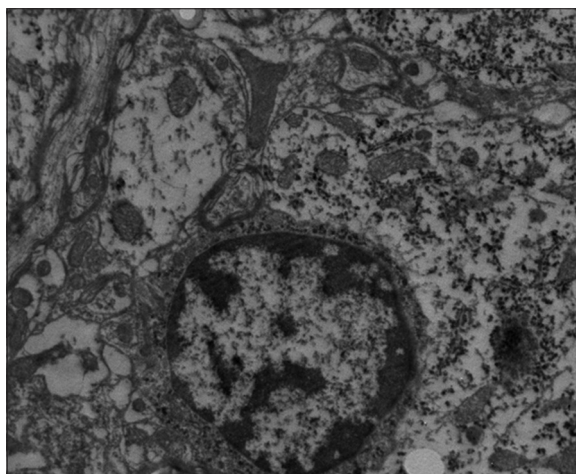Figure 1: Disintegration of thin myelin fibers in the SF group

Figure 2: Cytoplasmic edema and myelin disintegration are together in the precedex group

Figure 3: Slice demonstrating myelin disintegration and decrease of neurotubul number in the precedex group

Figure 4: Slices demonstrating myelin degeneration (myelin disintegration) in the precedex group

Figure 5: Slice showing the intact mitochondria crystal in the precedex group

agonist, it did not produce histopathological changes associated with neurotoxicity.[16,17,19]

Neurological damage seems not to be detected in the present study although there were reversible stress findings at the morphologic level in both groups, it was observed that the process has not reached an irreversible extent. Mitochondrial cristae were intact in both groups. Deformation in the mitochondria may cause permanent damage. In the present study, there was edema in the cytoplasm of the ganglion cells and focal disintegration in the myelin sheath of the myelinated axons in both groups. These findings indicate minimal structural damage. Observation of the findings in both groups supported the notion that the findings originated from the mechanical damage of the needle injected through the spinal area.

Many methods for neurotoxicity researches have been attempted, including single intrathecal injections and implanted intrathecal or epidural catheters.[21-23] In their study, Coombs and Fratkin[24] obtained objective findings in epidural and subarachnoid spaces of animal models that drug applied through intrathecal cannulation. These findings are remarkable fibrosis at spinal roots and medulla spinalis and additionally epidural granulations. Some of these findings were also met in the control group that serum physiologic applied, thus differentiating findings due to drug usage from findings due to chronic catheter application is quite difficult. Hence, we avoid catheter usage and chose to use single-dose application in the present study.

In this study, although the reduction was rare in the nonmyelinated axons, the reduction in the number of neurotubules and neurofilaments was more significant in the dexmedetomidine group. These observations indicate the deceleration of cellular transportation; however, this may represent a morphologic finding which corroborates the mechanism of action of dexmedetomidine. Similarly, in our

study, according to Işgüzar *et al*.'s study, intrathecal injections of dexmedetomidine at the dose of 10 µgr-1 produce antinociception but did not cause any histopathological sign of injury in the spinal cord.[25]

Interestingly, Hou *et al*. found that intrathecal injections of dexmedetomidine at low doses (0.75 and 1.50 µg/kg) can without engendering neurotoxicity, whereas a large dose of dexmedetomidine (3.00 µg/kg) can induce strong antinociceptive effects but significantly increased c-fos expression in the dorsal horn.[26] This dose is very small than our and işgüzar study.

Similarly, in our study, they administered the same dose of the intrathecal dexmedetomidine they found that no significant pathological effects of dex on spinal cord was observed at cellular level, and *in vitro* experiments indicated that dex might act as a preventer of the local anesthetics-induced neurotoxicity when used together with local anesthetics.[27]

In the study of Konakçı *et al*.,[28] 28 applied dexmedetomidine, there was moderate or severe demyelination of myelin sheaths in the white matter of the epidural area. This demyelination was thought to be related to the pH of dexmedetomidine.[25,28]

In the present study, scattered notching and invagination in the nuclear sheath of some neurons, and edema in the oligodendrial cells were observed in both groups. These findings are suggestive of reversible damage in the cells. Synaptic structures were more evident in the dexmedetomidine group, and this may be a morphologic finding corroborating the blockade of neural transmission.

Furthermore, dexmedetomidine treatment resulted in an attenuation of microglial activation and proinflammatory cytokine production both *in vivo* and *in vitro* following lipopolysaccharide stimulation.[29]

Konakci *et al*.[25,28] reported moderate or severe demyelinization of myelin sheaths in the white matter when 10 µg dexmedetomidine was administered through the epidural route. This dose was the same dose used in the present study. In other animal studies conducted in rats, rabbits, dogs, and sheep, intrathecal dexmedetomidine was administered at a dose of 2.5–100 µg.[16-20,26-28,30-32] The largest dose (100 µg) of intrathecal dexmedetomidine was used in a sheep model, and no neurologic deficits were found in a 7-day follow-up study.[16] In humans, the reported dose of epidural dexmedetomidine was within the range of 1.5–2.0 µg/kg. Fukushima *et al*.[33] administered 2.0 µg/kg of dexmedetomidine epidurally for the purpose of postoperative analgesia in humans and no

neurologic deficits were reported. Moreover, Maroof *et al*.[30,34] used approximately 1.5 µg/kg of dexmedetomidine epidurally no neurologic deficits were reported.

As a result of the present study, in addition to the evident synaptic structures containing minimal spherical presynaptic vesicles reversible findings in both groups were observed through the administration of a single-dose intrathecal dexmedetomidine (10 µg/kg) during the neurohistologic examination of the spinal cord specimens. Reversible neuronal findings, which are not lethal, were demonstrated after dexmedetomidine was administered through the intrathecal area.

Conclusions

These findings demonstrated that a single intrathecal dose of dexmedetomidine did not lead to histologic neurotoxicity; however, further investigations are needed for the evaluation of different doses and the timing, as well as to define possible behavioral effects.

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Conflicts of interest
There are no conflicts of interest.

References

1. Strebel S, Gurzeler JA, Schneider MC, Aeschbach A, Kindler CH. Small-dose intrathecal clonidine and isobaric bupivacaine for orthopedic surgery: A dose-response study. Anesth Analg 2004;99:1231-8.
2. Dobrydnjov I, Axelsson K, Thörn SE, Matthiesen P, Klockhoff H, Holmström B, *et al*. Clonidine combined with small-dose bupivacaine during spinal anesthesia for inguinal herniorrhaphy: A randomized double-blinded study. Anesth Analg 2003;96:1496-503.
3. Dobrydnjov I, Axelsson K, Samarütel J, Holmström B. Postoperative pain relief following intrathecal bupivacaine combined with intrathecal or oral clonidine. Acta Anaesthesiol Scand 2002;46:806-14.
4. De Kock M, Gautier P, Fanard L, Hody JL, Lavand'homme P. Intrathecal ropivacaine and clonidine for ambulatory knee arthroscopy: A dose-response study. Anesthesiology 2001;94:574-8.
5. Klimscha W, Tong C, Eisenach JC. Intrathecal alpha 2-adrenergic agonists stimulate acetylcholine and norepinephrine release from the spinal cord dorsal horn in sheep. An *in vivo* microdialysis study. Anesthesiology 1997;87:110-6.
6. Kalso EA, Pöyhiä R, Rosenberg PH. Spinal antinociception by dexmedetomidine, a highly selective α2-adrenergic agonist. Pharmacol Toxicol 1991;68:140-3.
7. Savola M, Woodley J, Kendig J, Maze M. Alpha2B adrenoreceptor activation inhibits nociceptor response in the spinal cord of the neonatal rat. Eur J Pharmacol 1990;183:740.
8. Gordh T Jr., Post C, Olsson Y. Evaluation of the toxicity of subarachnoid clonidine, guanfacine, and a substance P-antagonist on rat spinal cord and nerve roots: Light and electron microscopic observations after chronic intrathecal administration. Anesth Analg 1986;65:1303-11.
9. Guevara-López U, Aldrete JA, Covarrubias-Gómez A,

Hernández-Pando RE, López-Muñoz FJ. Absence of histological changes after the administration of a continuous intrathecal clonidine in Wistar rats. Pain Pract 2009;9:122-9.

10. Eisenach JC, De Kock M, Klimscha W. α2-adrenergic agonists for regional anesthesia. A clinical review of clonidine (1984-1995). Anesthesiology 1996;85:655-74.

11. Lawhead RG, Blaxall HS, Bylund DB. Alpha-2A is the predominant alpha-2 adrenergic receptor subtype in human spinal cord. Anesthesiology 1992;77:983-91.

12. Smith MS, Schambra UB, Wilson KH, Page SO, Hulette C, Light AR, *et al*. Alpha 2-adrenergic receptors in human spinal cord: Specific localized expression of mRNA encoding alpha 2-adrenergic receptor subtypes at four distinct levels. Brain Res Mol Brain Res 1995;34:109-17.

13. Yaksh TL, Jage J, Takano Y. Pharmacokinetics and pharmacodynamics of medullar agents. The spinal actions of α-2 adrenergic agonists as analgesics. In: Atikenhead AR, Benad G, Brown BR, Cousins MJ, Jones JG, Struninn L , *et al*., editors. Baillieres Clinical Anaesthesiology. Vol. 7. London: Bailliere Tindall; 1993. p. 597-614.

14. Fairbanks CA, Wilcox GL. Spinal antinociceptive synergism between morphine and clonidine persists in mice made acutely or chronically tolerant to morphine. J Pharmacol Exp Ther 1999;288:1107-16.

15. Hayashi Y, Maze M. Alpha 2 adrenoceptor agonists and anaesthesia. Br J Anaesth 1993;71:108-18.

16. Eisenach JC, Shafer SL, Bucklin BA, Jackson C, Kallio A. Pharmacokinetics and pharmacodynamics of intraspinal dexmedetomidine in sheep. Anesthesiology 1994;80:1349-59.

17. Lo WC, Harris J, Clarke RW. Endogenous opioids support the spinal inhibitory action of an alpha 2-adrenoceptor agonist in the decerebrated, spinalised rabbit. Neurosci Lett 2003;340:95-8.

18. Talke P, Xu M, Paloheimo M, Kalso E. Effects of intrathecally administered dexmedetomidine, MPV-2426 and tizanidine on EMG in rats. Acta Anaesthesiol Scand 2003;47:347-54.

19. Xu M, Kontinen VK, Kalso E. Effects of radolmidine, a novel alpha-2-adrenergic agonist compared with dexmedetomidine in different pain models in the rat. Anesthesiology 2000;93:473-81.

20. Horvath G, Joo G, Dobos I, Klimscha W, Toth G, Benedek G. The synergistic antinociceptive interactions of endomorphin-1 with dexmedetomidine and/or S(+)-ketamine in rats. Anesth Analg 2001;93:1018-24.

21. Kyttä J, Rosenberg PH, Wahlström T, Olkkola K. Histopathological changes in rabbit spinal cord caused by bupivacaine. Reg Anaesth 1982;5:85-8.

22. Bahar M, Rosen M, Vickers MD. Chronic cannulation of the intradural or extradural space in the rat. Br J Anaesth 1984;56:405-10.

23. Ravindran RS, Turner MS, Muller J. Neurologic effects of subarachnoid administration of 2-chloroprocaine-CE, bupivacaine, and low pH normal saline in dogs. Anesth Analg 1982;61:279-83.

24. Coombs DW, Fratkin JD. Neurotoxicology of spinal agents. Anesthesiology 1987;66:724-6.

25. Isgüzar O, Baris S, Bozkurt A, Can B, Bilge S, Türe H. Evaluation of antinociceptive and neurotoxic effects of intrathecal dexmedetomidine in rats. Balkan Med J 2012;29:354-7.

26. Hou J, Xia Z, Xiao X, Wan X, Zhao B. Neurotoxicity of intrathecal injections of dexmedetomidine into the rat spinal dorsal horn. Neural Regen Res 2012;7:1765-70.

27. Zhang H, Zhou F, Li C, Kong M, Liu H, Zhang P, *et al*. Molecular mechanisms underlying the analgesic property of intrathecal dexmedetomidine and its neurotoxicity evaluation: An *in vivo* and *in vitro* experimental study. PLoS One 2013;8:e55556.

28. Konakci S, Adanir T, Yilmaz G, Rezanko T. The efficacy and neurotoxicity of dexmedetomidine administered via the epidural route. Eur J Anaesthesiol 2008;25:403-9.

29. Bell MT, Agoston VA, Freeman KA, Puskas F, Herson PS, Mares J, *et al*. Presented at the 37th Annual Meeting of the Southern Association of Vascular Surgeons, Paradise Island, Bahamas. 2013.

30. Shimode N, Fukuoka T, Tanimoto M, Tashiro C, Tokunaga A, Noguchi K. The effects of dexmedetomidine and halothane on Fos expression in the spinal dorsal horn using a rat postoperative pain model. Neurosci Lett 2003;343:45-8.

31. Onttonen T, Pertovaara A. The mechanical antihyperalgesic effect of intrathecally administered MPV-2426, a novel alpha2-adrenoceptor agonist, in a rat model of postoperative pain. Anesthesiology 2000;92:1740-5.

32. Takano Y, Yaksh TL. Characterization of the pharmacology of intrathecally administered alpha-2 agonists and antagonists in rats. J Pharmacol Exp Ther 1992;261:764-72.

33. Fukushima K, Nishimi Y, Mori K, Takeda J. Effect of epidurally administered dexmedetomidine on sympathetic activity and postoperative pain in man. Anesth Analg 1996;82:S121.

34. Maroof M, Khan SA, Jain D, Khan RM, Maroof BS. Evaluation of effect of dexmedetomidine in reducing shivering following epidural anesthesia. Anesthesiology 2004;101:A495.

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