Research Article

Clinical and Histological Effects of the Intrathecal Administration of a Single Dose of Dexmedetomidine in Rabbits

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Background: There is experimental evidence that dexmedetomidine has neuroprotective effects. So, it could be expected that its intrathecal or epidural administration presents no harm. However, whether dexmedetomidine is neurotoxic to the spinal cord remains to be fully elucidated.

Objective: To evaluate the effect of preservative-free dexmedetomidine administered as a subarachnoid single injection on the spinal cord and meninges of rabbits.

Study Design: Research article.

Setting: Experimental research laboratory.

Methods: Twenty young adult female rabbits, each weighing between 3200 and 4900 g, and having a spine length between 36 and 40 cm, were divided by lot into 2 groups (G): 0.9% saline in G1 and preservative-free dexmedetomidine in G2 (dose of 10 µg). After intravenous anesthesia with ketamine and xylazine, the subarachnoid space was punctured at S1-S2 under ultrasound guidance, and a random 5 µl.cm-1 of spinal length (0.2 mL) of solution (saline or dexmedetomidine) was injected. The animals remained in captivity for 21 days under medical observation and were sacrificed by decapitation. The lumbosacral spinal cord portion was removed for immunohistochemistry to assess the glial fibrillary acidic protein (GFAP), and histology was assessed using hematoxylin and eosin (HE) stain.

Results: None of the animals had impaired motor function or decreased nociception during the period of clinical observation. None of the animals from the control group showed signs of injuries to meninges. In the dexmedetomidine group, however, 9 animals presented with signs of meningeal injury. The main histological changes observed were areas with meningeal thickening and lymphoplasmocitary infiltration in the pia-mater and arachnoid. Further histological examination also revealed adherence areas among the pia and arachnoid. There was no signal of injury in neural tissue in any animal of both groups.

Limitations: Evaluation of the possible analgesic effects of the intrathecal dexmedetomidine was not performed.

Conclusion: On the basis of the present results, dexmedetomidine administered in the subarachnoid space in a single dose of 10 µg is capable of producing histological changes over the meninges of rabbits.

Key Words: Anesthesia, spinal; dexmedetomine; injections, spinal; spinal cord; rabbits

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Alpha (α)-2-adrenoceptor agonists exhibit a wide range of effects, including sedation, anesthesia-sparing, analgesia, and sympatholysis. Based on these effects, the role of these drugs in anesthesia is growing. Dexmedetomidine shows a high ratio of specificity for the α2-adrenoceptor (α2/α1 1600:1) with a favorable profile in anesthesia and intensive care (1-3). It was introduced in clinical practice in the United States in 1999 and approved by the FDA only as a short-term (< 24 hours) sedative for mechanically ventilated adult intensive care unit (ICU) patients. Because of its analgesic effect, dexmedetomidine has been used off-label in various settings outside of the ICU (1,3).

The analgesic effects of dexmedetomidine are complex. Alpha2-agonists have an analgesic effect when injected via the intrathecal and epidural routes (4). Dexmedetomidine readily reduces blood pressure when intrathecally injected in sheep (5). When injected into the epidural space, dexmedetomidine rapidly diffuses into the CSF (5). Epidural administration of dexmedetomidine was found to be approximately 5 times more effective than systemic administration in producing an antinociception effect in a rodent model (4). In human beings, studies using epidural or intrathecal dexmedetomidine have been conducted without any report of neurological deficit (6-10). The addition of dexmedetomidine to ropivacaine intrathecally prolongs the duration of the motor and sensory blocks in patients undergoing lower limb surgery (11).

Cellular effects mediated by signaling pathways other than through α2-adrenoceptors have been reported, and have a role in neuroprotection, both *in vivo* and *in vitro* (12-15). There is experimental evidence that dexmedetomidine has neuroprotective effects by several mechanisms, including sympatholysis, preconditioning, and attenuation of ischemia reperfusion injury (16). There is also evidence that dexmedetomidine decreases cerebral blood flow (17), but its ratio with cerebral metabolic rate appears to be preserved (18). As dexmedetomidine has neuroprotective effects, it could be expected that its intrathecal or epidural administration presents no harm. However, whether dexmedetomidine is neurotoxic to the spinal cord remains to be fully elucidated. Dexmedetomidine appears to have neurotoxic effects in the spinal cord when intrathecally injected in rats (19), as well as a harmful effect on the myelin sheath when administered via the epidural route (20).

Establishing the safety of neuroaxial drugs is crucial before these drugs are given to humans. In order to administer dexmedetomidine via the neuroaxial route as a safe adjuvant agent, further studies and advanced pathologic investigations are demanded. Thus, the objective of this study was to determine the clinical and histological effects of injecting dexmedetomidine into the intrathecal space of rabbits.

Methods

After proper approval by the Botucatu Medical School Ethics Committee on animal experiments, adult female rabbits (n = 20) were obtained from the Experimental Animal Center at the State University of São Paulo at Botucatu Campus. The mean and standard deviation (SD) weight of the animals was 4.1 ± 0.4 kg (3.2 – 4.8 kg) for the control group and 4.3 ± 0.4 kg (3.8 – 4.9 kg) for the dexmedetomidine group. The mean length of the vertebral column was 39.2 ± 1.4 cm for the control group and 39.8 ± 0.6 cm for the dexmedetomidine group. All tests were performed in accordance with the guidelines of the International Association for the Study of Pain (21).

The rabbits were randomized by chance into 2 experimental groups according to the type of solution injected into the subarachnoid space. Ten rabbits were allocated to each group. Group 1 was defined as the control group, where 0.9% normal saline solution was intraspinally injected. Group 2 received 10 µg of preservative-free dexmedetomidine. The researchers administering the solution were blinded to the group treatment.

The animals were fasted 12 hours before the procedure, with water ad libitum. All rabbits were submitted to the same anesthetic technique, xylazine (3 mg.kg-1) and ketamine (10 mg.kg-1) administered IV. A 10-cm area around the site of the spinal puncture at the S1-S2 intervertebral space level was washed with water and soap, followed by hair removal and skin cleansing with 0.9% normal saline. The naked skin was submitted to antisepsis with a 2% chlorhexidine gluconate solution, and sterile fields were appropriately positioned. Subarachnoid puncture was guided by ultrasound (US) image (SonoSite – M-Turbo, USA), using the micro-linear transducer with 6 to 13 MHz of frequency. The puncture was performed through the median line, approximately 45° to the skin with a 25½"-gauge Quincke needle. Difficulties during the procedure were recorded. If a traumatic spinal tap was identified, as defined by the need for more than one attempt of puncture, the animal was immediately excluded from the study and no

solution was administered into the subarachnoid space. Once the needle was properly located and identified by US image, 5µl.cm-1 of spinal length (0.2 mL) of solution (saline or dexmedetomidine) was injected over 10 seconds through 1 mL disposable syringes.

Dexmedetomidine sterile preservative-free solution was synthesized by Hospira (San Jose, CA, USA), at pH 4.5 to 7.0. Dexmedetomidine hydrochloride was supplied in individual ampoules as a white powder that is freely soluble in water and has a pKa of 7.1. The 0.9% saline solution (Baxter Healthcare Corp., São Paulo, Brazil) administered to the control group had a pH of 5.0.

Clinical Evaluation and Outcomes

Animals in the study were evaluated one hour after the intrathecal injection and once a day for 21 days after the injection. Each animal was assessed regarding the following secondary outcomes: motor deficit and response to nociception.

Motor deficit was evaluated according to Drummond and Moore criteria (22), as follows: 0 – paraplegic, with no lower-extremity motor function; 1 – poor lower-extremity motor function (flicker of movement or weak antigravity movement only); 2 – some lower-extremity function with good antigravity strength but inability to draw legs under body and/or hop; 3 – ability to draw legs under body and hop but not normally; and 4 – normal motor function.

Nociception was assessed by reaction to painful pressure in lower and upper extremities and ears. To control for possible interference due to visual perception of the stimuli by the animals, one researcher was responsible for masking the animals with a nontransparent cloth comfortably positioned around their neck. Pressure nociceptive stimuli were elicited by the bilateral pinch of a skin fold over sacral, lumbar, and thoracic dermatomes, as well as the interdigital membranes of limbs and ears by a surgical clamp. The presence of pain was defined by the following: limb withdrawal, vocalization, and facial expression. Nociception was classified dichotomously into absent or present.

Spinal Cord Preparation and Staining

Histological analysis of the spinal cord and meninges of the rabbits was performed after the 21-day observation period. To obtain tissue samples, animals were first given a sodium pentobarbital IV then euthanized by decapitation. Thereafter, the lumbar and sacral segments of the spinal cord with the surrounding meninges were quickly removed within 3 minutes to minimize the risk of injuries to those tissues from ischemia and apoptosis. The anatomical pieces were fixed in a 10% formalin solution. After a 7-day incubation period, 0.5 cm thick histological sections were prepared starting 10 cm above the level of the spinal puncture to the end of the cauda equina. The histological sections were stained by hematoxylin and eosin (HE) and glial fibrillary acidic protein (GFAP) techniques and examined by optical microscopy. Two researchers (EMG and MAM), experienced in histological neurotoxicity assessment, unanimously classified each of the sections according to the presence or absence of histological injury. If any kind of lesion was identified, it was further specified. To investigate the possible dose-related gradient effect, injuries were stratified according to severity and extent as ascertained by consensus. Researchers blinded to the experimental groups performed all clinical and histological evaluations.

For the purpose of measuring the number of cells ImageJ (version 1.43), a stand-alone java based image analysis program developed at the U.S National Institutes of Health (available as open source at http://rsbweb.nih.gov/ij/), was used to recognize and count the nuclei (17). GFAP-stained images were captured by Nikon Coolscope II microscope and processed with a heterogeneous correction filter to address the uneven illumination of the microscope field (Fig. 1). Four images of the dorsal horn and 2 images from the ventral horn of the spinal cord were collected (Fig. 2), and the program in each image counted the number of nuclei.

The sample size was calculated according to Fleiss et al (23) estimating a proportion of histological neurotoxicity of 1 and 70% (24) in the control and dexmedetomidine group, respectively, so as to obtain a power value of 90% while setting the one-sided alpha level for statistical significance at 0.05.

Data Analysis

The R software Version 2.3.4 was used for the performance of statistical analysis (25). In order to evaluate the effectiveness of the randomization procedure and the comparability of the 2 study groups, we performed one-way ANOVA comparing group differences regarding animals' weights and the length of their vertebral column. One-sided Fisher's exact test was selected to compare the frequencies of the findings on primary and secondary outcomes between the dexmedetomidine and the control groups. Differences among mean numbers of cells were accessed by meant of Student's t-test. The alpha level for statistical significance was set at 0.05.

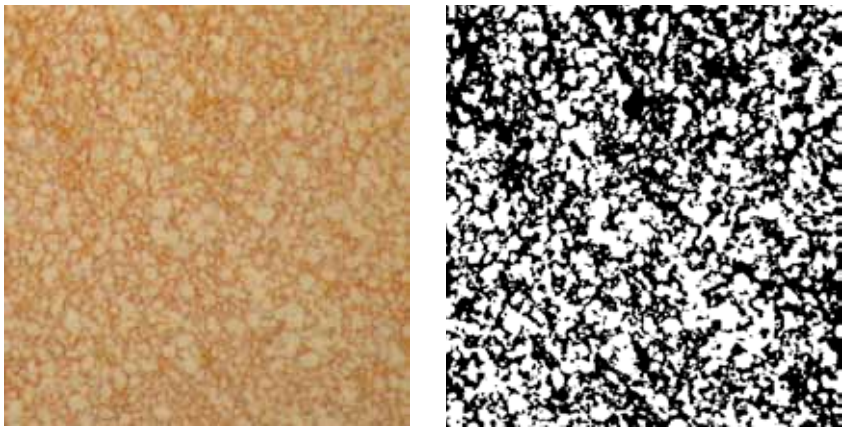


Fig. 1. *A- GFAP stained section of the spinal cord. B- GFAP stained section of the spinal cord after ImageJ filter application. GFAP: glial fibrilary acidic protein. ImageJ: stand-alone Java based image analysis program to recognize and count nuclei. GFAP stained images were captured by Nikon Coolscope II microscope and processed with a heterogeneous correction filter to address the uneven illumination of the microscope field (binary image).*

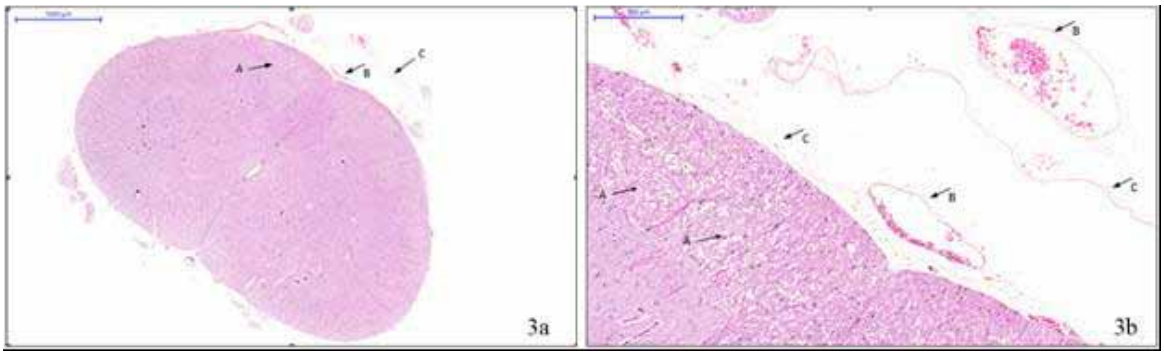
Fig. 2. *Spinal cord lumbar segment (1 cm above the spinal puncture). Low magnification (1000 µm) GFAP-stained section. Area of cell counting (squares). GFAP: glial fibrilary acidic protein.*

Results

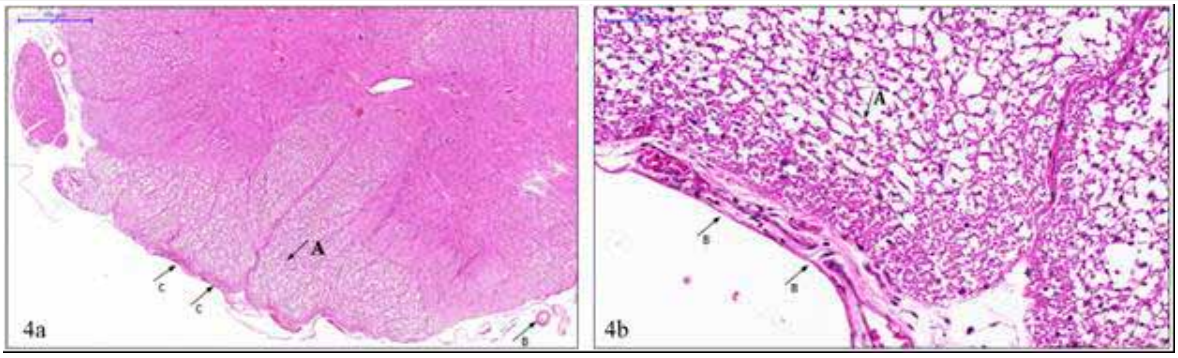
Both groups were similar in weight (P = 0.28) and length of the vertebral column (P = 0.23). None of the animals were excluded due to traumatic puncture or deaths in the study. The time of recovery from anesthesia in both groups was approximately 30 minutes. During the 21 days of observation, none of the animals had impaired motor function or decreased nociception.

Histological Effects after Spinal Dexmedetomidine

None of the animals from the control group showed macroscopic or microscopic signs of direct injuries to meninges, such as hemorrhage, infarct, vacuolization, necrosis, or meningeal thickness, during the necropsy (Figs. 3a and 3b). In the dexmedetomidine group, however, 9 animals presented with signs of meningeal injury. The main histological changes observed were areas



Figs. 3a and 3b. *Hematoxylin and eosin (HE) stained section – 3a - Low magnification (200 µm); 3b – HE stained section (1000 µm) - Normal nervous tissue (A), blood vessels (B), and meninges (C). All sections are from rabbits of the control group (G1). Spinal cord lumbar segment was taken from 1 cm above the spinal puncture.*



Figs. 4a and 4b. *Hematoxylin and eosin (HE) stained section- 4a – Low magnification (500 µm) showing normal nervous tissue (A) and blood vessels (B), with mild lymphoplasmocitary inflammatory infiltration between pia-mater and arachnoid with focal areas of adhesion between the meninges (C). 4b - HE stained section (100 µm), showing normal nervous tissue (A) and mild lymphoplasmocitary inflammatory infiltration between pia mater and arachnoid with focal adhesion areas between the meninges (B). Sections are from rabbit C6 of the dexmedetomidine group (G2). Spinal cord lumbar segment was taken from 1 cm above the spinal puncture.*

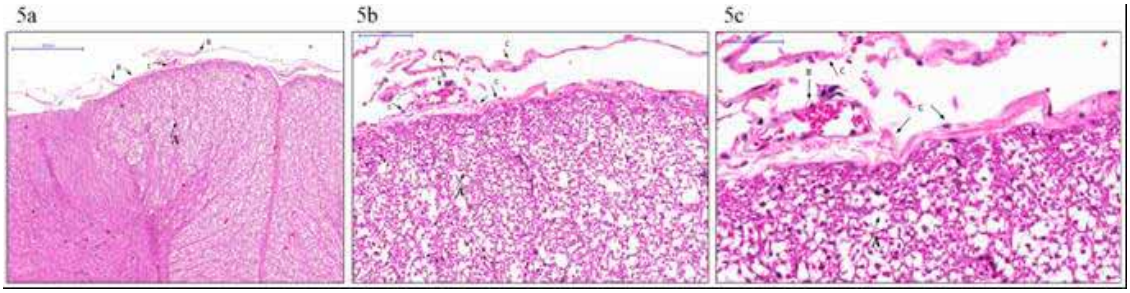
with meningeal thickening and lymphoplasmocitary infiltration in the pia-mater and arachnoid, as well as around the blood vessels. Further histological examination also revealed adherence areas among the pia and arachnoid. There was no signal of injury in neural tissue in any animal of both groups (Figs. 4a and 4b; 5a-c).

Intrathecal Dexmedetomidine and Glial Activation

Histological analyses in the anterior and dorsal horn indicated no differences in the quantification of GFAP (cellular counting) between groups ($P > 0.05$) (Fig. 6).

Discussion

Dexmedetomidine is a highly selective, short acting central α2-adrenoceptor agonist (1-3). Behavioral studies in animals have demonstrated an inhibition of nociceptive responses by intrathecally (26) and epidurally (4) administered dexmedetomidine. The spinal antinociception induced by α2-agonists is mediated by an inhibition of synaptic transmission within the dorsal horn of the spinal cord (27), especially via a direct suppression of the activity of dorsal horn neurons (28), via an activation of the descending noradrenergic inhibitory system (29), and via an activation of spinal cholinergic neurons (30). As dexmedetomidine was



Figs. 5a-c. *Hematoxylin and eosin (HE) stained section- 5a – Low magnification (500 µm); 5b - HE stained section (100 µm); 5c - HE stained section (50 µm), showing normal nervous tissue (A), perivascular lymphoplasmocitary inflammatory infiltration (B), and arachnoid and pia-mater thickening (C). Sections are from rabbit C7 of the dexmedetomidine group (G2).). Spinal cord lumbar segment was taken from 1 cm above the spinal puncture.*

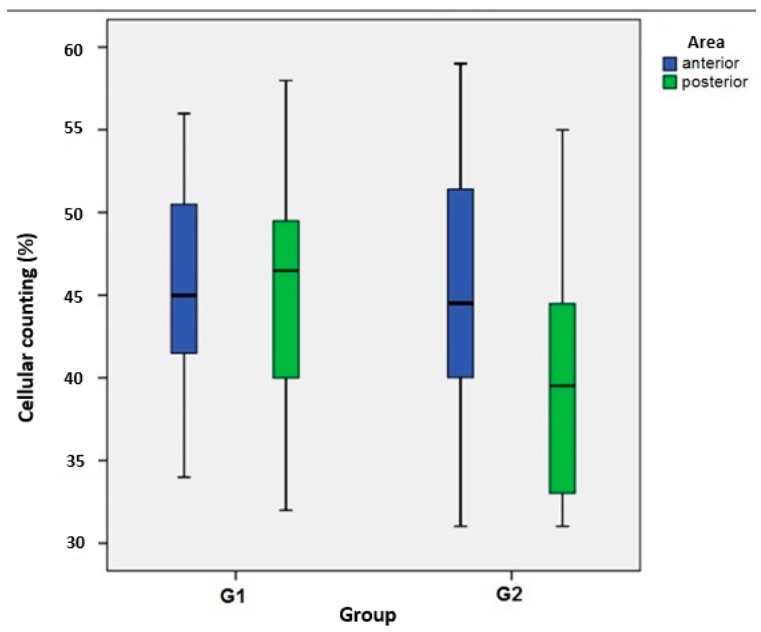


Fig. 6. *Quantification (%) of GFAP (cellular counting) between groups were similar (P > 0.05). GFAP: glial fibrilary acidic protein.*

reported to have neuroprotective effects (16), it could be expected that its intrathecal administration would not be harmful. However, this study showed that intrathecal dexmedetomidine (10 µg) produced histological changes over the meninges of rabbits. The main histological changes consisted of meningeal adherence and lymphocytic infiltrates in the blood vessels.

Other authors found that dexmedetomidine (10 µg) can produce moderate or severe demyelinization of myelin sheaths in the white matter when administered via the epidural route in rabbits (20). They credit this effect to be related to vasoconstriction of the medullary blood vessels (31) and pH of dexmedetomidine.

Although dexmedetomidine has been used as an adjunctive drug associated with local anesthetic via an intrathecal route (9,11,32), its commercial preparation (Precedex®) it is not prepared to be used in the intrathecal or epidural space. The effects of dexmedetomidine over the meninges could be related to the pH of dexmedetomidine solution (4.5 – 7.0). However, 0.9% saline is usually in a pH range similar to dexmedetomidine commercial preparation. Moreover, the solution is preservative-free and contains no additives or chemical stabilizers. Thus, we do not think that the histological changes could be related to these compounds and it is more likely that the meninges react to dexmedetomidine rather than to the pH.

One may speculate that the histological changes may be a result of trauma rather than a specific effect of the drug. However, the present study was a blinded, randomized, controlled trial in which potential confounding issues due to lesions induced by the spinal puncture procedure in the dexmedetomidine group were controlled by comparison to the control group. The experimental model selected a single intraspinal dose similar to the usual spinal anesthesia procedures used in humans. This technique displays less risk of complications than

other models in which implantable intrathecal catheters are used. Furthermore, fast removal and fixation of the anatomical piece, as well as the comparison to controls submitted to the same procedure, makes our findings unlikely due to cord extraction-related ischemic injuries or other procedure-related mechanisms.

The antinociception produced by the intrathecal administration of dexmedetomidine is dose-dependent. In an experimental model in rats, there appears to be no significant or transient effect when 1 to 3 µg of dexmedetomidine was injected via this route. However, rats given 10 µg of intrathecal dexmedetomidine experienced prolonged and profound antinociception (26). Thus, the dose of dexmedetomidine administrated in the intrathecal space in this study was chosen to be 10 µg.

Hou et al (19) showed that intrathecal injections of dexmedetomidine at low doses (0.75 and 1.5 µg.kg-1) can relieve pain without engendering neurotoxicity, whereas a large dose of dexmedetomidine (3 µg.kg-1) can induce strong anti-inflammatory and antinociceptive effects but significantly increase c-Fos expression in the dorsal horn within 7 hours of administration in rats. c-Fos is a protein that is expressed after noxious stimuli; therefore, it has been used as a marker for neural activation following tissue injury and nociception in the spinal cord (33).

The prolonged clinical observation (21 days) enabled the evaluation of chronic effects that dexmedetomidine could also cause to the meninges. Results of previous studies with this same methodology, however in a different animal model (dogs), showed that the drug primarily damages the nervous tissue, where clinical and histological alterations are immediately observed after the agent administration (34,35). However, when the meninges are damaged, a longer interval allows for the inflammatory reaction, which causes the nervous tissue lesion later. Nevertheless, another study using the same methodology with subarachnoid amitriptyline (36) depicted extensive adhesive arachnoiditis 21 days after spinal injection.

The present study suggests the potential meningeal damage due to the intrathecal administration of dexmedetomidine. The histological changes over the meninges of rabbits found in our study were meningeal adherence and lymphoplasmocitary infiltrates in the blood vessels. The same changes can be found in early stages of adhesive arachnoiditis (AA). Arachnoiditis is a severe progressive disorder characterized by an inflammatory process leading to fibrosis of the arachnoid and subarachnoid space (37). In 1958, Joseph and Denson (38) conducted experiments whereby they injected different quantities of detergents in the intrathecal space of monkeys. Two animals developed histological changes, but only one of the animals showed clinical signs of the disease. Different researchers, studying different animal models, have reported similar findings, suggesting that clinical findings do not always correlate with the degree of the histological changes (35,39-41). AA has an insidious onset and the neurologic symptoms and signs are likely to occur after an extensive period, within months or even years after exposure to the noxious agent. This may be the reason why the animals in the present study did not manifest any clinical signs during the 21 day experimental period.

There is no sign of nervous tissue injury when the hematoxylin and eosin method was used in this study, as well as there was no difference regarding the glial marker GFAP in the animals that received dexmedetomidine into the subarachnoid space versus the animals in the control group. In a relatively short period of time, the microglial cell has gone from a strongly contested component of the central nervous system (CNS), to being recognized as one of the main players in the response to brain injury. The microglial cell is the representative of the immune system within the nervous system (42). The most characteristic feature of microglial cells is their rapid activation in response to even minor pathological changes in the CNS. Qualitative and quantitative analysis of GFAP has shown this biomarker to be a sensitive and specific indicator of the neurotoxic condition (43). Therefore, the similarity of the results in the animals of both groups regarding the GFAP suggest that the dexmedetomidine may not have either a deleterious effect over the spinal nervous tissue, as well as any protective effect over the nervous tissue in the present animal model.

Previous studies found a strong correlation between the drug concentration and the neurotoxicity. The purpose of our study was to evaluate the possible clinical effects in the nervous tissue and meninges. As such, we did not evaluate the possible analgesic effects of the intrathecal dexmedetomidine, but rather used a dichotomous clinical evaluation instead of a scalar measure for the comparison of motor function and sensibility to painful stimulation. Since the classification scheme used was quite stringent (the slightest deficit would be classified as positive), it is likely that the comparisons were even more rigorous than scalar comparisons.

Conclusion

On the basis of the present results, dexmedetomidine administered in the subarachnoid space in a single dose of 10 µg is capable of producing histological changes in the meninges of rabbits. It seems it is reasonable to re-evaluate the clinical use of dexmedetomidine into the subarachnoid space since the risks seem to be larger than the benefits. Further studies are necessary to elucidate the mechanism of the meningeal toxicity observed and to evaluate the clinical long-term outcome in rabbits and in different animal models. Despite the negative results observed in the present experimental model, more research is necessary to determine the safety of dexmedetomidine in humans.

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