Basic Science Report

Neurotoxicity of Subarachnoid Preservative-Free S (+)-Ketamine in Dogs

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Free full manuscript: www.painphysicianjournal.com **Background:** Subarachnoid S(+)-ketamine is a matter of much debate as the results regarding its toxicity are contradictory.

Objectives: Our objective was to investigate possible histopathological alterations after subarachnoid administration of different doses of preservative-free S(+)-ketamine to dogs.

Study design: A randomized, blind, prospective experimental study.

Setting: Center for Research on Pain at the Federal University of Maranhão, Brazil.

Methods: Sixteen adult mongrel dogs of both sexes, each weighing 11 to 20 kg were divided into 3 groups: Group I (n=6), 0.7 mg/kg-1 S(+)-ketamine; Group II (n=6), 0.5 mg/kg-1 S(+)-ketamine, and a control group, Group III, (n=4), 0.9% NaCl. All substances were administered in one mL volume doses. The animals were kept in captivity for 2 weeks; after this period, they were put down and lumbar and sacral portions of the spinal cords were removed for histological examination using conventional light microscopy.

Results: There were histological alterations in the spinal cords of the test subjects in the control group. Comparison showed significant histological abnormalities in Groups I and II when compared to the control group, including gliosis, axonal edema, central chromatolysis, lymphocyte infiltration and fibrous thickening of the dura mater.

Limitations: Test subjects received only a single dose each. The observation period was not very long, less than a month.

Conclusions: Subarachnoid administration of S(+)-ketamine without preservative caused histological lesions on the spinal cord and meninges in the dogs studied. S(+)-ketamine should not be given to clinical patients in this way until further evaluation of the significance of this toxicity has been conducted.

Key words: S(+)-ketamine. subarachnoid, neurotoxicity, dog, histopathology

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etamine is a fast-acting anesthetic agent with analgesic properties which produces depression of the central nervous system, promoting a dissociative effect that might cause hallucinations. Ketamine is an arylcycloalkylamine and occurs as a racemic mixture of 2 optically active isomers which possess different pharmacological properties. S

(+)-ketamine, the levorotatory isomer, is on average twice as potent and has a better therapeutic index than the dextrorotatory isomer R (-)-ketamine (1,2). Ketamine is administered both in pediatric anesthesia and to adult and elderly patients, and is also frequently used in veterinary anesthesia (2-5). Interest in the pharmacological properties of ketamine, as well as in its different types of application, such as postoperative analgesia, treatment of chronic pain of somatic, neuropathic or visceral origin, and specific diseases such as fibromyalgia, has been growing (6-13).

Ketamine can be administered by different routes: intravenous, intramuscular, oral, rectal, intranasal, transdermal, subcutaneous, and even intra-articular. However, intravenous administration is the most frequent since the therapeutic plasma concentration is reached more rapidly (3,14,15). Neuroaxial use of ketamine has been reported since the 1970s, including the peridural administration of the racemic drug with preservative (16,17). During the initial studies, the authors observed some suggestive cases of neuronal lesions that led to the interruption of ongoing studies. Subsequently, it was discovered that the substance with neurotoxic potential was the preservative (benzethonium chloride or chlorbutanol) and not the racemic ketamine itself (16,17).

In humans, the intrathecal application of racemic ketamine with and without a preservative is limited by the lack of consistent data regarding the risk of central nervous system toxicity, with its use being restricted to cases of chronic pain in oncologic patients resistant to treatment with opioids (18,19). Animal studies have shown that racemic ketamine without preservative is the most indicated for intrathecal application to humans since the neurotoxic potential of this route of administration was also attributed to the preservative (20,21).

The levorotatory enantiomer of ketamine reemerged in anesthesia practice, offering new possibilities for the control of postoperative pain and for the treatment of chronic painful processes (5,22,23). The results demonstrated the efficacy of postoperative analgesia induced with S (+)-ketamine administered intravenously, with this analgesia being dose dependent. The results were even more promising when morphine was administered in combination (24). With respect to the intrathecal use of S (+)-ketamine, a reduction in the total dose of the local anesthetic and a shorter time of motor block were observed during the perioperative period (25).

Subarachnoid administration of S (+)-ketamine has also been associated with less intense side effects when compared to racemic ketamine administered by the same route, without signs of damage to the spinal cord (26). However, important histopathological alterations, especially central chromatolysis and gliosis were observed in the spinal cord of a patient who received intrathecal S (+)-ketamine without preservative for the treatment of cancer pain, thus representing the first report of neurotoxicity of S (+)-ketamine in humans (27). Despite its demonstrated efficacy, subarachnoid S (+)-ketamine has been a matter of much debate since the results regarding its toxicity are contradictory, even when the drug is used without preservatives (26).

The objective of the present experimental study was to histologically evaluate the neurotoxicity of S (+)-ketamine after subarachnoid administration of different doses to dogs.

METHODS

Study Design and Experimental Groups

Sixteen adult mongrel dogs of both sexes (7 males and 9 females) weighing 11 to 20 kg and with a length of the spine of 59 to 75 cm, were used in this study. The animals were obtained from the Zoonosis Center of the Municipality of São Luís, at the Campus of Maranhão State University (UEMA), after approval for the study by the Ethics Committee on Animal Research of UEMA was granted. Dogs that were seropositive for visceral leishmaniasis were excluded. No other research was carried out on these dogs at the same time.

A randomized, blinded design was used. The dogs were maintained under constant environmental conditions with free access to food and filtered water, and were allowed to adapt for 5 days. The dogs were then divided into 3 groups that received different concentrations of the drug by the subarachnoid route in a constant volume of 1 mL: Group I (n = 6), 0.7 mg/kg S (+)-ketamine; Group II (n = 6), 0.5 mg/kg S (+)-ketamine; Group III (control, n = 4), physiological saline (0.9% NaCl).

Animal preparation and injection technique

All dogs were weighed on a mechanical scale and submitted to a 12-hour fast with free access to water. Anesthesia was induced by intravenous administration of 0.005 mg/kg fentanyl and 2 mg/kg etomidate. Anesthesia was maintained with intermittent doses of fentanyl (0.003 mg/kg) if necessary, and the dogs were monitored using heart rate, blood pressure and ECG and the depth and frequency of breathing. Fluids (0.9% saline) were administered at 5 mL/kg/h. Next, the dogs were placed in sternal recumbency on a steel table and the distance between the occipital protuberance and base of the tail was measured for determination of the length of the spine (28-31).

The skin was disinfected with water and soap and an area measuring approximately 10 cm in diameter was shaved around the site of the subarachnoid puncture. Next, the area was again cleaned with sterile saline and antisepsis was performed with 2% chlorhexidine gluconate. A surgical drape with a window was placed for exposure of the puncture site around the L5-L6 and L6-L7 intervertebral spaces.

The site of the subarachnoid puncture was determined by palpation of the 2 tuberosities of the iliac bone and spinous process of the last lumbar vertebra and localization of the lumbosacral space immediately below. The L5-L6 and L6-L7 intervertebral spaces were identified by sliding the index finger along the midline in the direction of the head. Next, a disposable 22gauge 88 mm length Quincke needle was introduced on the midline of the intervertebral space at an angle of 45° to the skin, with the bevel of the needle facing rostrally, and after seeing cerebrospinal fluid, the prepared solutions were injected.

The previously established solutions were injected with disposable 3 mL syringes (volume of 1 mL) after randomization of the groups. The difficulties encountered during puncture, as well as the color of cerebrospinal fluid leaking from the needle, were recorded.

S (+)-ketamine hydrochloride without preservative, dissolved in sterile water as a vehicle, was used in this study. Each ampule contained 1 mL, corresponding to 50 mg S (+)-ketamine base.

After injection, the dogs were taken off the steel table and placed in dorsal recumbency. Clinical observation and monitoring was continued until recovery from subarachnoid and intravenous anesthesia.

After recovery from anesthesia, the dogs remained in captivity for 2 weeks and then were humanely killed after intravenous anesthesia with sodium pentobarbital and intravenous injection of 10% potassium chloride. Death was confirmed by cardiac arrest, absence of central pulse, asystole and the complete absence of reflexes.

Removal of the Spinal Cord and Histopathological Analysis

The lumbosacral region was exposed for identification and removal of the lumbar and sacral portions of the spinal cord. The lumbar and sacral portions of the spinal cord were removed within 3 minutes of death (28-31). The specimens were fixed in 10% formalin for microscopic examination. The collected material was sent to a pathology service of the University Hospital, and was processed as follows: removal of excess formalin under running water for 15 minutes, dehydration in an increasing ethyl alcohol series (70, 80 and 90%), clearance in xylene, and embedding in paraffin. Cross-sections of spinal cord tissue and meninges were cut approximately 5 cm above the site of spinal puncture to the end of the cauda equina at 1-cm intervals. The spinal tissue sections were embedded in paraffin blocks (an average of 4 blocks per dog with multiple fragments) and stained with hematoxylin-eosin as well as Masson's trichrome for the detection of fibrosis (blue) (32).

For histological analysis, the slides were examined by conventional light microscopy. The results of the analysis of spinal cord tissue and meninges sections were classified as normal when no alterations were observed. When alterations were detected they were described and recorded on a protocol chart. The pathologist who analyzed the slides was unaware as to which group the slide belonged.

Statistical Analysis

Kruskal-Wallis nonparametric analysis of variance were used for the evaluation of homogeneity of the groups in terms of weight and spine length. For the histological lesions, multiple comparisons were made between groups using Fisher's exact test. A P value < 0.05 was considered to be significant in all tests.

RESULTS

Statistical analysis showed that the 3 groups were homogenous in terms of body weight and spine length. Normality of the data was tested using a Shapiro-Wilke's test. Data are reported as the mean and standard deviation (Table 1).

None of the dogs was excluded from the study due to hemorrhagic complications associated with subarachnoid puncture (not observed) or puncture difficulties (observed in 5 dogs). There was no case of bradycardia or respiratory arrest during anesthesia or recovery from anesthesia.

Histopathological analysis

Histopathological analysis revealed various alterations in Groups I and II, including gliosis, axonal edema, central chromatolysis, lymphocyte infiltration detected by hematoxylin-eosin staining, and fibrous thickening of the dura mater demonstrated by staining with Masson's trichrome (Fig. 1). In Group II, only

	Group I	Group II	Group III	Р
Body weight (kg)	16.7 ± 2.0	13.2 ± 1.55	14.4 ± 2.51	0.10
Spine length (cm)	70.7 ± 4.0	64.2 ± 3.5	66.0 ± 6.26	0.17

Table 1. Body weight and spine length in dogs receiving subarachnoid S (+)-ketamine and in control dogs.

Group I: 0.7 mg/kg S (+)-ketamine; Group II: 0.5 mg/kg S (+)-ketamine; Group III (control): 0.9% saline. *P*=statistical significance (Kruskal-Wallis test).



Fig. 1. Photomicrographs of spinal sections (HE, 400X) obtained from dogs receiving a subarachnoid injection of S (+)-ketamine and evaluated 15 days later. A-Presence of axonal edema. B-Presence of central chromatolysis. C-Subpial lymphocyte infiltrate. D-Fibrous thickening of the dura mater. E- Enlarged view of axonal degeneration. F- Vasculitis accompanied by intense lymphocyte infiltration around the vessel.

one dog showed a small focal area of thickened dura mater. Group I dogs also had axonal degeneration, hemorrhagic subpial injury and vasculitis. In Group III, histopathological examination was normal in all dogs. Significant differences were observed for Groups I and II when compared to the control group. There were no differences between Groups I and II (Table 2).

Discussion

In the present study, the dog was chosen as the experimental model because of its availability for study, the anatomical similarity of its spine to the human spine, and the presence of well-established models for this type of experiment (28-31). The spine of dogs consists of 7 cervical, 13 thoracic, 7 lumbar, 3 fused sacral and 5 caudal or coccygeal vertebrae (33). The neurotoxicity of preservative-free racemic ketamine injected into the intrathecal space has been demonstrated in other animal species such as monkeys, rats, pigs and rabbits (20,21,34-38). However, a database search revealed no studies investigating the neurotoxicity of intrathecal injection of S (+)-ketamine or racemic ketamine in dogs.

In our study, a constant volume of 1 mL S (+)-ketamine was injected into the intrathecal space, irrespective of body weight and spine length of the dogs, in order to avoid changes related to dispersal of the solution in the groups as reported by other investigators (29,31). It is well established that increasing anesthetic dose and volume can change the subarachnoid spinal fluid dispersal (39,40). S (+)-ketamine doses of 0.5 and 0.7 mg/kg were used. In a study on rabbits, a dose of racemic ketamine of 1.5 mg/kg was injected into the subarachnoid space (20). In another study on swine, racemic ketamine without preservatives was injected at a dose of 25 mg/d (21), and a recent study reported the injection of 0.7 mg/kg S (+)-ketamine without preservative into the subarachnoid space of rabbits (38). There are case reports of subarachnoid administration of racemic ketamine in adult humans using doses of 0.05, 0.5 and 0.75mg/kg (25) and 67.2 mg/d (13), and of S (+)-ketamine at doses of 20 to 50 mg/d (26,27,41,42). The ideal dose for spinal administration has not been established. However, the doses used in the present study could potentially be applied to adult humans since, according to the cited studies, the doses administered to animals and human adults ranged from 0.5 to 0.7 mg/kg assuming an average weight of 60 kg for adults.

The anesthetic was injected as a single dose in this study. In contrast, in another study on rabbits S (+)-ketamine was administered through a subarachnoid catheter for 7 consecutive days, a fact that might have caused different types of damage such as necrotizing lesions around the central canal (38).

The neurotoxic potential of intrathecal ketamine has been questioned since some animal studies found that preservative-free ketamine caused no important alterations (20,37). In addition, there are case reports in which subarachnoid administration of preservativefree S (+)-ketamine caused no neurological alterations suggestive of neurotoxicity (26,41,42). In 2 case reports with post-mortem histopathological analysis of the spinal cord, the authors observed neurotoxic lesions (18,19); however, these patients received racemic ketamine with preservatives and the alterations were at-

Neurological injury	Group I n	Group II n	Group III n	GIII/GI P	GIII/GII P	GI/GII P
Gliosis	6	2	0	0.048	0.46	0.06
Axonal edema	6	5	0	0.048	0.047	1.0
Subpial injury	1	0	0	1.0	1.0	1.0
Central chromatolysis	3	1	0	0.2	1.0	0.5
Lymphocyte infiltration	3	6	0	0.2	0.0048	0.1
Axonal degeneration	1	0	0	1.0	1.0	1.0
Vasculitis	1	0	0	1.0	1.0	1.0
Dura mater thickening	5	1	0	0.047	0.2	1.0

Table 2. Histopathological analysis of the spinal cord of dogs receiving subarachnoid S (+)-ketamine and control dogs using hematoxylin-eosin staining and Masson's trichrome.

Group I: 0.7 mg/kg S (+)-ketamine; Group II: 0.5 mg/kg S (+)-ketamine; Group III (control): 0.9% saline. n= number of dogs. *P*= statistical significance (Fisher's exact test).

tributed to the use of benzethonium chloride, a fact also demonstrated in animal studies (21,37). Recently, Vranken and colleagues (27) published the first case report of histopathological abnormalities suggestive of neurotoxicity in a male patient after repeated intrathecal injections of S (+)-ketamine for 28 days.

We have observed that subarachnoid administration of a single dose of S (+)-ketamine without preservative to dogs resulted in histopathological abnormalities in all dogs of Group I, and we observed focal lesions in only one dog in the group receiving a dose of 0.5 mg/kg. No histopathological alterations were observed in the control group. These results were significant when comparing Groups I and II with the control group, especially regarding the findings of gliosis, axonal edema and subpial lymphocyte infiltration. Similar results have been reported in studies on rabbits receiving preservative-free S (+)-ketamine, with the observation of axonal edema and a subpial lymphocyte infiltrate (38), and in swine which had gliosis after injection of racemic ketamine with preservative (benzethonium chloride) (21). Central chromatolysis was detected in 3 dogs of Group I and one dog of Group II. Although not significant when compared to the control group, this type of injury has also been reported in other studies on animals and humans (27,38). One dog of Group I had vasculitis accompanied by lymphocyte infiltration of spinal tissue, a finding also detected in a patient receiving continuous intrathecal infusion of ketamine with preservative (19). The spinal surface is the main site of contact of the anesthetic with nervous tissue and is therefore more vulnerable to the agent administered (43). The frequent observation of subpial lymphocyte infiltration in the 2 experimental groups might suggest an acute toxic effect on the tissue exposed to S (+)-ketamine.

Fibrous thickening of the dura mater was observed in 5 dogs of Group I. This finding has not been reported in other studies investigating subarachnoid S (+)-ketamine, probably because of differences in the staining technique of the slides, since in the present study we used Masson's trichrome, a stain specific for the detection of fibrosis (32). Adhesive arachnoiditis is characterized by the presence of arachnoid fibrosis accompanied by other abnormalities that depend on the cause triggering the process (44). Thus, arachnoiditis has been described as a progressive inflammatory process which is initially characterized by radiculitis of the pia mater and arachnoid membrane, accompanied by nerve root edema (45). The present results do not permit us to conclude that S (+)-ketamine is the cause of arachnoiditis since no fibrosis was observed in the pia mater despite the presence of fibrous thickening of the dura mater and nerve root edema in some dogs. The thickening might have been caused by puncture since fibrosis was detected in the outer meninges, but not in the control dogs and only one dog of Group II had a small focal area of thickened dura mater.

NMDA receptor blockade has been related to necrosis and cell apoptosis after injection of ketamine into the rat brain (46,47). The signs of neurotoxicity found in this study might be attributed to excessive antagonism of glutamate NMDA receptors, which are responsible for excitatory processes in the brain and spinal cord, by S (+)-ketamine.

No significant difference in lesions was observed between Group I and Group II; however, in view of the changes observed in both groups, it is not possible to state that the dose of 0.5 mg/kg is safe for subarachnoid use. In this respect, studies involving a larger number of animals, a longer period of clinical observation, and subsequent or continuous injections might produce more intense lesions.

It should be emphasized that the administration of S (+)-ketamine into the subarachnoid space caused neurotoxic alterations in the spinal cord of dogs which, like other animal species, might be more sensitive to the drug than humans, a fact that impairs extrapolation of animal findings to humans (48). We suggest that S (+)-ketamine should not be given to clinical patients by this route until further evaluation of the significance of this toxicity.

CONCLUSION

The administration of a single dose of preservative free S (+)-ketamine into the subarachnoid space causes histopathological alterations in dogs suggestive of central nervous system toxicity.

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