Ain-Shams Journal of Anesthesiology

ORIGINAL ARTICLE Open Access

Histopathological and analgesic effects of intrathecal dexmedetomidine, racemic ketamine, and magnesium sulfate in rats

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Abstract

Background: This study aims to investigate the histopathological and analgesic effects of intrathecal administration of dexmedetomidine, preservative-free racemic ketamine, and magnesium sulfate in Sprague Dawley rats. This study included 40 male Sprague Dawley rats weighing between 240 and 260 g. After the intrathecal catheterization, the rats were randomly divided into four groups. Following the baseline measurements, no drugs were administered in the control group (group C). Simultaneously, 0.02 ml (1 μgr/kg) of dexmedetomidine was administered in group D, 0.02 ml (1 mg/kg) preservative-free racemic ketamine in group K and 0.02 ml (0.05 mg/kg) magnesium sulfate in group M via intrathecal route. Concomitantly, the hot-plate test was used to measure the analgesic effect of drugs. For histopathological evaluation, the rats were sacrificed to obtain the medulla spinalis.

Results: The hot-plate test revealed that the mean response time was 6.3 ± 1.2 s in baseline measurements without medication. However, prolongation in the mean response times of the drug-administered groups to the hot-plate test was also observed. Upon histopathological examination, myelin degeneration was detected in all study groups. No inflammation was observed in rats in group D, whereas inflammation was noted in only two rats in group K. Concerning the presence of red neurons, the only group that differed from the control group belonged to group K.

Conclusions: Dexmedetomidine, preservative-free racemic ketamine, and magnesium sulfate have an analgesic effect when administered intrathecally in rats. Of these drugs, preservative-free racemic ketamine stands out as the most histopathologically safe drug.

Keywords: Dexmedetomidine, Racemic ketamine, Magnesium sulfate, Intrathecal, Histopathology

Background

Various adjuvants have been used not only to potentiate the effect of local anesthetic but also to reduce the side effects in regional anesthesia (Staikou & Paraskeva, 2014). Although several studies are investigating the analgesic effects of these adjuvants (Albrecht et al., 2013; Beltrutti et al., 1999; Erdivanli et al., 2013), studies examining the histopathological effects are relatively scarce.

Dexmedetomidine is a new generation drug and is a highly selective α2-adrenergic receptor (α2-AR) agonist that is associated with sedative, analgesic, and sympatholytic effects. The role of the intrathecally administered dexmedetomidine in alleviating both acute and chronic pain has already been studied (Hayashi & Maze, 1993; Konakci et al., 2008). Dexmedetomidine has several neuroprotective effects including sympatholysis, preconditioning, and reducing ischemic reperfusion injury (Dahmani et al., 2005). On the contrary, the intrathecal administration of dexmedetomidine has been demonstrated to have neurotoxic effects on the spinal cord in rats and rabbits (de Pereira Cardoso et al., 2016; Hou et al., 2012).

The popularity of ketamine has been increasing again in recent years. The mechanism of action of the spinal

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analgesic effect of intrathecal injection of ketamine remains controversial. Ketamine noncompetitively inhibits N-methyl-D-aspartate (NMDA) receptors in the dorsal horn (Staikou & Paraskeva, 2014); it may also interact with opioid receptors, monoaminergic neural systems and suppress myelinated nerve conduction (Iida et al., 1997). Although ketamine has been used for many years, the potential for direct neurotoxic effects that can occur when it is administered intrathecally has not been clarified (Staikou & Paraskeva, 2014; Vranken et al., 2006). Several substances, such as chlorobutanol and benzethonium chloride, added to ketamine have been claimed to be potentially neurotoxic, but studies conducted with both a mixture of racemic ketamine and preservative-free S (+)-ketamine have shown conflicting results (Lizarraga et al., 2008; Rojas et al., 2012; Vranken et al., 2006).

Although magnesium (Mg) sulfate does not have a direct analgesic effect, it inhibits calcium influx into the cell by blocking NMDA receptors, which play a key role in both initiating and sustaining central sensitization, and has an antinociceptive effect (Na et al., 2011). Mg sulfate shows this effect at the spinal level and reduces the need for analgesics after intrathecal administration (Albrecht et al., 2013; Kroin et al., 2000). However, the histopathological effects of Mg observed in several studies have revealed contradictory results (Chanimov et al., 1997; Ozdogan et al., 2013; Simpson et al., 1994; Staikou & Paraskeva, 2014).

This study aimed to examine the histopathological and the analgesic effects of intrathecal administration of dexmedetomidine, preservative-free racemic ketamine, and Mg sulfate in rats.

Fig. 1 The flowchart of the study

Methods

Animals and ethical statement

After obtaining approval from the animal trials ethics committee Akdeniz University, 40 male Sprague Dawley rats (240-260 g) were provided from Akdeniz… University Experimental Animal Application and Research Center. All interventions were performed following the guidelines of the International Association for the Study of Pain (Zimmermann, 1983). Additionally, standard laboratory conditions (12 h a day and 12 h night lighting, 22–24 °C room temperature) and separate cages were provided. The rats were fed ad libitum. Moreover, all tests were performed at the same time (from 8:30 to 11.30 AM) so that the diurnal rhythm did not affect the drugs. The flowchart of the study in Fig. 1 was followed.

Intrathecal catheterization

The rats were anesthetized by intraperitoneal xylazine hydrochloride (15 ml/kg) and ketamine (50 mg/kg) injection. Lumbar areas were shaved before the intrathecal catheterization. After sterilization with povidone-iodine solution, we performed skin and subcutaneous dissection and inserted an intrathecal polyethylene catheter (ID 0.28 mm, OD 0.61 mm; Becton Dickinson, Philadelphia, USA), using the modified Yaksh method (Yaksh & Rudy, 1976). Following the catheterization procedure, if neurological damage or motor paralysis develops, we decided to exclude these rats from the study. We randomly divided the subjects into four groups (ten rats in each group), using a randomization scheme generated by software available online (https://www.graphpad.com/quickcalcs/randMenu). We started the hot-plate test 1 day after catheterization. Following the baseline measurements, no drugs were administered in the control group (group C). Also, we administered 0.02 ml (1 μgr/kg) dexmedetomidine in group D, 0.02 ml (1 mg/kg) preservative-free racemic ketamine in group K, and 0.02 ml (0.05 mg/kg) Mg sulfate in group M via intrathecal route.

Hot-plate test

We used a 55 °C platform for hot-plate (HP) testing and measured the latency of the movement of the hind paws of the rats placed on the platform. The HP response times were measured for 4 days at 15, 30, 45, 60, 90, and 120 min after baseline measurement by a researcher who had no information whether the drug was administered. As a result, three measurements were made at each measurement time, and the meantime was recorded as the HP response time (Fig. 2).

Histopathological examination

On the fifth day of the study, the rats were sacrificed to remove the medulla spinalis for histopathological examination. Samples were fixed in a 10% formalin solution and embedded in paraffin blocks. Then, 5-μm-thick sections of the samples were prepared using a microtome. After deparaffinization, sections were stained with hematoxylin and eosin. Subsequently, a pathologist evaluated the histopathological parameters such as myelin degeneration, inflammation, and the presence of red neurons under the light microscope using a blinded method (Fig. 3).

Statistical analysis

Statistical analysis was performed using SPSS for windows 13.0 (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to analyze the distributions of HP test results. Moreover, the student's t test was used to compare the drug-administered groups and the control

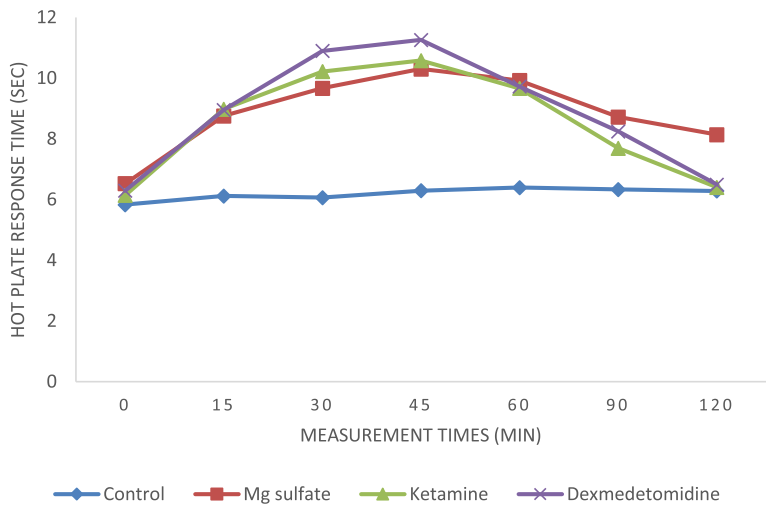

Fig. 2 Hot-plate response times of study groups

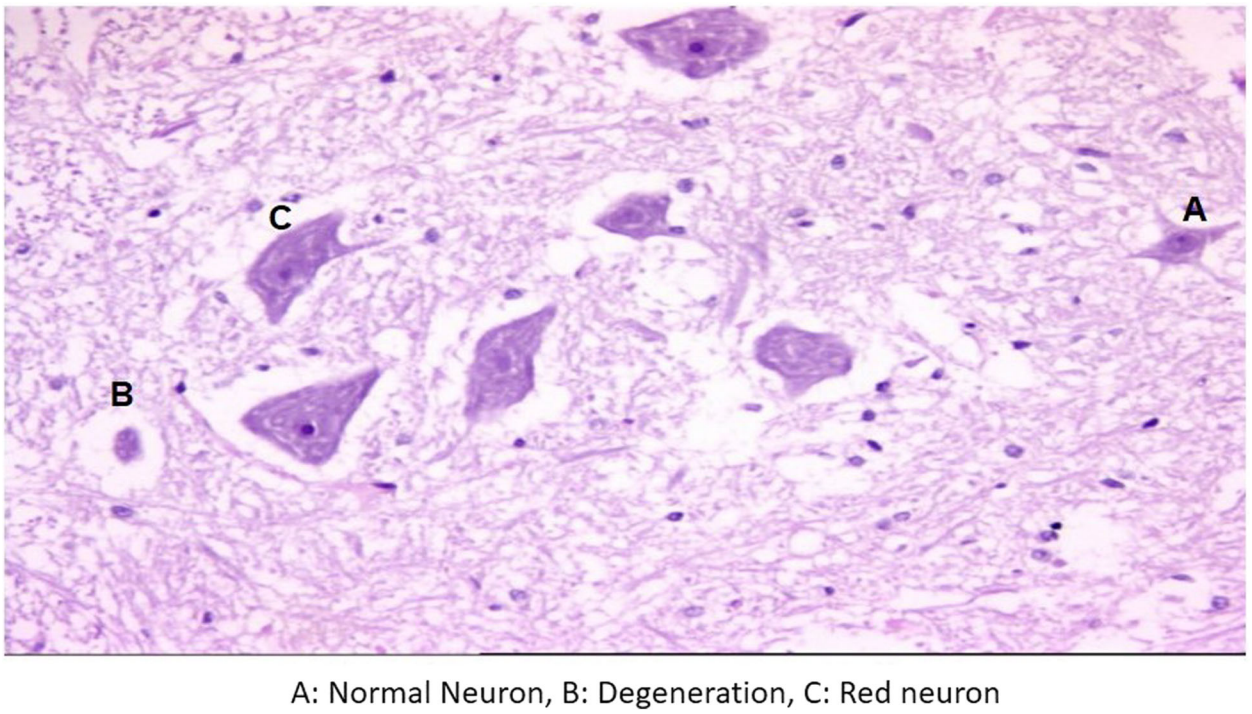

Fig. 3 The histopathology of the spinal cord section of a rat (hematoxylin- and eosin-stained section, × 200)

group in terms of HP testing, whereas the chi-square test was used to compare histopathological findings. Fundamentally, *P* values < 0.05 were considered significant.

Results

Based on the analysis, the mean weight of 40 rats included in the study was 249.9 ± 6.1 g. No rats were excluded from the study due to traumatic puncture, neurological deficit, motor paralysis, or death.

HP test results

At baseline, the HP test response times of the rats were similar (Table 1). Afterward, we observed prolongation of HP test response times in all adjuvant drug groups. In addition, we found that the HP test response times of the rats in group K and group D returned to their basal values at 120 min, while it was still longer in the rats in group M (Fig. 2).

Histopathological findings

Myelin degeneration was detected in all the study groups (p = 0.09) (Table 2). There were no differences between group C and group M in terms of inflammation. Inflammation was detected in only two rats in group K, whereas no inflammation was observed in group D (Table 3). When we examined the study groups in terms

Table 1 Hot-plate test results of the study groups

Measurement time	Control (n = 10)	Mg sulfate (n = 10)	Ketamine (n = 10)	Dexmedetomidine (n = 10)
T0	6.1 ± 1	6.3 ± 1.1	6.3 ± 1.2	6.4 ± 1.3
T1	6.1 ± 0.6	8.8 ± 1*	9 ± 1.5*	9 ± 1.9*
T2	6.1 ± 0.4	9.7 ± 0.9*	10.2 ± 1.3*	10.9 ± 1.8*
T3	6.2 ± 0.6	10.3 ± 1.3*	10.5 ± 0.9*	11 ± 1.4*
T4	6.2 ± 0.4	9.9 ± 1*	9.6 ± 1.4*	9.7 ± 1.1*
T5	6.3 ± 0.5	8.7 ± 1.2*	7.7 ± 1.2*	8.3 ± 0.4*
T6	6.3 ± 0.5	8.3 ± 1.3*	6.4 ± 1	6.5 ± 0.6

Data expressed as mean ± SD (s)
*Different from the control group (p < 0.001)
T0 baseline, *T1* 15 min after baseline, *T2* 30 min after baseline, *T3* 45 min after baseline, *T4* 60 min after baseline, *T5* 90 min after baseline, *T6* 120 min after baseline

Table 2 Comparison of study groups in terms of Myelin degeneration

	Absent	Mild	Severe
Control (n = 10)	0	7	3
Magnesium sulfate (n = 10)	0	7	3
Ketamine (n = 10)	0	9	1
Dexmedetomidine (n = 10)	3	7	0

p = 0.09

Table 4 Comparison of study groups in terms of presence of red neurons

	Absent	Present
Control (n = 10)	2	8
Magnesium sulfate (n = 10)	1*	9
Ketamine (n = 10)	9	1
Dexmedetomidine (n = 10)	6	4

*Different from the control group (p = 0.002)

of the absence of red neurons, we found a difference only between group C and group K (Table 4).

Discussion

We administered dexmedetomidine, preservative-free racemic ketamine, and Mg sulfate intrathecally to rats and examined their analgesic and histopathological effects. The ideal drug for neuroprotection was preservative-free racemic ketamine. In addition, we found prolongation in HP test response times of all three adjuvant drugs compared to the control group.

Dexmedetomidine produces a nociceptive effect due to NMDA receptor activation at the spinal cord level. NMDA receptors are located in the superficial dorsal horn, most commonly in lamina II. Thus, activation of these receptors plays a major role in the transmission of nociceptive information (Horvath et al., 2001). Furthermore, intrathecal administration of α2-AR agonists exerts an antinociceptive activity by reducing the release of glutamate from the primary afferent nerve terminals (Ueda et al., 1995) and by suppressing the activity of wide dynamic range neurons because of harmful stimuli (Murata et al., 1989). Because sedative-acting α2-AR is mostly present at the supraspinal level, the sedative effect is rarely seen in intrathecal administration (Konakci et al., 2008). Furthermore, because α2-AR agonists cross the blood-brain barrier poorly, their side effects associated with intrathecal or epidural administration are minimal, and effective local antinociception occurs (Konakci et al., 2008). In this study, the longer HP response times in the dexmedetomidine-administered group are similar to the literature. However, the widespread use of dexmedetomidine is limited due to the side effects such as hypotension and bradycardia, which are caused by systemic absorption and redistribution after spinal administration (Staikou & Paraskeva, 2014). In our study, we found that myelin degeneration and red neurons occurred with the use of multiple doses of 1 μg/kg dexmedetomidine. This may be due to the vasoconstriction of medullary blood vessels and the commercial form of dexmedetomidine having a pH of 4.5–7.0 (de Pereira Cardoso et al., 2016). Therefore, the neuroprotective effect may not be indicated.

Table 3 Comparison of study groups in terms of inflammation

	Absent	Mild	Moderate	Severe
Control (n = 10)	1	6	1	2
Magnesium sulfate (n = 10)	4	2	3	1
Ketamine (n = 10)	8†	2	0	0
Dexmedetomidine (n = 10)	10*	0	0	0

*Different from the control group (p < 0.001)
†Different from the control group (p = 0.001)

It is worth noting that intrathecally administered ketamine changes the perception of pain at the spinal level in both animals and humans (Schnoebel et al., 2005). However, this analgesic effect is reported to be seen in chronic pain rather than in acute pain (Pelissier et al., 2008). A study by Kosson et al., 2008 did not achieve analgesic effects with a single dose of ketamine. Additionally, Lizarraga et al., 2008 argue that ketamine causes analgesia by the effect of local anesthesia. Nevertheless, we detected prolonged HP response times with 1 mg/kg ketamine. This may be due to both the local anesthetic effect of ketamine and the modulation of central sensitization by specific NMDA blockade (Rojas et al., 2012). Again, Beltrutti et al., 1999 reported that the analgesic effect can be indicated at high doses, but in this case, there is a potential for neurotoxicity. A study in rabbits reported that multiple doses of intrathecally administered preservative-free S (+)-ketamine are neurotoxic (Vranken et al., 2006). In contrast, neurotoxicity was not detected in a study of dogs using single-dose preservative-free S (+)-ketamine (Rojas et al., 2012) and in a study of sheep using single-dose ketamine (Lizarraga et al., 2008). Unlike all these studies, in our study, multiple doses of preservative-free racemic ketamine were administered to rats, but neither inflammation nor presence of red neurons was observed. Hence, intrathecally administered preservative-free racemic ketamine may not be neurotoxic.

The specific role of Mg sulfate in protecting from central sensitizing caused by peripheral nociception stimulation has been studied previously (Liu et al., 2001). Mg sulfate inhibits calcium entry into the cell by blocking

NMDA receptors, thereby demonstrating the antinociceptive effect (Na et al., 2011). In this study, HP response times were significantly prolonged, which is similar to the literature. Besides, Chanimov et al., 1997 reported that both rats without Mg sulfate and rats administered with 6.3% Mg sulfate intrathecally showed a slight vacuolization in the ganglion cells in gray matter, whereas the rats administered with 12.6% Mg sulfate intrathecally were moderately vacoulized. Again, Ozdogan et al., 2013 administered 15% of Mg sulfate intrathecally in both single doses and repeated doses daily for 7 days. They detected neurodegeneration in both groups, but more in the recurrent dose group. In this study, repeated doses of 15% Mg sulfate were used. And as a result, similar to the control group, we detected myelin degeneration, inflammation, and presence of red neurons in group M. Based on this, the neuroprotective effect of intrathecally used Mg sulfate may not be mentioned.

Conclusions

All of the adjuvant drugs that were applied multiple times intrathecally in this study showed an analgesic effect. In addition, among the drugs that were tested, preservative-free racemic ketamine is the safest adjuvant drug in terms of histopathological results. However, the response of the animal spinal cord and the human spinal cord to drugs may be different. Therefore, further studies are required for the safe use of these adjuvant drugs in humans. Nevertheless, this study of rats can shed light on human studies.

Abbreviations

α2-AR: α2-Adrenergic receptor; NMDA: N-Methyl-D-aspartate; Mg: Magnesium; HP: Hot plate

Acknowledgements

Not applicable.

Authors' contributions

EO is the guarantor and done the concepts, design, definition of intellectual content, literature research, clinical studies, experimental study, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing, and manuscript review. ZB, BK, AT, IEG, and NK have done the concepts, design, definition of intellectual content, literature research, data acquisition, manuscript preparation, manuscript editing, and manuscript review. All authors have read and approved the manuscript.

Funding

None.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the animal trials ethics committee of Akdeniz University (date 14 January 2004—number 2004/4). Rats were provided from Akdeniz University Experimental Animal Application and Research Center. All interventions were performed in accordance with the guidelines of the International Association for the Study of Pain.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 21 April 2021 Accepted: 26 November 2021
Published online: 18 December 2021

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