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The Potentiation of the Analgesic Effect of Intrathecally Coadministered Magnesium Sulphate and Bupivacaine in Duration of Sensory Blockade in Rats

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= Abstract =

Background: Based on previously reported articles, magnesium sulphate seemed to cause a motor paralysis, but not complete analgesia when administered intrathecally alone, but is likely to have a partial analgesic effect. Accordingly, we tested a hypothesis that magnesium sulphate might potentiate the analgesic effect when coadministered intrathecally with bupivacaine.

Methods: Eighteen male Sprague-Dawley rats were allocated into three groups of six animals each. The duration of sensory blockade was determined by observing the period when the animal did not vocalize and/or withdraw (struggle) while forceps-pinch tests were applied to a hindlimb paw. The six animals in each of the following three groups were injected intrathecally with 0.03 ml of the different test substances: (group 1) 16.7% magnesium sulphate {50% magnesium sulphate (0.01 ml) + 0.9% sodium chloride (0.02 ml)}; (group 2) 50% magnesium sulphate (0.01 ml) + 0.5% bupivacaine (0.02 ml); (group 3) 0.33% bupivacaine {0.5% bupivacaine (0.02 ml) + 0.9% sodium chloride (0.01 ml)}.

Results: Sensory blockade in the hindlimbs was observed only in group 2 and lasted for 12 to 14 minutes, while there were no sensory blockades in group 1 and group 3.

Conclusions: Magnesium sulphate potentiated the analgesic effect of bupivacaine when coadministered intrathecally with bupivacaine in rats. These results suggest that intrathecal administration of magnesium sulphate may be a useful adjunct to spinal bupivacaine anesthesia. **(Korean J Anesthesiol 2001; 41: S 33~S 38)**

Key Words: Anesthetic techniques: intrathecal. Pharmacology: bupivacaine; magnesium sulphate.

INTRODUCTION

The magnesium ion blocks the ion channel of the N-methyl-D-aspartate (NMDA) receptor in a voltage-dependent fashion[1] and increased extracellular magnesium concentrations in vitro causes noncompetitive NMDA blockade.[2] At normal values of the resting potential, the pore of the NMDA channel is blocked by Mg^{2+}. Thus, even when glutamate binds to the receptor, the blocked channel prevents ionic flow (and an excitatory postsynaptic potential). The block can be relieved by depolarization, which presumably displaces the Mg^{2+} from the pore. When the pore is unblocked, cations (i.e., Na^{+}, K^{+}, and Ca^{2+}) can

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readily flow through the channel.[3]

In rats, after intrathecal administration of either magnesium sulphate or lidocaine, vocalization with tail-pinching was recognized after the administration of 12.3% magnesium sulphate (10 μl), but was not observed after the administration of 8% lidocaine (10 μl) during paralysis.[4] These results suggest that magnesium sulphate caused motor paralysis, but not complete analgesia.

Other studies[5,6] showed the same result that intrathecal administration of magnesium sulphate alone did not provide a satisfactory analgesia. These studies[5,6] also revealed potentiation of the analgesic effect when magnesium was coadministered intrathecally with opioids. But, no study that investigated the effects of magnesium sulphate on the analgesic effect of intrathecal bupivacaine has been published. Accordingly, we tested a hypothesis that magnesium sulphate might potentiate the analgesic effect when coadministered intrathecally with bupivacaine. In this study, we investigated the impact of intrathecal magnesium sulphate on the sensory and motor profiles in the rats hindlimbs under the intrathecal subanalgesic dose of bupivacaine.

METHODS

This study was approved by the Animal Care Committee of Yonsei University. Experiments were performed on 18 male Sprague-Dawley rats, weighing between 310 and 380 g, divided randomly into three groups of six animals each. The animals were anesthetised with ketamine (20 mg, IM). An intrathecal catheter was inserted using a modification of Bahar's method.[7] Briefly, with the rat in the prone position, the back was shaved and a line joining both iliac crests was defined. A point 7 cm rostral from the midline of this line was identified, and a midline incision was made at this point. The fascia and muscles were retracted, and then two spinous processes were resected. A 2 mm hole was drilled with a ball-shaped diamond drill between the resected spinous processes. When the dura was exposed, it was tensed and torn gently with fine-pointed forceps until CSF leaked out. A polyethylene tubing (PE-10) catheter was inserted caudally 1.5 cm from this point. The caudal tip of the catheter lay at the spinal level between vertebrae T13 and L1. A catheter connector (Epidural Minipack, Portex Co) was connected at 15 cm from the inserted catheter end. The wound was closed with 3-0 silk sutures. We used only catheterised rats in which flaccid paralysis of the hindlimbs was observed after intrathecal administration of 35 μl of 2% lidocaine on the day of surgery. Rats showing postoperative neurological deficits, inflammation or weight loss were excluded.

For assessment of neurological functions, the animals were allowed to breathe room air and the following observations were made from the time of commencement of the study: (a) The duration of sensory blockade was determined by observing the period when the animal did not vocalise and/or withdraw (struggle) while forceps-pinch tests were applied to a hindlimb paw,[8-10] (b) The duration of motor blockade was determined by observing the period when the animals were not able to walk on all four limbs. Forceps-pinch tests were applied at every five seconds for the measurement of sensory blockade onset, and at every one minute for the measurement of sensory blockade duration.

Intrathecal injections were performed at least 48 h after recovery from surgery, recovery being defined as the ability to walk steadily, drink and feed. The rat was restrained while intrathecal injections were made and then freed to move around its cage.

During the experiment itself, 0.03 ml of the different test substances were injected intrathecally using a 50-μl syringe in the six animals in each of the following three groups: (group 1) 16.7% magnesium sulphate {50% magnesium sulphate (0.01 ml) + 0.9% sodium chloride (0.02 ml)}; (group 2) 50% magnesium sulphate (0.01 ml) + 0.5% bupivacaine hydrochloride (plain, Astra) (0.02 ml); (group 3) 0.33% bupivacaine {0.5% bupivacaine (0.02 ml) + 0.9% sodium chloride (0.01 ml)}. The dead space volume of catheter and connector were flushed with physiological saline after each injection. The details concerning the experimental groups and the substances injected intrathecally are summarized in Table 1.

Kruskal-Wallis test was used to examine the differences among the three groups. And then, each two groups were

separately examined by Wilcoxons signed rank-sum test for multiple comparisons. All statistical test was done with SAS 6.12 package. The level of statistical significance was defined as $P < 0.05$ (Kruskal-Wallis test) or $P < 0.05/3$ (Wilcoxons signed rank-sum test).

RESULTS

In group 2, the mean onset of the sensory blockade was 57.5 seconds after the intrathecal injection. Sensory blockade in the hindlimbs was observed only in group 2. Duration of sensory blockade lasted for 12 to 14 minutes in group 2, while there were no sensory blockades in group 1 and group 3 (Table 2). There were no differences among the three groups in the onset of motor blockade.

Table 1. Experimental Groups and Intrathecally Injected Drugs

Experimental group	Injected drugs
Group 1 (n = 6)	50% magnesium sulphate 10 μl + 0.9% sodium chloride 20 μl
Group 2 (n = 6)	50% magnesium sulphate 10 μl + 0.5% bupivacaine 20 μl
Group 3 (n = 6)	0.5% bupivacaine 20 μl + 0.9% sodium chloride 10 μl

There was no difference between group 1 and group 2 in the duration of motor blockade. The duration of motor blockade of group 3 was significantly shorter than that of group 1 or group 2 (Table 3).

DICUSSION

When magnesium sulphate alone was administered intrathecally, there were vocalization (struggle) and withdrawal of the hindlimbs in response to the forceps-pinch test, suggesting incomplete analgesia. After 0.33% bupivacaine (0.03 ml) injection, there was a motor blockade for 5.8

Table 2. Onset and Duration of Sensory Blockade in Group 2

Number of rats	Onset (sec)	Duration (min)
1	40	14
2	55	12
3	45	12
4	65	14
5	80	12
6	60	14
Mean	57.5	13
SD	14.4	1.1

There were no sensory blockades in group 1 and group 3

Table 3. Onset and Duration of Motor Blockade

Number of rats	Onset (sec)			Duration (min)		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
1	20	20	30	206	138	8
2	20	20	30	213	162	5
3	15	30	25	210	220	5
4	30	15	20	220	209	5
5	30	20	20	188	268	7
6	20	25	20	210	271	5
Mean	22.5	21.7	24.2	207.8	211.3	5.8*
SD	6.1	5.2	4.9	10.8	54.2	1.3

The duration of motor blockade of group 3 was significantly shorter than that of group 1 and group 2 (*$P < 0.01$)

minutes, but no sensory blockade in the hindlimbs to which the forceps-pinch test was applied. But, when the above mentioned subanalgesic dose of bupivacaine was coadministered with magnesium sulphate, a definite sensory block was demonstrated, suggesting potentiation of the analgesic effect.

Many of the previous studies showed inconsistent results on the sensory blocking effect of intrathecal magnesium,[4,9,10] although most of them agreed that intrathecal magnesium caused a motor blockade. Because of differences in study design, it is difficult to compare our results with those of previous studies, but our results are similar to those of Karasawa's study in that intrathecal magnesium alone caused a motor blockade but not complete sensory blockade.

Searching for the adequate intrathecal dose of magnesium was difficult. In Karasawa's study, vocalization was observed after intrathecal injection of magnesium sulphate 12.3% (10 μl) and loss of transient vocalization was observed in two-thirds of rats receiving magnesium sulphate 24.6% (10 μl) when forceps-pinch tests were applied to their tail.[4]

In our previous pilot study, in which 50% magnesium sulphate (15 μl) and 0.5% bupivacaine (20 μl) were coadministered intrathecally, the duration of the sensory blockade lasted for about 20 minutes in hindlimbs. But because most of the rats became lethargic, assessment of neurological functions was difficult and the results were unreliable. When 50% magnesium sulphate (5 μl) and 0.5% bupivacaine (20 μl) was coadministered intrathecally, the duration of the sensory blockade lasted up to 4−5 minutes in the hindlimbs. But, duration of sensory blockade was variable. Therefore, we chose 50% magnesium sulphate (10 μl) for the intrathecal coadministration with 0.9% sodium chloride (20 μl) or 0.5% bupivacaine (20 μl).

We tried to find the subanalgesic dose of bupivacaine. And when 0.33% bupivacaine (30 μl) was administered intrathecally, sensory blockade to the nociceptive stimulus of the forceps-pinch test developed for about 5 minutes over the animal's trunk, but it did not produce a sensory block in the hindlimbs. Therefore we chose 0.33% bupivacaine (30 μl) and measured the sensory blockade in the animal's hindlimbs.

The safety of intrathecally administered magnesium has been mentioned in several previous studies. After the same dose {(magnesium sulphate 6.3% (20 μl) or 12.6% (20 μl)} was given as a series of 15 injections on alternate days for one month, there were no lasting neurological consequences, and the spinal cords showed identical histologic changes in animals receiving saline injections or those with an intrathecal catheter without any injections.[10] In dogs, an intrathecal injection of 45−60 mg of magnesium sulphate did not produce spinal cord abnormalities on histopathologic examination.[11] In a case in which a patient was inadvertently administered 1,000 mg of magnesium sulphate intrathecally, there was a motor blockade lasting for 5 hours and followed by a complete recovery.[12] Although we did not perform histopathologic examinations on our study animals, we could not find serious neurological sequellae after recovery from intrathecal magnesium. Therefore, intrathecal magnesium sulphate appears to have a good safety profile, although more studies in large animals would be desirable before clinical trials.

Local anesthetics block both the propagation of the neural action potential, as well as generation of an action potential, by a selective effect on sodium channels that prevents the depolarization of the nerve membrane.[13] Therefore, low concentration of local anesthetics may serve to diminish nociceptive transmission.[14] Experimental evidence for the involvement of excitatory amino acids in nociception came from behavioural studies. Intrathecal administration of NNDA in the spinal subarachnoid space of mice produced a hyperalgesic effect in the tail-flick and hot-plate tests.[15,16] Intrathecal injection of competitive NMDA receptor antagonist 2-amino-5-phosphonopentanoate (AP5) produced analgesic effects in rats.[17] Despite these studies showed analgesic effect of magnesium on acute pain model, the mechanism of analgesic effect of magnesium is still under debate. But, it is considered that magnesium, which is an antagonist of the NMDA receptor and also is a physiological calcium channel blocker, has analgesic properties in pain conditions.[18-20] Because interference with calcium influx is important for the release of neurotransmitters and other substances implicated in nociception,

calcium channel blockade seems to play an important role in the analgesic effect of magnesium. The NMDA receptor is an amino acid receptor responsible for excitatory synaptic transmission. The NMDA blockade may serve to diminish nociceptive transmission. Therefore if local anesthetics and magnesium are coadministered intrathecally, the potentiation of the analgesic effect may appear (in our pilot study, other local anesthetics such as lidocaine and tetracaine each showed potentiation of the analgesic effect when coadministered intrathecally with magnesium).

And we could not confirm the catheter tip position accurately before injecting the study solutions, but at postmortem dissection, the catheters were located at the spinal level between vertebrae T13 and L1.

In conclusion, intrathecal magnesium sulphate potentiated the analgesic effect of intrathecal bupivacaine in rats. These results suggest that intrathecal administration of magnesium sulphate may be a useful adjunct to spinal bupivacaine anesthesia

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REFERENCES

1. Nowak L, Bregestovski P, Ascher P: Magnesium gates glutamate activated channels in mouse central neurones. Nature 1984; 307: 462-5.
2. Harrison NL, Simmonds MA: Quantitative studies on some antagonists of N-methyl D-aspartate in slices of rat cerebral cortex. Br J Pharmacol 1985; 84: 381-91.
3. Byrne JH: Postsynaptic potentials and synaptic integration. In: Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR, eds: Fundamental neuroscience. San Diego: Academic Press, 1999; 353-4.
4. Karasawa S, Ishizaki K, Goto F: The effect of intrathecal administration of magnesium sulphate in rats. Anaesthesia 1998; 53: 879-86.
5. Kroin JS, McCarthy RJ, Von-Roenn N, Schwab B, Tumen KJ, Ivankovich AD: Magnesium sulphate potentiates morphine antinociception at the spinal level. Anesth Analg 2000; 90: 913-7.
6. McCarthy RJ, Kroin JS, Tumen KJ, Penn RD, Ivankovich AD: Antinociceptive potentiation and attenuation of tolerance by intrathecal co-infusion of magnesium sulphate and morphine in rats. Anesth Analg 1998; 86: 830-6.
7. Bahar M, Rosen M, Vicker MD: Chronic cannulation of the intradural or extradural space in the rat. Br J Anaesth 1984; 56: 405-10.
8. Yaksh TL, Rudy TA: Studies on the direct action of narcotics in the production of analgesia in the rat. J Pharmacol Exp Ther 1977; 202: 411-28.
9. Bahar M, Chanimov M, Grinspun E, Koifman I, Cohen ML: Spinal anaesthesia induced by intrathecal magnesium sulphate: an experimental study in a rat model. Anaesthesia 1996; 51: 627-33.
10. Chanimov M, Cohen Ml, Grinspun Y, Herbert M, Rief R, Kaufman I, et al: Neurotoxicity after spinal anaesthesia induced by serial intrathecal injections of magnesium sulphate: an experimental study in a rat model. Anaesthesia 1997; 52: 223-8.
11. Simpson JI, Eide TR, Schiff GA, Clagnaz JF, Hossain I, Tverskoy A, et al: Intrathecal magnesium sulphate protects the spinal cord from ischemic injury during thoracic aortic cross-clamping. Anesthesiology 1994; 81: 1493-9.
12. Lejuste MJ: Inadvertent intrathecal administration of magnesium sulphate. S Afr Med J 1985; 68: 367-8.
13. Åkerman B, Arweström E, Post C: Local anesthetics potentiate spinal morphine antinociception. Anesth Analg 1988; 67: 943-8.
14. Penning JP, Yaksh TL: Interaction of intrathecal morphine with bupivacaine and lidocaine in the rat. Anesthesiology 1992; 77: 1186-200.
15. Dickenson AH: A cure for wind up: NMDA receptor antagonists as potential analgesics. Trends Pharmacol Sci 1990; 11: 307-9.
16. Aanonsen LM, Wilcox GL: Nociceptive action of excitatory amino acids in the mouse: effects of spinally administered opioids, phencyclidine and sigma agonist. J Pharmacol Exp Ther 1987; 243: 9-19.
17. Cahusac PM, Evans RH, Hill RG, Rodriquez RE, Smith DA: The behavioural effects of an N-methylaspartate receptor antagonist following application to the lumbar spinal cord of conscious rats. Neuropharmacology 1984; 23: 719-24.
18. Miranda HF, Bustamante D, Kramer V, Pelissier T, Saavedra H, Paeile C, et al: Antinociceptive effects of calcium channel blockers. Eur J Pharmacol 1992; 217:

137-41.
19. Wong CH, Dey P, Yarmush J, Wu W, Zbuzek VK: Nifedipine-induced analgesia after epidural injection in rats. Anesth Analg 1994; 79: 303-6.
20. Koinig H, Wallner T, Marhofer P, Andel H, Hörauf K, Mayer N: Magnesium sulfate reduces intra- and postoperative analgesic requirements. Anesth Analg 1998; 87: 206-10.