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 and increased extracellular magnesium concentrations in vitro causes noncompetitive NMDA blockade. At normal values of the resting potential, the pore of the NMDA channel is blocked by Mg²⁺. 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 Mg²⁺ from the pore. When the pore is unblocked, cations (i.e., Na⁺, K⁺, and Ca²⁺) can

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readily flow through the channel.3

In rats, after intrathecal administration of either magnesi-
sum sulphate or lidocaine, vocalization with tail-pinching
was recognized after the administration of 12.3% magnesi-
sum 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 studies5,6 showed the same result that intrathecal
administration of magnesium sulphate alone did not pro-
vide a satisfactory analgesia. These studies5,7 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 pub-
lished. 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 magne-
sium 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
grams, divided randomly into three groups of six animals each.
The animals were anesthetized 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.5,6 (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-pinç 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% magnesi-
um 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 Wilcoxon's 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 (Wilcoxon's 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>
<thead>
<tr>
<th>Experimental group</th>
<th>Injected drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n = 6)</td>
<td>50% magnesium sulphate 10μl + 0.9% sodium chloride 20μl</td>
</tr>
<tr>
<td>Group 2 (n = 6)</td>
<td>50% magnesium sulphate 10μl + 0.5% bupivacaine 20μl</td>
</tr>
<tr>
<td>Group 3 (n = 6)</td>
<td>0.5% bupivacaine 20μl + 0.9% sodium chloride 10μl</td>
</tr>
</tbody>
</table>

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).

**DISCUSSION**

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>
<thead>
<tr>
<th>Number of rats</th>
<th>Onset (sec)</th>
<th>Duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>12</td>
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<td>3</td>
<td>45</td>
<td>12</td>
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<tr>
<td>4</td>
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<td>14</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>14</td>
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</table>

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

<table>
<thead>
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<th>Number of rats</th>
<th>Onset (sec)</th>
<th>Duration (min)</th>
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<tbody>
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<tr>
<td>2</td>
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</tr>
<tr>
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<td>15</td>
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<td>5</td>
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<td>20</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>25</td>
</tr>
</tbody>
</table>

Mean 57.5 13

SD 14.4 1.1

There were no sensory blockades in group 1 and group 3

<table>
<thead>
<tr>
<th>Number of rats</th>
<th>Onset (sec)</th>
<th>Duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>2</td>
<td>213</td>
<td>162</td>
</tr>
<tr>
<td>3</td>
<td>210</td>
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<tr>
<td>5</td>
<td>188</td>
<td>268</td>
</tr>
<tr>
<td>6</td>
<td>210</td>
<td>271</td>
</tr>
</tbody>
</table>

Mean 22.5 21.7 24.2

SD 6.1 5.2 4.9

<table>
<thead>
<tr>
<th>Number of rats</th>
<th>Onset (sec)</th>
<th>Duration (min)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>207.8</td>
<td>211.3</td>
</tr>
<tr>
<td>2</td>
<td>210</td>
<td>271</td>
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<td>3</td>
<td>209</td>
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<td>268</td>
</tr>
<tr>
<td>6</td>
<td>210</td>
<td>271</td>
</tr>
</tbody>
</table>

Mean 207.8 211.3 5.8

SD 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 subanalgiesic dose of bupivacaine was
coadministered with magnesium sulphate, a definite sen-
sory block was demonstrated, suggesting potentiation of
the analgesic effect.

Many of the previous studies showed inconsistent re-
sults on the sensory blocking effect of intrathecal magne-
sium,¹⁹,²⁰ although most of them agreed that intrathecal magnesium caused a motor blockade. Because of differ-
ces 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 magne-
sium 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 co-
administered 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 block-
ade 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 subanalgiesic dose of bupivacaine.
And when 0.33% bupivacaine (30 μl) was administered
intrathecally, sensory blockade to the noicceptive 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% bu-
pivacaine (30 μl) and measured the sensory blockade in
the animals 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 conse-
quences 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 neu-
rological sequelae after recovery from intrathecal mag-
nesium. 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 noicceptive transmission.¹⁴ Experimental
evidence for the involvement of excitatory amino acids in
noicception came from behavioural studies. Intrathecal
administration of NMDA 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-phosphopentanoate (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 neuro-
transmitters 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
