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ORIGINAL ARTICLE

Does Epidural Magnesium Sulfate Cause Medulla Spinalis Injury in Rabbits?

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Abstract

Introduction: Magnesium is an intracellular ion that has analgesic properties through calcium regulation and N-methyl-D-aspartate receptors. However, the safety of neuroaxial magnesium is not proved. The aim of this experimental study is to investigate the possible neurotoxicologic effects of epidural magnesium sulfate (MgSO4) on medulla spinalis in rabbits.

Methods: After ethic committee's approval, 18 male Albino New Zealand rabbits were enrolled into the study. Epidural catheter was inserted into the sacral canal under ketamine. The development of motor and sensorial block 5 min after the administration of 1 mL of 1% lidocaine verified the placement of the catheter. Group Control (n=6): 0.20 mL isotonic saline was administered through epidural catheter. Group M150 (n=6): One mL of 150 mg.mL-1 MgSO4 (~ 0.6 mmol elemental magnesium) (pH=6.20) was administered through epidural catheter, then catheter was flushed with 0.20 mL isotonic saline. Group M450 (n=6): One mL of 450 mg.mL-1 MgSO4 (~ 1.8 mmol elemental magnesium) (pH=6.10) was administered through epidural catheter then catheter was flushed with 0.20 mL isotonic saline. Catheter's placement was localized by laminectomy. Spinal sections were taken between 5 cm rostral and caudal segments from the tip of the catheter. The sections were stained both hematoxylin-eosin and Cresyl violet. The slides were examined using a light microscope.

Results: Nissl body loss, vacuolization, myelin irregularity, gliosis, and fibrosis in gray and white matter samples were assessed. There were no signs of histological tissue damage. There was no statistically significant histopathological difference between groups.

Discussion and Conclusion: This is the first study that investigates spinal cord injury after epidural magnesium administration to our knowledge. These results are important since epidural route is the second most common route for MgSO4. In this study, we report that, even relatively higher doses of epidural MgSO4 did not cause any spinal cord injury. Further studies need to be performed to adapt these findings to clinical practice.

Keywords: Magnesium sulfate; medulla spinalis; rabbit.

Magnesium is an intracellular ion that has analgesic properties through calcium regulation and N-methyl-D-aspartate receptors[1]. The trials which investigate the analgesic efficiency of intravenous magnesium have conflicting results[2,3]. Magnesium passes poorly across the blood–brain barrier[1]. In animal[4] and human[5,6] studies, magnesium's analgesic efficiency has been shown.

However, the safety of neuroaxial magnesium is not proved. It has been shown that intrathecal (IT) magnesium can cause neurotoxicity in rabbits[7]. And also, there are two case reports about its side effects (disorientation and continuous periumbilical pain)[8,9].

We previously showed that epidural 150 mg magnesium sulfate ($MgSO_4$) did not produce any motor or sensory blockade[10] and epidural 150, 300, and 450 mg $MgSO_4$ increased cerebrospinal fluid and blood ionized Mg levels and epidural 450 mg $MgSO_4$ resulted with motor blockade (unpublished data). The limitation of these studies was the lack of histolog-

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ical investigations. Therefore, we conducted an experimental study to investigate the possible neurotoxicologic effects of epidural $MgSO_4$ on medulla spinalis in rabbits.

Materials and Methods

After ethic committee's approval (19/2015), 18 male Albino New Zealand rabbits (2000–3000 g) with normal motor activity were enrolled into the study. The rabbits were kept under standard laboratory conditions (12 h day-12 h night cycle, room temperature between 20–22°C and 50–60% humidity) until the experiment. All experiments were performed in accordance with the Declaration of Helsinki.

After intravenous (IV) access was established, anesthesia for catheter placement was induced with IV 50 mg/kg ketamine (Ketalar, Pfizer İlaçları Ltd.Şti, İstanbul, Türkiye). Spontaneous ventilation was preserved. Under ketamine anesthesia, the operative site was infiltrated subdermally with 1% lidocaine (Jetmonal, Adeka İlaç ve Kimyasal Ürünleri San. ve Tic. A.Ş, Samsun, Türkiye). Epidural catheter (Portex®, SIMS Portex Ltd, Hythe, England) was inserted into the sacral canal according to the method described previously by Arkan et al.[11]

The subjects without any neurological sequel enrolled into the study. Development of motor and sensorial block 5 min after epidural administration of 1 mL of 1% lidocaine verified the placement of the catheter.

Twenty-four hours after catheter placement, animals were assessed for any signs of replacement of epidural catheter, infection, neurological, or clinical impairment.

99.5% pure $MgSO_4$ (Sigma-Aldrich Corporation, Steinheim, Germany) was dissolved in distillate water by heating and mixing with vortex (Reax top, Heidolph Instruments GmbH and Co. KG, Schwabach, Germany). The solutions were prepared as 150 $mg.mL^{-1}$ and 450 $mg.mL^{-1}$ $MgSO_4$. The pH of the solutions was controlled with a pH meter device (InoLab® 720, WTW Wissenschaftlich-Technische Werkstätten GmbH, Munich, Germany).

Study Groups

The rabbits were randomized into three groups.

Group Control (n=6): 0.20 mL isotonic saline was administered through epidural catheter.

Group 150 (n=6): One mL of 150 $mg.mL^{-1}$ $MgSO_4$ (~0.6 mmol elemental magnesium) (pH=6.20) was administered through epidural catheter, then catheter was flushed with 0.20 mL isotonic saline.

Group 450 (n=6): One mL of 450 $mg.mL^{-1}$ $MgSO_4$ (~1.8 mmol elemental magnesium) (pH=6.10) was administered through epidural catheter, then catheter was flushed with 0.20 mL isotonic saline.

Histological Evaluation

The animals were sacrificed 1 day after drug administration with high-dose intraperitoneal sodium thiopental (120 $mg.kg^{-1}$). After thoracic incision, left atriums of the animals was cannulated (18 G cannula, Mediflon® IV cannula, Eastern Medikit Ltd., India) and transcardiac 10% formaldehyde perfusion was performed[12].

Catheter's placement was localized by laminectomy. Medulla spinalis and nerve roots were kept in +4oC fixation solution. Spinal sections were taken between 5 cm rostral and caudal segments from the tip of the catheter.

- Nissl body loss,
- Nucleus-cytoplasm membrane irregularity, vacuolization,
- Myelin irregularity,
- Gliosis in gray matter, necrosis-Stellosis,
- Fibrosis in gray matter, non-specific infection, and neurophagy were assessed with light microscopy.
    - 0 – No neurotoxicity (–)
    - 1 – Minimal neurotoxicity (±) (1–3 neurons)
    - 2 – Mild neurotoxicity (+) (3–10 neurons)
    - 3 – Moderate neurotoxicity (++) (10–20 neuron)
    - 4 – Severe neurotoxicity (+++) (>20 neuron).

Tissue samples were fixed in 10% formalin in phosphate buffer for 3 days. They were then rinsed under running tap water overnight to remove the fixative. Tissue samples were processed for embedding in paraffin wax by routine tissue protocol. 5 µm thick sections were cut using a Leica/Reichert-Jung rotary microtome (RM, 2255) (Cologna, Germany). After deparaffinization in xylene, the sections were rehydrated through a graded ethanol series, stained both hematoxylin (01562E, Surgipath, Bretton, Peter Borough, Cambridgeshire) and eosin (01602E, Surgipath, Bretton, Peter Borough, Cambridgeshire) and Cresyl violet. The sections were dehydrated through a graded ethanol series, cleared in xylene, and mounted in Entellan (UN 1866, Merck, Darmstadt, Germany). The slides were examined using a light microscope (Euromex, Oxion) (Arnhem, Nederland), and the histopathological appearance of tissues in the different groups was compared.

Statistics

Statistical analysis was performed using SPSS for Windows software program 15.0 version. Values were reported as mean±SD. Friedman and Wilcoxon tests were used in group comparisons. Kruskal–Wallis and Mann–Whitney U tests were used for comparing three groups. $P<0.05$ was accepted as statistically significant.

Table 1. Neurotoxicity scores of the animals.

Group	No	Score
Control	1	0
Control	2	0
Control	3	0
Control	4	0
Control	5	0
Control	6	0
Group 150	1	0
Group 150	2	0
Group 150	3	0
Group 150	4	0
Group 150	5	0
Group 150	6	0
Group 450	1	0
Group 450	2	0
Group 450	3	0
Group 450	4	0
Group 450	5	0
Group 450	6	0

Results

All animals survived until the end of the study.

All medulla spinalis sections were stained with hematoxylin eosin and cresyl violet and scored. We could not detect any statistically significant difference between groups (Table 1).

Gray and white matter samples from the groups were also assessed for Nissl body loss, vacuolization, myelin irregularity, gliosis, and fibrosis in gray matter. There were no signs of histological tissue damage (Fig. 1).

Discussion

In this study, we investigated the possible neurotoxicologic effects of epidural magnesium. We could not detect any histological findings of neurotoxicity in rabbits.

Many studies had been shown that IT and epidural $MgSO_4$ have analgesic effects[13,14]. However, the optimal dose for neuroaxial magnesium is not clear. In many clinical trials, 50 or 100 mg is chosen for IT and epidural routes. However,

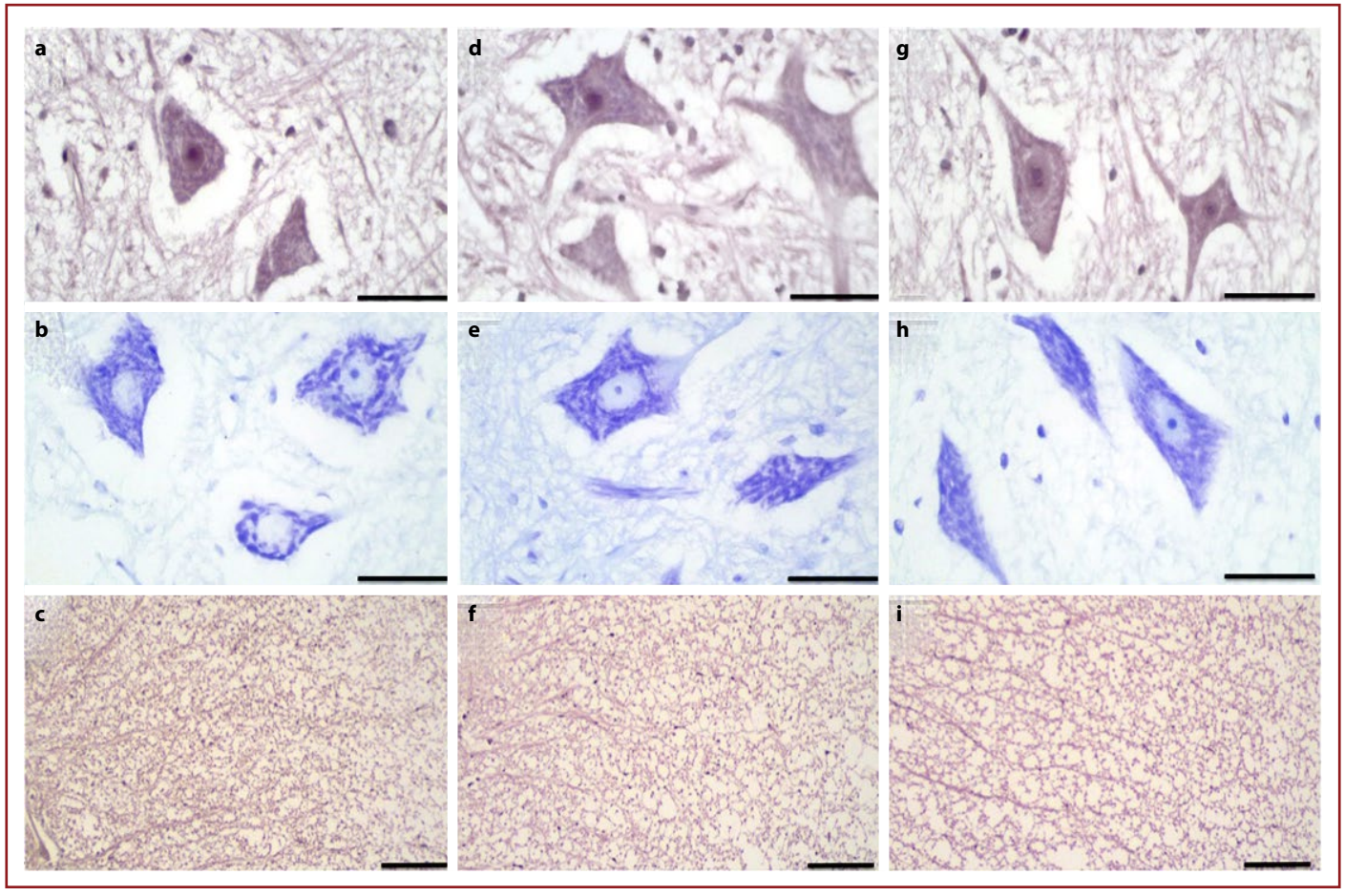

Figure 1. (a and b, d and e, g and h) anterior horn (Multipolar motor neurons) and bar =50 mm (×40), **(c, f, i)** white matter (Myelinated axon sections) and bar =150 mm (×10). **(a-c)**: Control group, **(d-f)**: Group 150, **(g-i)**: Group 450. **(a, d, g, c, f, i)**: Hematoxylin eosin, **(b, e, h)**: Cresyl violet.

when the pharmacokinetics of the drugs administered IT or epidurally is considered, it is unlikely that the same dose can have the same effects.

We previously showed that 150 and 450 mg epidural $MgSO_4$ did not change the plasma magnesium levels[10] and epidural 150 and 450 mg $MgSO_4$ increased spinal magnesium levels by 13% and 200%, respectively, and 450 mg caused motor block (unpublished data). Hence, we used the same doses to assess neurotoxicologic effects of epidural magnesium.

Magnesium is commonly used for it is analgesic effects as an adjuvant to local anesthetics. Relatively lower doses of $MgSO_4$ are used for this purpose. In a case report, 700 mg IT $MgSO_4$ was administered accidently and motor block continued until 8 h after the injection[15]. Ozdogan et al.[16] used IT 0.02 mL of 15% $MgSO_4$, and they showed that it caused significant neurodegeneration in rats. Even though 450 mg $MgSO_4$ resulted with 200% increase in cerebrospinal fluid (CSF) ionized magnesium levels, we could not detect any neurotoxicity. The investigators did not measure the CSF magnesium levels before histopathological evaluation. This difference could be due to direct injection of $MgSO_4$ caused a greater increase in CSF magnesium levels. Another possible could be the histological investigation method (they used an electron microscope). However, we think if epidural magnesium had caused such gross injury, we could also detect it with the light microscope.

Chanimov et al.[17] could not find any histological injury after IT 4.6 mg.kg-1 $MgSO_4$ and only moderate vacuolization in gray matter after 9.2 mg.kg-1 in rat. In this study, the investigators used relatively higher doses for IT injection. Even though they used rat and give $MgSO_4$ intrathecally, their findings are similar to ours.

Goodman et al.[8] reported two inadvertent epidural magnesium administrations (8.7 and 9.6 g) without any neurological symptoms. Dror et al.[9] also reported a patient that had periumbilical continuous burning pain after inadvertent epidural magnesium infusion, but she did not have any neurological sequel. These case reports with extremely high doses of epidural $MgSO_4$ injections without any neurological sequels can support our study's results. However, obviously it is impossible to perform any histological assessment in these patients. Our study is the first one to investigate the histopathological changes in medulla spinalis after epidural $MgSO_4$ administration as far as we know.

Another experimental use of IT $MgSO_4$ is protection from spinal cord injury. Several studies showed that IT magnesium is neuroprotective[18-20]. Nevertheless, contrarily to these findings, Saeki et al.[7] could not find any advantageous effects of $MgSO_4$ in spinal cord injury. They also reported that IT $MgSO_4$ has neurotoxicity risk.

A limitation of this study is we did not control the sensorial and motor block after epidural magnesium injections. However, as mentioned previously in two different studies with epidural magnesium administration, we also established sensorial and motor block effects of the same doses (10 and unpublished data).

Conclusion

As we reviewed the literature, we find contemporary findings about neurotoxicological effects of neuroaxial $MgSO_4$. However, this is the first study that investigates spinal cord injury after epidural magnesium administration to our knowledge. We think our results are really important since epidural route is the second most common route for $MgSO_4$ IV route in clinical practice. In this study, we report that even relatively higher doses of epidural $MgSO_4$ did not cause any spinal cord injury. However, further studies need to be performed to adapt these findings to clinical practice since the most of the clinical trials investigate the analgesic effects not the complications after neuroaxial $MgSO_4$.

Ethics Committee Approval: After ethic committee's approval (19/2015), 18 male Albino New Zealand rabbits (2000–3000 g) with normal motor activity were enrolled into the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: A.A., N.G.; Design: A.A., E.Ö., N.G.; Supervision: N.G., A.B.; Materials: N.G., A.B.; Data Collection or Processing: A.A., E.Ö., H.A.E., Y.E., S.B.; Analysis or Interpretation: N.G., A.B., N.E., H.K.; Literature Search: A.A., E.Ö., H.A.E., N.G.; Writing: A.A., E.Ö., H.A.E.; Critical Review: N.G., A.B.

Conflict of Interest: None declared.

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