Magnesium: does it reduce ischemia/reperfusion injury in an adnexal torsion rat model?

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Aim: The aim of the present study was to assess the protective effects of magnesium sulfate \((\text{MgSO}_4)\) on ischemia/reperfusion (I/R) induced ovarian damage in a rat ovarian torsion model.

Methods: Forty-two female Sprague Dawley rats were included in the study. They were divided into six groups as Group 1, sham; Group 2, bilateral ovarian torsion; Group 3, bilateral ovarian torsion–detorsion; Group 4, \text{MgSO}_4–sham; Group 5, \text{MgSO}_4–bilateral ovarian torsion; Group 6, bilateral ovarian torsion–\text{MgSO}_4–detorsion. Both torsion and detorsion periods lasted 3 hours. In Groups 4, 5 and 6, \text{MgSO}_4 \((600 \text{ mg/kg})\) was administered by intraperitoneal route 30 minutes before sham operation, torsion and detorsion, respectively. At the end of the study period, both ovaries were removed. One of the ovaries was used for histopathological analyses and the other for biochemical analyses.

Results: In the torsion–detorsion group, all the histopathological scores were higher compared to the sham and torsion only group \((p<0.05)\). Administration of \text{MgSO}_4 only caused significant decrease in the inflammatory cell scores of the torsion–detorsion group \((p<0.05)\). \text{MgSO}_4, whether given before torsion or before detorsion, suppressed malondialdehyde levels when compared to the untreated groups \((p<0.01\) and \(p<0.001,\) respectively). Glutathione peroxidase activities were significantly higher in the \text{MgSO}_4 applied torsion and detorsion groups than Groups 2 and 3 \((p<0.05,\) for both). Administration of \text{MgSO}_4 also caused an increase in glutathione levels in the torsion and detorsion groups compared to the torsion only and detorsion only groups \((p<0.05,\) for both). Also, total oxidant status levels decreased in the \text{MgSO}_4 applied torsion and detorsion groups compared to the untreated corresponding ones \((p<0.01\) and \(p<0.001,\) respectively). \text{MgSO}_4 significantly decreased the Oxidative Stress Index levels in the torsion–detorsion group compared to Group 2 \((p<0.001)\).

Conclusion: Histopathological and biochemical analysis revealed that prophylactic treatment with \text{MgSO}_4 reduces the changes observed in I/R injury in a rat model.

Keywords: ovarian torsion, ischemia/reperfusion injury, magnesium sulfate

Introduction

Ovarian torsion has been described as rotation of the adnexa around its vascular axis. It is a rarely encountered surgical emergency and requires prompt diagnosis and treatment. Clinical presentation is non-specific and there are often significant delays between the onset of symptoms and operative intervention. For all age groups, traditional management was resection of the ischemic-appearing ovary because of the theoretical risk of thromboembolism, the concern of associated malignancy and the belief that a grossly blue–black and hemorrhagic adnexa was non-viable. Long-term analysis of the cases treated by detorsion revealed that the method is safe and can save the ovary. So, management has changed and today laparoscopic detorsion as soon as possible is the preferred method in premenopausal women.
Reperfusion by detorsion protects the ovary from ischemic damage. However, during the reperfusion period tissue damage continues because of oxygen-derived free radicals. Due to its results, the torsion–detorsion process is named ischemia/reperfusion (I/R) injury. Several methods have been tried to prevent this injury. Pharmacologic conditioning with different drugs, having antioxidant and anti-inflammatory properties, has been tested to determine their preventive effect on I/R injury.

Magnesium is found in large amounts both in intracellular and extracellular spaces of the body. It is important for numerous physiological events and is used as a therapeutic agent in many areas of medicine like neurology, cardiology, gastroenterology and obstetrics. Magnesium is a powerful antioxidant and a potent anti-inflammatory agent. It inhibits endotoxin-related inflammatory molecule upregulation. Magnesium is an L-type calcium channel blocker and in this way may decreases calcium overload of the tissue which is an important factor in tissue damage. Magnesium also provides cellular protection by stabilizing the cell membrane potential and decreasing energy need.

Despite all these important effects, nothing is known about the effect of magnesium on I/R injury of the ovary. We aimed to explore whether MgSO₄ could prevent I/R injury in rat ovaries.

Materials and methods

Approval of the study was provided by Firat University local ethics committee for animal research and adhered to guidelines prepared according to EU Directive 2010/63/EU. Experiments were done in a laboratory of the same university. By performing daily vaginal smears, animals showing regular cycles were selected. Forty-two female Sprague Dawley rats weighing between 200 and 250 g, aged 10–12 weeks, were taken and allocated randomly into six groups:

- Group 1: sham (n=7)
- Group 2: bilateral ovarian torsion (n=7)
- Group 3: bilateral ovarian torsion–detorsion (n=7)
- Group 4: MgSO₄–sham (n=7)
- Group 5: MgSO₄–bilateral ovarian torsion (n=7)
- Group 6: bilateral ovarian torsion–MgSO₄–detorsion (n=7)

Operations were done under anesthesia. Ketamine hydrochloride (50 mg/kg Ketalar; Eczacibasi, Istanbul, Turkey) and xylazine hydrochloride (7 mg/kg Rompun; Bayer, Istanbul, Turkey) were used intramuscularly. Under anesthesia, the animals were placed in prone position on the operating table. The surgical field was shaved, and antisepsis was performed. Two imaginary points, 1–1.5 cm lateral from the point where the last rib of the animal joined with the vertebrae, were identified on both sides of the back of the animal. New points 1–1.5 cm caudal to the first points were found and incisions measuring nearly 1 cm were made. Through passing the muscular layer, the abdominal cavity was reached. The encountered fat layer was carefully removed from the incision out of the abdomen. Next to the fat tissue, the ovary was observed. In Group 1, after 3 minutes the ovary was put back into the abdomen using the same incision and the incision was closed. This procedure was repeated for both sides. After a 3-hour period, euthanasia was performed and both ovaries were taken. In Group 2, bilateral ovarian ischemia was induced by putting a knot that can be opened easily just below the ovary, instead of applying vascular clips. A No 1 silk suture was used for this procedure (Figure 1). Both ovaries were removed 3 hours after placing the knot. In Group 3, after performing ischemia for 3 hours, sutures were removed, and the ovaries were reperfused for 3 hours before removal. In Group 4, MgSO₄ (600 mg/kg) was given intraperitoneally 30 minutes before performing a sham operation. In Group 5, 30 minutes after intraperitoneal MgSO₄ application, ischemia was induced, and the ovaries were taken out after a 3-hour ischemia. In Group 6, an ischemia period lasting 3 hours was performed and MgSO₄ was administered intraperitoneally 30 minutes before reperfusion. After the 3-hour reperfusion period both ovaries were taken out. In all groups, one ovary was taken for biochemical analysis and the remaining ovary for histopathological examination. Ovarian tissue samples taken for biochemical analysis were stored at −80°C.

Histological examination

Tissues were fixed in formalin, dehydrated by alcohol, cleared in xylene and put in paraffin blocks. Fragments 5 µm thick were cut and hematoxylin and eosin staining was performed. They were analyzed and photographed by the same pathologist in a blind manner using a Leica DM...
Biochemical analysis

The ovarian tissues were homogenized with phosphate buffer (50 mmol/L, pH 7.0) for 3 minutes on ice. They were centrifuged at 10,000 rpm for 10 minutes at 4°C to determine malondialdehyde (MDA) and glutathione (GSH) levels and catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities. The modified method of Ohkawa et al was used to determine MDA concentrations and results were expressed as nmol/g protein.11 Tissue GSH levels were measured by the dithio-nitrobenzoic acid recycling method described by Ellman and the results were given as nmol/mg protein.12 CAT activities were determined according to the method of Aebi and results were given as k/g protein, where k is the first-order rate constant.13 GSH-Px activities were determined by the method of Paglia and Valentine.14 The oxidation of NADPH was followed spectrophotometrically at 340 nm and results were given as U/g protein. SOD activities were measured by the method of Sun et al and a slightly modified method by Durak et al.15,16 Tissue SOD activities were given as U/g protein. Protein concentrations were determined according to the method of Lowry et al.17 Total oxidant status (TOS) and total antioxidant status (TAS) were determined using commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey) on an Advia 2400 analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA).18,19 The TOS measurement method was based on the oxidation of ferrous ion-o-dianisidine complex to ferric ion by the oxidants present in the sample. The assay method was calibrated with hydrogen peroxide and results were expressed as µmol H₂O₂ Eq/g protein. The TAS measurement method was based on the reduction of dark blue–green colored 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical to the colorless reduced 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) form with the antioxidants present in the sample. The assay was calibrated with Trolox and results were given as mmol Trolox Eq/g protein. The Oxidative Stress Index (OSI) was defined as the percent ratio of TOS to TAS levels.

Statistical analysis

Results are given as the mean ± SD. One-way analysis of variance and post hoc Tukey tests were used for statistical analyses. For statistical analyses, SPSS version 22.0 (IBM Corporation, Armonk, NY, USA) was utilized. p<0.05 was considered statistically significant.

Results

All the torsioned and detorsioned ovaries showed blue–black discoloration macroscopically. Histopathologic evaluation scores are given in Table 1.

This evaluation revealed that ovaries were normal in the sham and MgSO₄–torsion groups (Figure 2A and D, respectively). Compared to the sham group, increase in edema (black star) and hemorrhage (black arrow) was not statistically significant in the torsion group (Figure 2B) (p>0.05). Increase in these parameters was significant in the torsion–detorsion group compared to the sham group (Figure 2C) (p<0.05).

Decrease in interstitial edema, hemorrhage and inflammatory cells was observed in the MgSO₄–torsion group compared to the torsion group (Figure 2E) but differences were not significant (p>0.05).

A significant increase in edema, hemorrhage and inflammatory cells (green arrow) was observed in the torsion–detorsion group compared to the torsion group (p<0.05).

In the torsion–MgSO₄–detorsion group (Figure 2F), we observed a statistically insignificant decrease in interstitial edema and hemorrhage and a statistically significant decrease in inflammatory cells compared to the torsion–detorsion group (p>0.05 and p<0.05, respectively).

Levels of TOS, TAS, OSI, MDA and GSH and activities of SOD, CAT and GSH-Px of ovarian tissues for each group are presented in Table 2.

Both adnexial torsion and torsion–detorsion caused significant increase in tissue MDA levels (p<0.05 and p<0.001, respectively). MDA levels were statistically lower in the torsion–MgSO₄ group than in the torsion group (p<0.01)
and were statistically lower in the torsion–MgSO₄–detorsion group than in the torsion–detorsion group (p<0.001).

Torsion–detorsion caused a significant decrease in activity of GSH-Px (p<0.05) and the level of GSH (p<0.001) compared to the sham group. Administration of MgSO₄ caused a higher tissue activity of GSH-Px and level of GSH in the torsion–MgSO₄ group compared to the torsion group (p<0.05, for both). We also demonstrated a higher GSH-Px activity and GSH level in the torsion–MgSO₄–detorsion group than in the torsion–detorsion group (p<0.05, for both).
Table 2 Ovarian tissue TOS, TAS, OSI, MDA and GSH levels and SOD, CAT and GSH-Px activities of all groups

<table>
<thead>
<tr>
<th>Markers</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOS (μmol H₂O₂ Eq/g protein)</td>
<td>7.01±0.48</td>
<td>10.10±1.68**</td>
<td>12.71±3.95***</td>
<td>7.78±1.00</td>
<td>6.39±0.65***</td>
<td>6.36±0.30***</td>
</tr>
<tr>
<td>TAS (nmol Trolax Eq/g protein)</td>
<td>0.50±0.09</td>
<td>0.45±0.03</td>
<td>0.35±0.12***</td>
<td>0.52±0.04</td>
<td>0.42±0.07</td>
<td>0.37±0.02</td>
</tr>
<tr>
<td>OSI (arbitrary unit)</td>
<td>1.44±0.36</td>
<td>2.22±0.39</td>
<td>3.90±0.67***</td>
<td>1.50±0.27</td>
<td>1.54±0.26</td>
<td>1.71±0.14***</td>
</tr>
<tr>
<td>MDA (nmol/g protein)</td>
<td>6.29±0.79</td>
<td>8.33±0.86***</td>
<td>10.17±1.00***</td>
<td>4.73±1.15</td>
<td>5.88±0.40***</td>
<td>6.37±1.92***</td>
</tr>
<tr>
<td>SOD (U/g protein)</td>
<td>41.02±12.93</td>
<td>42.99±9.48</td>
<td>35.93±6.61</td>
<td>43.49±10.19</td>
<td>45.02±13.35</td>
<td>49.63±12.29</td>
</tr>
<tr>
<td>CAT (k/g)</td>
<td>4.04±0.87</td>
<td>3.04±0.69</td>
<td>2.41±0.46***</td>
<td>4.12±0.97</td>
<td>3.62±0.85</td>
<td>3.14±0.80</td>
</tr>
<tr>
<td>GSH-Px (U/g protein)</td>
<td>43.03±7.67</td>
<td>32.25±4.38</td>
<td>29.94±5.50***</td>
<td>48.79±11.90</td>
<td>44.39±5.77***</td>
<td>42.55±7.64***</td>
</tr>
<tr>
<td>GSH (nmol/mg protein)</td>
<td>3.14±0.52</td>
<td>2.03±0.30***</td>
<td>1.84±0.15***</td>
<td>3.23±0.54</td>
<td>2.77±0.34***</td>
<td>2.58±0.47***</td>
</tr>
</tbody>
</table>

Notes: Group 1, sham; Group 2, bilateral ovarian torsion; Group 3, bilateral ovarian torsion–detorsion; Group 4, MgSO₄–sham; Group 5, MgSO₄–bilateral ovarian torsion; Group 6, bilateral ovarian torsion–MgSO₄–detorsion. Data are expressed as the mean ± standard deviation. *p<0.05; **p<0.01; ***p<0.001. Group 2 vs Group 1; †Group 3 vs Group 1; ‡Group 5 vs Group 2; §Group 6 vs Group 3.

Abbreviations: TOS, total oxidant status; TAS, total antioxidant status; OSI, Oxidative Stress Index; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; MgSO₄, magnesium sulfate.

Torsion caused an insignificant decrease but torsion–detorsion caused a statistically significant decrease in the activity of catalase (p<0.05). Administration of MgSO₄ caused a statistically insignificant increase in catalase levels in all the applied groups. Changes in the activity of SOD were not significant in any of the groups (p>0.05).

We also demonstrated that TOS levels were higher in the torsion and torsion–detorsion groups when compared to the sham group (p<0.05 and p<0.001, respectively), whereas TOs levels were lower in the MgSO₄ applied torsion and detorsion groups when compared to the torsion only and torsion–detorsion only groups (p<0.01 and p<0.001, respectively). OSI levels were higher in the torsion–detorsion group when compared to the sham group (p<0.001). MgSO₄ administration caused significantly lower OSI levels in the MgSO₄-treated torsion–detorsion group compared to the torsion–detorsion only group (p<0.001).

Discussion

Through the advances in organ-preserving surgeries in many fields of medicine, not only ischemic injuries but also reperfusion injuries became important. Studies investigating the prevention of these damages have gained speed. Torsion caused ischemic changes, which can be demonstrated by biochemical analysis, in the ovary. Detorsion, as soon as possible, is an important step in the prevention of ovarian damage but alone it does not prevent ovarian damage. By detorsion, reperfusion is established, and reactive oxygen species are created. In an organism, there is a balance between the production and destruction rate of free radicals and it is known as “oxidative balance”. An imbalance that occurs in favor of free radicals is called “oxidative stress”. These radicals cause lipid peroxidation in the cell. It is known that the primary mechanism underlying tissue damage is lipid peroxidation and inhibiting or decreasing lipid peroxidation can be a basic strategy in order to prevent tissue injury. MDA levels show oxidative stress and increase through lipid peroxidation. Literature examination revealed that MDA levels were increased in many studies related to I/R injuries. In our study, we determined increased levels of MDA in both the torsion and torsion–detorsion groups compared to the sham group. Significant reduction in MDA levels was observed when MgSO₄ was given before torsion and detorsion. As a result of this, we can conclude that MgSO₄ can decrease lipid peroxidation and so the tissue injury. Other studies also proved that magnesium deficiency enhances lipid peroxidation and supplementation reduces lipid peroxidation. To prevent the damage caused by reactive oxygen radicals, different biological defense systems are present in cells. CAT, SOD and GSH-Px are the best-known ones. In our study, application of MgSO₄ caused statistically non-significant increases in tissue levels of SOD and CAT.
magnesium dependent, the antioxidant property of magne-
sium might be also related to the GSH pathway.

OSI is a marker for the degree of oxidative stress and
is the combined ratio between TOS and TAS. In our study,
torsion–detorsion caused a significant increase in the level of
TOS and OSI. Administration of MgSO\(_4\) caused significantly
lower tissue levels of TOS and OSI in the torsion–MgSO\(_4\)–
detorsion group.

Histologic examination revealed that torsion–detorsion
caused a statistically significant increase in the scores of
edema, hemorrhage and inflammatory cells. Administra-
tion of MgSO\(_4\) caused a decrease in all scores but only the
decrease in inflammatory cells was significant. Inflammatory
cells are important in tissue damage.

The effects of magnesium on I/R injury of different
organs have been investigated. Studies demonstrated that
administration of magnesium reduced renal I/R injury in rat
experimental models.\(^7\) Kao et al demonstrated that MgSO\(_4\)
significantly reduced the oxidative stress and inflammatory
response caused by bilateral lower limb I/R in rats.\(^8\) Data
from different studies support that brain damage caused by
hypoxia and radiation-induced oxidative stress of the spinal
cord could be diminished by MgSO\(_4\).\(^20,29\) Kim et al showed
that administration of magnesium before reperfusion of
liver transplantation significantly reduced blood lactate
levels.\(^30\) Kaptanoglu et al demonstrated that administration of
magnesium reduced the I/R injury on fetal rat skin.\(^31\) A free
radical reducing effect of magnesium in a coronary occlu-
sion reperfusion model was demonstrated by Garcia et al.\(^32\)
It was also demonstrated that magnesium reduces the lactate
and MDA levels after cerebral ischemia.\(^33\) It was shown that
application of Lomodex–MgSO\(_4\) prior to detorsion increases semen quality and fertility.\(^34\) There are many other
beneficial effects of magnesium. It is known that magnesium
can inhibit thrombus formation.\(^35\) Ravn et al demonstrated
that magnesium can inhibit arterial thrombus formation.\(^36\)
Magnesium also increases red cell deformability.\(^37\) After
the discovery of the importance of calcium in ischemic cell
death, calcium antagonists have come into focus for protec-
tion from ischemic damage. Magnesium is a physiological
calcium antagonist. It competes with calcium ions for ion
channels or receptor binding. Inhibition of calcium entry
into the cell by magnesium may serve an additional benefit
in ovarian I/R injury.

Magnesium also causes blood vessel dilatation. It can
reduce infarct size in ischemia.\(^38\) We did not try to explore
these effects of magnesium in our study, but these properties
of magnesium may provide additional benefits in the case of
torsion–detorsion induced ovarian damage.

**Conclusion**

This is the first study to investigate the effects of magnesium
on I/R induced injury of the ovary. Magnesium is an easily
accessible inexpensive drug. Obstetricians and gynecolo-
gists are familiar with it and can use the drug safely. In
the light of previous reports and our study, we can enounce
that MgSO\(_4\) may save the ovary from I/R injury. A limitation of
our study is the absence of long-term influences of MgSO\(_4\).
Further studies are needed to define the best effective dose,
duration, other mechanisms of action and long-term effects
before its usage in clinical practice.

**Author contributions**

ECK and SBK designed the study after a detailed literature
search for ischemia/reperfusion injury and effects of magne-
sium. OB, ECK and FGB performed the experiments. FGB
performed the biochemical analyses, and wrote the results
and methods of analysis. NK performed the histologic evalu-
ation, wrote the results and also took the photographs of the
tissue specimens. ECK wrote the article with input from all
authors. ECK arranged and submitted the article.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**

1. Poonai N, Poonai C, Lim R, Lynch T. Pediatric ovarian torsion: case
   Minimal surgery for the twisted ischaemic adnexa can preserve ovarian
3. Carden DL, Granger DN. Pathophysiology of ischaemia-reperfusion
4. Türk E, Karaca İ, Ozcinar E, et al. The effect of hypothermia on
5. Ozkisacik S, Yazici M, Gursoy H, Culhaci N. Does gradual detorsion
   protect the ovary against ischemia-reperfusion injury in rats? Pediatr
   ovarian protection than enoxaparin sodium in a rat ovarian torsion
   protects ovary against ischemia-reperfusion injury in rats: evaluation of
8. Kao MC, Jan WC, Tsai PS, Wang TY, Huang CJ. Magnesium sulfate miti-
gates lung injury induced by bilateral lower limb ischemia-reperfusion
9. Lee CY, Jan WC, Tsai PS, Huang CJ. Magnesium sulfate mitigates acute
10. Fawcett WJ, Hasbey EJ, Male DA. Magnesium: physiology and phar-
11. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues
12. Ellman G. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82:
    70–77.


