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Magnesium Sulfate Improves Sperm Characteristics Against Varicocele in Rat

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Abstract

Objective: The main purpose of this study was to determine the effect of magnesium sulfate on sperm characteristics in unilateral varicocele-induced male Wistar rats.

Materials and Methods: Sixty male Wistar rats were randomly divided into 6 experimental groups. The control group (1) received no medications and surgery. The Sham group (2) had not received any medications; abdominal cavity was opened but no varicocele was induced. Varicocele group (3): abdominal cavity was opened, varicocele-induced and no medications were applied. In group 4 abdominal cavity was opened, varicocele-induced and animal received magnesium sulfate (25 mg/kg) for 6 weeks. The groups 5 and 6 were similar to group 4, except animals received 50 and 100 mg/kg of magnesium sulfate, respectively. At the end of the weeks 3 and 6, the abdomen was opened, semen samples were collected from the Caudal epididymis to determine epididymis weight, sperm mortality, mobility and sperm count.

Results: According to the results, experimental unilateral varicocele significantly diminished sperm mortality, mobility and sperm count compared to control and sham group ($P<0.05$). Administration of magnesium sulfate as a medication dose dependently (25, 50 and 100 mg/kg) decreased sperm mortality and increased mobility and sperm count in experimental varicocele in rat compared to varicocele group at weeks 3 and 6 ($P<0.05$).

Conclusion: According to the results, it seems, magnesium sulfate might improve sperm characteristics during varicocele.

Keywords: Magnesium sulfate, Rat, Varicocele

Introduction

Varicocele is an abnormal dilatation and stasis of veins of the pampiniform plexus that drain the testis. It occurs within the spermatic cord in 15%-20% of the male population and causes infertility in approximately 50% of male (1). The pathophysiology of testicular damage in varicocele is not completely understood, however, gross testicular alterations associated with varicocele are well documented. The effect of the varicocele varies, but often results in a generalized impairment of sperm production, characterized by abnormal sperm quality, ranging from oligozoospermia to complete non-obstructive azoospermia (2).

There are several causes leading to male infertility, such as diseases, oxidative stress or nutritional insufficiency of trace elements such as selenium and zinc (3). Magnesium (Mg^{2+}) is the second most prevalent intracellular action and is involved in the metabolic activity of the cell (4). Magnesium has numerous physiological functions in the body, in health and disease. For instance it is reported Mg^{2+} can protect against vanadium-induced lipid peroxidation in the hepatic tissue (5). Recently, it is revealed Mg^{2+} deficiency impairs reproductive functions (6). However, scarce information exists on role of Mg^{2+} in fertilization. It believes Mg^{2+} has prominent role in the human reproductive system and in semen as well as fertilization (7). Seminal plasma plays an important role in providing nourishment and protection to sperm and acts as a buffer as well as a medium for sperm motility. Semen is composed of lipids, ions such as citrate, calcium, Mg^{2+}, K^+, Na^+, zinc and chloride), proteins, oxidative enzymes that protect sperm from oxidative stress (8).

Varicocele repairs improve intratesticular temperature, but fertility will reverse only in about one half of the patients. Perhaps changes appear semen concentration that leads to infertility. Although many infertile people have varicocele, its relationship with male infertility still remains unexplained (9). To best of our knowledge, no report exists on role of magnesium sulfate ($MgSO_4$) on experimental unilateral varicocele-induced rat. In this regard, based on literature review, our hypothesis was that perhaps administration of $MgSO_4$ might have positive effect on fertility in unilateral varicocele in rat. So, we investigated the possible role of dietary $MgSO_4$ on sperm mortality, mobility and sperm count on experimental unilateral varicocele-induced rat.

Materials and Methods

Study Animals

To survey possible effects of $MgSO_4$ on sperm character-

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istics in experimental unilateral varicocele-induced rat, sixty male Wistar rats (230-250 g) were allocated into 6 treatment groups. The rats were housed individually under standard laboratory conditions according to European community suggestions for laboratory animals at a temperature of 21 ± 2°C, relative humidity of 55%-60% and a 12 hours light period. All animals had free access to chow pellets and fresh water. All experimental procedures were carried in accordance with the Guide for the Care and Use of Laboratory Animals to Investigate Experimental Pain in Animals (10). Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and the current laws of the Iranian government.

Experimental Creation of Varicocele

All surgical procedures were performed under anesthesia by intraperitoneal (IP) injection of 60 mg/kg ketamine hydrochloride 10% and 10 mg/kg xylazine hydrochloride 2%. Then experimental varicocele was created (11,12). The upper left abdominal quadrant was approached through a midline laparotomy incision. Then to reveal the left kidney, the abdominal contents were packed to the right side. Herein, the renal and adrenal veins and the left spermatic vein inserts into the left renal vein. Surrounding fat and connective tissues of the left renal vein cleared to the insertion of the spermatic and adrenal veins. With a midline incision the left renal vain was exposed and after fine dissection of proximal left renal vein, left renal vein was tied using a silk suture (4-0). At the point of medial to insertion of the adrenal and spermatic vain into the renal, a metal probe (diameter ranging from 0.4-0.85 based on size of renal vein) was placed. The ligature was made around the probe, then probe removed and the vain allowed expanding within the boundary of ligature. This procedure leads to decrease renal vain diameter to one half. The midline incision of the abdominal wall and the anterior abdominal muscles were repaired, separately (2).

Study Design

Sixty male wistar rats were randomly divided into 6 experimental groups (n = 10). The control group (group 1) had not received any medication or surgery. The Sham group (group 2) had not received any medication, abdominal cavity was opened without inducing varicocele. Varicocele group (group 3): abdominal cavity was opened, varicocele-induced and no medication was applied. In group 4 abdominal cavity was opened, varicocele-induced and animal received $MgSO_4$ (Poole, Dorset, UK; 25 mg/kg) as medication, once a day for 6 weeks. The groups 5 and 6 were similar to group 4, except the animals received 50 and 100 mg/kg of $MgSO_4$, respectively. At the end of the third and sixth weeks, the abdomen was opened, the left testis extracted. Animals in the other groups, received the equal volume of distilled water once a day for 6 weeks. At the end of the study, rats fasted overnight and euthanized, peritoneum opened and testes were taken out for investigate sperm characteristics.

Sperm Characteristics

At the end of the weeks 3 and 6, semen samples were collected from the Cauda epididymis carefully separated from the testis and placed in a Petri dish containing Ham's F10. Epididymal caudal was minced with scissors to release sperm and then was placed in the incubator for 15 minutes. Approximately 10 μL of the diluted sperm suspension was transferred to each counting chamber of the hemocytometer and allowed to stand for 5 minutes. The cells which settled during this time were counted by a light microscope at 200× magnification. The sperm heads were counted and expressed as million/ml of suspension (13).

Statistical Analysis

Data were prepared in Excel, the parametric data analyzed with one way analysis of variance (ANOVA) using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). Data were expressed as mean values ± standard error of mean (SEM). P values < 0.05 were considered to denote significant differences between groups.

Results

Effects of $MgSO_4$ on epididymis weight, sperm mortality, mobility and sperm count after 3 and 6 weeks in experimental varicocele in rat is presented in Tables 1 and 2.

According to the results, experimental unilateral varicocele significantly diminished sperm mortality, mobility and sperm count compared to varicocele group at weeks 3 and 6 ($P = 0.04$). Administration of $MgSO_4$ as a medication dose dependently (25, 50 and 100 mg/kg) decreased sperm mortality and increased mobility and sperm count after 3 weeks in rat suffering from varicocele compared to group 3 ($P = 0.036$).

The same results observed at week 6 post $MgSO_4$ admin-

Table 1. Effect of Different Levels of Magnesium Sulfate on Sperm Characteristics After 3 Weeks in Experimental Unilateral Varicocele-Induced Rat

Groups	Epididymis Weight	Mortality (%)	Mobility (%)	Sperm Count
Control	0.28	48.6[b]	50.5[a]	5 660 000[a]
Sham	0.3	49.4[b]	53.1[a]	5 720 000[a]
Varicocele	0.27	67.38[a]	27.2[c]	4 000 000[b]
$MgSO_4$ (25 mg/kg)	0.32	46.75[b]	51[a]	4 840 000[a]
$MgSO_4$ (50 mg/kg)	0.3	47.38[b]	47.34[b]	5 200 000[a]
$MgSO_4$ (100 mg/kg)	0.28	46.56[b]	48.02[b]	5 840 000[a]

Abbreviation: $MgSO_4$: magnesium sulfate.
There are significant differences between groups with different superscripts in a column (a, b and c; $P < 0.05$).

Table 2. Effect of Different Levels of Magnesium Sulfate on Sperm Characteristics After 6 Weeks in Experimental Unilateral Varicocele-Induced Rat

Groups	Epididymis Weight	Mortality (%)	Mobility (%)	Sperm Count
Control	0.28	48.6[b]	51.2[a]	5 560 000[a]
Sham	0.3	49.4[b]	57.04[a]	5 720 000[a]
Varicocele	0.21	65.57[a]	21.65[c]	2 500 000[b]
$MgSO_4$ (25 mg/kg)	0.3	47.76[b]	49.77[b]	5 580 000[a]
$MgSO_4$ (50 mg/kg)	0.28	49[b]	57.74[a]	5 200 000[a]
$MgSO_4$ (100 mg/kg)	0.25	47.3[b]	54.43[a]	5 370 000[a]

Abbreviation: $MgSO_4$: magnesium sulfate.
There are significant differences between groups with different superscripts in a column (a, b and c; $P<0.05$).

istration which $MgSO_4$ at doses of 25, 50 and 100 mg/kg diminished sperm mortality while improved mobility and sperm count in experimental varicocele rat in comparison to the control as well as sham groups ($P=0.001$). However, no significant difference was observed for sperm characteristics among control and sham groups during the study ($P=0.06$).

Discussion

To the best of our knowledge, there are limited studies describing the role of $MgSO_4$ on sperm characteristics in experimental varicocele in rat. As observed, administration of different levels of $MgSO_4$ improved sperm mortality, mobility and sperm count after 3 weeks in rat suffering from varicocele. Also, the results continued until week 6 post-treatment.

Semen contains high concentrations of calcium, magnesium, zinc, and copper in bound and ionic forms. The testicular plasma, that is, the fluid composed of the secretions originating in the seminiferous tubules, tubuli recti, rete testis, and ductuli efferentes, and the epididymal plasma serve as a nutrient medium in which maturation of the developing spermatozoa takes place (14). In a study it is revealed Mg^{2+} supplements are of considerable benefit and show no harmful effects in patients receiving cisplatin treatment (15).

The diagnosis of male infertility routinely begins with a basic semen analysis, which measures various semen parameters including semen volume, color, pH, liquefaction time, viscosity, sperm count and motility, sperm morphology, concentration of round cells and polymorphonucleocytes, sperm agglutination and sperm viability (8). A relationship reported between varicoceles and semen parameters.

Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) and the antioxidants that scavenge surplus free radicals (16). ROS are natural products of cellular metabolism which, in physiological amounts, are essential requirements of spermatozoa for sperm processes leading to successful fertilization, such as capacitation, hyperactivated motility and acrosomal reaction (17). However, studies have shown that 30%-80% of male factor infertility cases are due to ROS mediated sperm damage (8).

ROS are generally produced as a byproduct of enzymatic reactions in oxidative phosphorylation, which is used to produce energy in the form of ATP (18). Animal model indicate a relation between varicocele and semen oxidation where ROS levels increased and antioxidant capacity decreased in the semen of animal with varicocele (19). These changes lead to abnormal sperm function and the infertility (20). Low levels of ROS are critical for normal fertilization, capacitation, hyperactivation and motility (21).

Surgical correction of the varicocele is associated with decreased oxidative stress; Mostafa and colleagues reported that varicocelectomy results in a significant reduction in ROS levels and also an increase in the antioxidant capacity of semen in infertile men (20).

Sperm membranes contain large amounts of unsaturated fatty acids which provide fluidity, a process that is necessary for membrane fusion (16). However, this also makes spermatozoa vulnerable to ROS attack. Seminal fluid is an important source of antioxidants in semen, as the lack of cytoplasm and DNA compaction in spermatozoa leaves very little room for translation or for antioxidant defenses. Lipid peroxidation has also been associated with a decrease in sperm motility (22).

The role of Mg^{2+} in spermatozoa quality is as yet not clear. Depletion of intracellular Mg^{2+} is known to affect all functions dependent on this ion, including glycolysis, protein synthesis, respiration, and reproduction (14). It is reported the number of spermatogonia A, preleptotene spermatocytes, mid pachytene, spermatocytes and step 7 spermatid increased in Mg^{2+} treated animals. There have been reports of Mg^{2+} deficiency induced morphological changes up to 40% of the spermatids (6).

Recently it is reported Mg^{2+} has positive role in control of ROS generation where administration of $MgSO_4$ decreases superoxide dismutase in bile duct ligation-induced liver injury in male Wistar rats (23). Mg^{2+} complexes with phospholipids reduce fluidity of the membrane and decreases membrane permeability, with parallel polarizing electrostatic effects. Conversely, it has been shown that Mg^{2+} deficiency increases permeability and promotes fragility of the heart membrane. However, to best of our knowledge, no report exists on role of $MgSO_4$ on sperm characteristics in experimental varicocele in rat. So, we were not able to compare our results with it. These results can be used as base information on effect of $MgSO_4$ on sperm characteristics in experimental varicocele in rat. Finally, the authors recommend merit researches needed to identify direct cellular and molecular signaling pathways of $MgSO_4$ on sperm characteristics.

Conclusion

According to the results, experimental unilateral varicocele diminished sperm mortality, mobility and sperm count in rat. Administration of $MgSO_4$ as a medication dose dependently (25, 50 and 100 mg/kg) decreased sperm mortality and increased mobility and sperm count in experimental varicocele in rat. It seems $MgSO_4$ might improve sperm characteristics during varicocele.

Ethical issues

All protocol of the study proved by ethic committee of Islamic Azad University, Science and Research Branch, Tehran, Iran.

Conflict of interests

The authors declare that they have no conflict of interest.

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