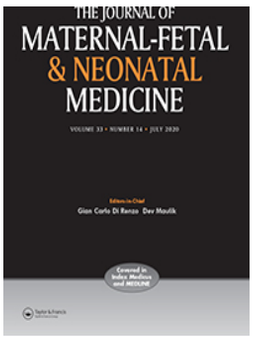

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ORIGINAL ARTICLE

Evaluation of magnesium sulfate effects on fetus development in experimentally induced surgical fetal growth restriction in rat

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ABSTRACT

Objective: The objective of this study was to evaluate the effect of magnesium sulfate in the prevention of fetal growth restriction due to the impaired uterine blood supply in the rat model.

Methods: A total number of 24 female rats were used in this study. They were mated overnight and randomly divided into control and treatment groups. After anesthesia and incising abdominal midline in day 17 of gestation, the uterine artery was occluded by an atraumatic clamp for 60 min. The rats of the control group received normal saline after surgery and the rats of treatment group received magnesium sulfate subcutaneously. The laparotomy was repeated on day 21 of gestation, and the number of alive and dead fetuses was counted in each horn. The viability of fetuses was evaluated. The weight of the placenta and fetuses and the distance between the head and tail as well as back to the abdomen of the fetuses were also measured. Samples of the amniotic fluid (AF) were collected during both surgeries for biochemical analyses of the glucose, urea, lactate, and pyruvate levels by an AutoAnalyzer.

Results: Among the total fetuses in ischemic horn, only 50% survived in the control group. Dead fetuses had less body consistency and had a dark color. In contrary, only 7.6% of the fetuses in the treatment group were absorbed and 92.4% were completely healthy and developed. Parameters related to placenta weight, fetus weight, fetus length, and fetus width had significant differences and those of the treatment group were higher. Glucose and lactate levels of the AF in the treatment group were significantly lower and urea level was significantly higher than the control group in day 21 of gestation. The changes in pyruvate levels were not significant.

Conclusion: In conclusion, magnesium sulfate may counteract with the effects of temporary uterine ischemia in pregnant rats and prevent intrauterine growth restriction.

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Embryology; fetal growth retardation; magnesium sulfate; pregnancy; rats

Introduction

Fetuses which do not reach their potential growth inside the uterus are in danger of pre- and post-delivery events including stillbirth, preterm birth, and negative health consequences either in neonatal or long-term periods. For this reason, recognition and monitoring of fetal growth restriction (FGR) are important parts of the prebirth surveillance. Despite many risk factors can lead to stillbirth, the most important factor is fetal growth restriction [1,2]. This phenomenon is also known as intrauterine growth restriction (IUGR) or small for gestational age fetus [3]. Generally, FGR is the inability of the fetus to reach its full genetic growth potential, which occurs in up to 8% of all pregnancies and is the second major cause of infant mortality after stillbirth. There is not any available treatment for this disorder [4].

Fetal growth depends on placenta function, maternal nutrition, and many other factors. Restriction of blood supply to the gravid uterus reduces the delivery of metabolic nutrients to the placenta and growing fetus. In addition, any reduction in uterine blood supply will lead to insufficient oxygen delivery to the fetus. The causal relationship between hypoxia and FGR can be justified by decreasing in growth stimulator factors or synthesis ability as a result of low oxygen consumption by the fetus [5,6].

Many investigations have been done for FGR in different species of laboratory animals such as the mouse, guinea pig, rabbit, rat, and domestic animals like sheep. The rat is mostly used because of its short-term 21 days

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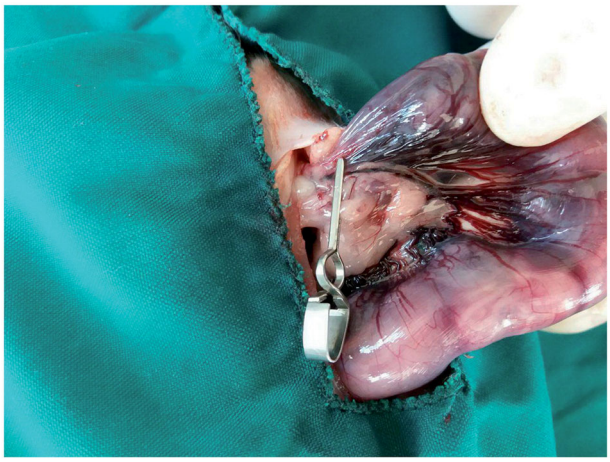

Figure 1. Experimental fetal growth restriction by temporary occluding of the uterine artery by an atraumatic clamp.

of gestation period and a large number of offspring in each pregnancy as well as the capability to easy manipulation during surgery. The researchers have used different methods for FGR in the rat, including permanent occlusion of uterine artery [7], temporary occlusion of the uterine artery for 60 min [8], dexamethasone injection, feeding restriction [9,10], and protein restriction [11].

Magnesium has known vasodilatory and anti-inflammatory effects [12,13]. In this regard, we assumed that magnesium sulfate, because of its improving effects on blood supply and oxygen carrying capacity by hemoglobin, can compensate reduced blood supply and prevent FGR when the uterine artery is temporarily occluded. The objective of this study was to evaluate the effect of magnesium sulfate in the prevention of FGR due to the impaired uterine blood supply in the rat model.

Materials and methods

Animals

Twenty four adult healthy female Wistar rats weighing 250 ± 20 g were used in this study. They were kept in separate cages in a room with appropriate ventilation and the environmental temperature was kept in 22–25 °C. The light/dark cycle was 12 h. The rats were fed by semisynthetic pellets and free access to the water was available. The 1-week period of acclimatization was considered. They were treated according to the guidelines as recommended by the National Research Council's criteria (NIH No. 86-23) and the study follows the principles of the Declaration of Helsinki.

Study design and surgery

The female rats were kept with male ones in the same cage to mate overnight. After detecting the vaginal plaque early in the morning which indicates successful mating, they were randomly divided into two equal groups including control and treatment. When the vaginal plaque was not detectable, the rats were allowed to mate one another night.

All rats were anesthetized by intraperitoneal injection of ketamine 5% (Alfasan, Woerden, Holland) and xylazine 2% (Alfasan, Woerden, Holland) combination at the doses of 80 and 5 mg/kg, respectively, at the day 17 of pregnancy. After shaving the abdomen from xiphoid to pubis and aseptic preparation, the area was draped and laparotomy was done by a ventral midline incision. The horns of the gravid uterus and its blood vessels were identified and the number of implantations was counted in both horns. The uterine artery of the left horn was occluded by atraumatic bulldog clamps (Codman® 20–1200 DRAKE Aneurysm Clip, 16 mm) for 60 min to induce ischemia (Figure 1). The abdomen was temporarily closed with towel clamps and the vital parameters of the rat were checked until removal of the clamp. The clamp was removed 60 min later and the abdomen was closed in two layers with the simple continuous pattern using 4-0 polyglactin 910 (Coated Vicryl, Ethicon Inc, US) and 3-0 polyamide (Monofil Polyamide, SUPA, Tehran, Iran) sutures.

The treatment group received 270 mg/kg 10% magnesium sulfate (Pasteur Institute, Tehran, Iran) subcutaneously immediately after abdominal closure, and then 27 mg/kg every 20 min for 4 h [13]. The control group received the same volume of normal saline (Shahid Ghazi Co, Tabriz, Iran). A single dose of 50 mg/kg cefazolin (Cefazolin-Exir®, Exir Pharmaceutical Co, Iran) was injected intraperitoneally after surgery.

Sampling and evaluation method

The rats were again anesthetized and went under laparotomy at 21 days of gestation (total pregnancy length is 21 days in rat). After cesarean section, the number of alive, absorbed, and dead fetuses was counted in each uterine horn and compared to the number of implantations at day 17 of gestation. The absorption rate of the fetuses was calculated by implantation number at day 17 minus the number of absorbed fetuses at day 21. Mortality percent was computed similarly based on dead fetuses. Fetus viability percent was calculated according to the number of live fetuses. Macroscopic parameters such as color (pink or purple = normal, brown or dark

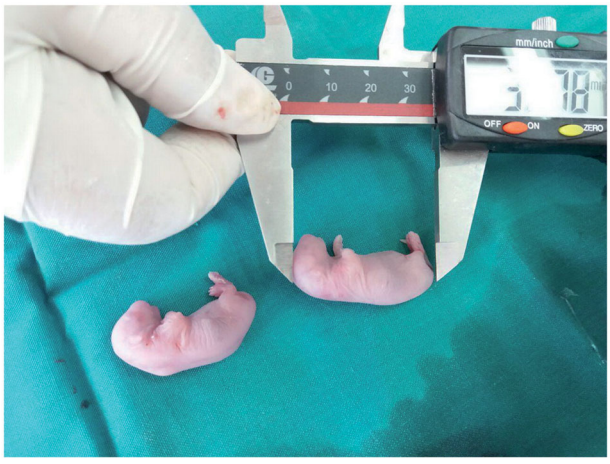

Figure 2. Measuring the length of the fetuses by a digital caliper on day 21 of gestation.

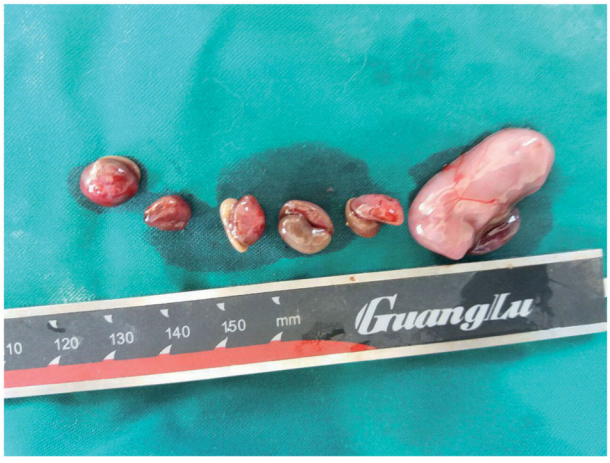

Figure 3. Absorbed fetuses along with their placenta in the control group in comparison to an alive one.

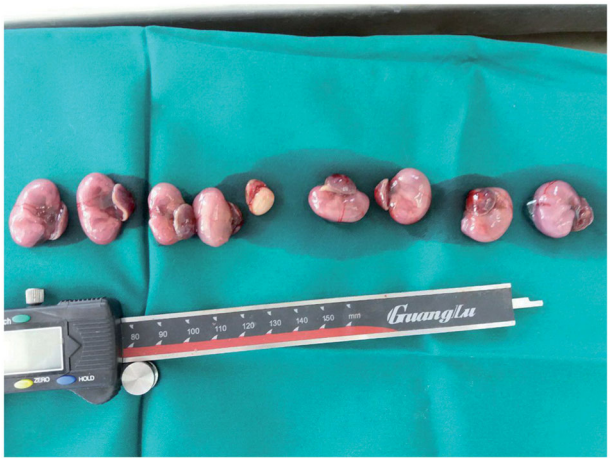

Figure 4. Alive fetuses and attached placenta in the treatment group. Only one fetus is absorbed.

green = abnormal), body consistency (normal, dried, or wet), size, and body anomalies were evaluated. The rats were euthanized by an intracardiac overdose of sodium thiopental (VUAB Pharma Inc. Czech Republic). In all alive and dead fetuses, they were weighted by a digital balance and the head to tail and back to abdomen distances were measured using a digital caliper (Figure 2). The weight of the placenta was also recorded. The liver and brain to the body weights ratios were calculated. It is worthy to mention that weights and body dimensions were not measured in absorbed fetuses, but the measurements were done in alive and dead fetuses because the dead fetuses had reached nearly to their ultimate growth similar to alive ones.

Table 1. Viability, absorption, and mortality rates (%) of the fetuses in experimental groups.

	Control group	Treatment group
Total number of the fetuses	60	78
Viability rate (%)[a] ($p = .001$)	50	92.4
Absorption rate (%)[a] ($p = .009$)	25	7.6
Mortality rate (%)[a] ($p = .005$)	25	0

[a]Indicates significant difference ($p < .05$).

Biochemical analyses

Samples of the amniotic fluid (AF) were collected from all fetuses during the first surgery and from alive and dead fetuses during the second surgery and transferred in a liquid nitrogen tank into a −20 °C freezer. But, it was not collected from absorbed fetuses in the second surgery because they were shrunk and had not any fluid. The samples were diluted to 1:20 with distilled water in order to biochemical analyses by an AutoAnalyzer (Hitachi 917, Roche Diagnostics, Indianapolis, IN, USA) using glucose and urea (Pars Azmun, Tehran, Iran), lactate (Lactate Gen2, Roche Diagnostic GmbH, Mannheim, Germany), and pyruvate (Greiner Diagnostic GmbH, Bahlingen, Germany) commercial kits.

Statistical analysis

Minitab software (version 16.2.0, Minitab Inc, State College, PA, USA) was used for statistical analysis. Regarding two experimental groups, all numerical data were analyzed by Student *t*-test method. $p < .05$ was considered as significant difference.

Results

Among the total fetuses in ischemic horn which were counted in the first surgery, 15 fetuses were dead and another 15 fetuses were absorbed in the control group, and only 30 fetuses survived. Dead fetuses had less body consistency and had a dark color (Figure 3). The closer the fetuses to the horn tip, the more

Table 2. Measurements of fetus and placenta parameters in experimental groups (mean ± SD).

	Control group	Treatment group
Fetus weight (gr)[a] ($p = .000$)	1.49 ± 0.73	2.86 ± 0.94
Placenta weight (gr)[a] ($p = .018$)	0.44 ± 0.18	0.56 ± 0.95
Fetus length (mm)[a] ($p = .000$)	23.38 ± 7	32.28 ± 5
Fetus width (mm)[a] ($p = .001$)	9.53 ± 2.46	12.14 ± 1.44
Liver to body weight ratio (%) ($p = .460$)	6.99 ± 2.77	7.68 ± 1.94
Brain to body weight ratio (%) ($p = .677$)	5.21 ± 0.99	5.37 ± 1.12

[a]Indicates significant difference ($p < .05$).

congestion and underdevelopment they had. In contrary, only six fetuses of the treatment group were absorbed and 72 fetuses were completely healthy and developed (Figure 4). The viability, absorption, and mortality rates of the fetuses are presented in Table 1. Parameters related to the placenta weight, fetus weight, fetus length, and fetus width had significant differences and those of the treatment group were higher (Table 2).

Biochemical findings of the AF are presented in Figure 5(a–d). The glucose level of the AF in the treatment group was decreased over time and had a significant difference ($p = .000$) with the control group in day 21 of gestation. Urea concentration of the AF had an increasing pattern in both groups, with significant difference ($p = .005$) in day 21 of gestation. The AF lactate level of the control group was significantly higher than the treatment group ($p = .0001$). Finally, the changes of pyruvate levels were not significant ($p = .246$).

Discussion

In this current study, administration of magnesium sulfate to the pregnant rats which encountered uterine ischemia in day 17 of gestation significantly increased viability rate of the fetuses and encouraged their growth in comparison with the control group. In addition, the absorption and mortality rates of the fetuses in the treatment group were significantly lesser than the control group. The fetal and placental weights of the treatment group were greater than of those in the control group.

FGR can be either symmetric in which the whole body is smaller or asymmetric that is manifested by the normal size of the vital organs like brain and heart, but the restricted growth of the liver, muscles, and fat tissue. If the causative agent is severe or presents for a long time, asymmetric FGR can alter to symmetric one [14]. In our study, the growth restriction was symmetric and the brain and liver to body ratios had no significant difference in the groups. So, the 60 min of ischemia is a long period for this species and the protective mechanisms could not preserve the vital organs.

The mineral magnesium is the second most abundant intracellular cation and has a role in several important biochemical reactions [15]. Supplementation of magnesium can prevent hypertension in the early stages [16]. Van Laecke et al. [17] have reported that hypomagnesemia may affect vascular stiffness in patients undergoing renal transplantation. Magnesium deficiency may alter the mechanical properties of the arteries in young animals and may be the mechanism that contributes to increased blood pressure, atherosclerosis, and other cardiovascular diseases [18]. The results of a study on forearm blood flow showed that magnesium causes endothelium-dependent vasodilatation without any change in general hemodynamics [19]. In another study, magnesium caused concentration-dependent dilation in the brain's arterioles. The authors concluded that magnesium can be effective in the treatment of delayed brain ischemia or vascular spasm following subarachnoid hemorrhage [20].

Change in extracellular level of magnesium can affect the production and release of nitric oxide which changes the smooth muscle tone of the arteries and regulates the blood pressure [21]. In addition, *in vitro* researches demonstrated that magnesium acts as calcium canal antagonist [22]. Low extracellular magnesium level stimulates intracellular calcium metabolism by inositol-triphosphate and reduces calcium-ATPase. Therefore, calcium intake by the sarcoplasmic reticulum diminishes that leads to intracellular calcium accumulation which is an important factor in vasoconstriction [23]. Intracellular magnesium has a role in ATP production and glucose intake by insulin and regulation of vascular tone [24,25]. Other possible mechanisms of magnesium include anti-inflammatory, antioxidant, and cellular growth regulation [26]. Animals with magnesium deficiency have shown high levels of angiotensin I—which is a vasoactive agent [27]. Alpha and beta-adrenergic vascular sensitivity reduce during normal pregnancy. In some pregnant women, the inability to reduce alpha-adrenergic vascular contractility may be important in the pathogenesis of gestational hypertension or other physiological conditions associated with placental blood flow. There is considerable evidence that magnesium sulfate is an arterial dilatant in humans and animals [28,29]. In pregnant ewes, magnesium sulfate significantly reduces the mean arterial blood pressure and systemic vascular resistance and

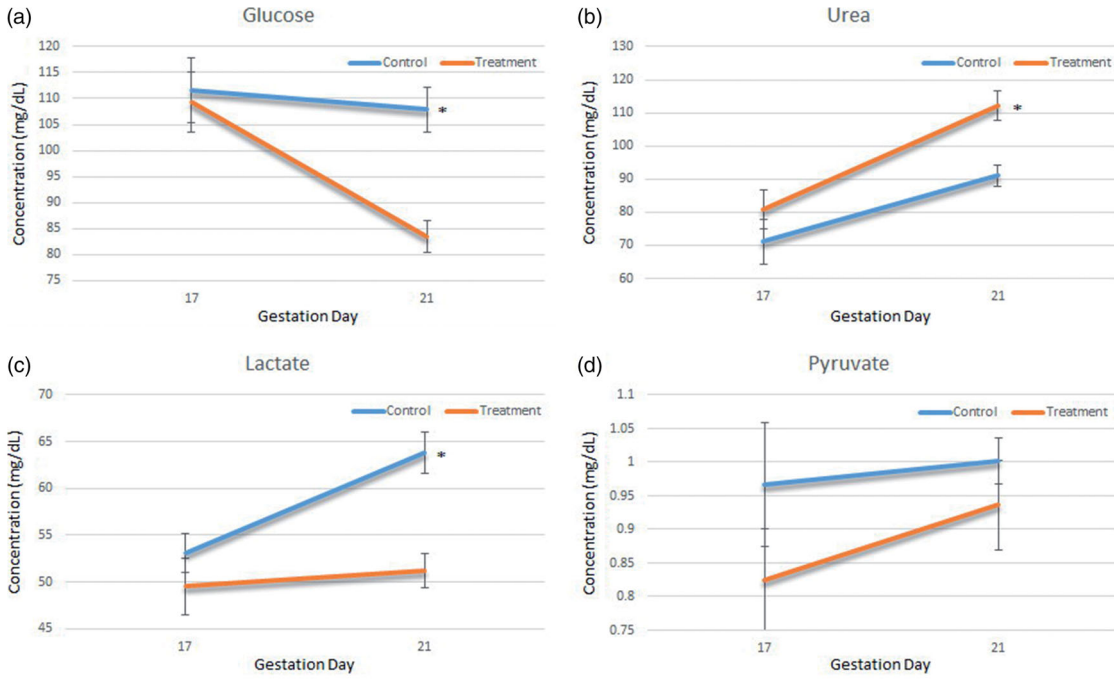

Figure 5. Glucose (a), urea (b), lactate (c), and pyruvate (d) levels in the amniotic fluid of control and magnesium sulfate groups in days 17 and 21 of gestation (mean ± SD). Asterisk (*) indicate significant difference ($p < .05$) between groups in similar time.

prevented cardiac output decrease during alpha-2-adrenergic stimulation. Meanwhile, magnesium sulfate has also been shown to overcome uterine blood flow decrease when stimulating alpha 1-adrenergic or angiotensin II [30]. Several studies on the arteries and veins isolated from the umbilical cord show that magnesium deficiency may be responsible for spasm of the umbilical and placental arteries [31,32]. We believe that increased placenta weight, fetus weight, fetus length, and fetus width in magnesium sulfate treated group of this study is due to increase in blood flow of the uterine and placenta by mechanisms that discussed earlier, which has overcome the effects of temporary ischemia. However, the exact blood flow level of the placenta was not evaluated by Doppler ultrasound or similar methods, which could be a limitation of this study.

Amniotic fluid produced from maternal, fetal, and placental tissues and its metabolic profile is the result of metabolite synthesis/degradation, fetal maturation, and biochemical exchanges. It shows the physiological processes of fetal development and is a valuable material for fetal health diagnostics [33]. Our results showed a decrease in AF glucose levels in the treatment group, while unaffected in the control group. The decrease in the glucose level overlaps with increasing fetal energy demand during the pregnancy progression [33]. On the other hand, magnesium can encourage normal fetal development and increase its energy needs, so that little glucose is secreted into the amniotic fluid. Albeit urea concentration of AF was increased in both groups, its level in the treatment group was significantly higher than those of the control group in day 21 of gestation. Lower contents of urea in malformation samples may be related to the fetal inability to synthesize urea, kidney underdevelopment, and/or prerenal causes such as deficient blood perfusion [34]. The origin of the AF lactate is fetus metabolism and its concentration increases in fetal distress conditions including hypoxemia [35]. So, a significant increase in AF lactate level of control group reflects compromised blood supply in this group, while magnesium sulfate could overcome this situation. Pyruvate levels were increased in both groups; however, the differences were not significant. Pyruvate is amongst the major sources of acetyl-CoA for Krebs cycle [33].

In conclusion, magnesium sulfate may neutralize the effects of uterine ischemia in pregnant rats and prevent fetal intrauterine growth restriction caused by temporary occlusion of the uterine artery. However, different doses of magnesium sulfate are needed in future studies. Surgical FGR induction in different pregnancy days is also recommended.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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