The effects of magnesium sulphate on the contractile activity of uterus in an animal model of preeclampsia

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Summary

Purpose: This study was undertaken to evaluate the effects of magnesium sulfate ($MgSO_4$) on the contractile activity of the uterus in a pregnant rat model of preeclampsia induced by N-nitro-arginine methyl ester (L-NAME). *Materials and Methods:* Twenty-eight, 160-220 gram, three to four month old female Sprague-Dawley rats were used in this study. After conception was confirmed by vaginal smears on the first day of pregnancy, the animals were allocated into four groups according to the chemicals fed in their drinking water as control (nothing administered), L-NAME (50 mg/kg L-NAME), $MgSO_4$ (600 mg/kg $MgSO_4$), and $MgSO_4$ + L-NAME group (600 mg/kg $MgSO_4$ + 50 mg/kg L-NAME). The pregnant uterus strips were isolated on the 19th day and the contractile activity of uterus was examined by applying 0, 0.1, 0.2, 0.4, 0.8, and 2.5 mIU/ml oxytocin to each group and responses are recorded accordingly. *Results:* There were no statistically significant differences regarding fetal parameters and peak amplitudes of the oxytocin stimulated pregnant rat myometrial strips among groups. In L-NAME group at 0 and 0.1 mIU/ml oxytocin, the contraction frequency in a ten-min period was statistically lower than the control group ($Z = -2.850$, $p = 0.004$; $Z = -2.902$, $p = 0.004$, respectively). In $MgSO_4$ group only at 0 mIU/ml oxytocin, the frequencies of the contractions in ten-min period were statistically lower than the control group ($Z = -2.973$, $p = 0.003$). In L-NAME + $MgSO_4$ group at 0, 0.1 and 0.2 mIU/ml oxytocin concentrations the frequencies of the contractions in ten-min period were statistically lower than the control group ($Z = -4.018$, $p = 0.000$; $Z = -3.237$, $p = 0.001$; $Z = -2.902$, $p = 0.004$, respectively). In L-NAME + $MgSO_4$ given group at each oxytocin concentrations, the frequencies of the contractions in ten-min period were lower but not statistically different than the L-NAME group. *Conclusion:* $MgSO_4$ has no significant effect on the amplitude of spontaneous or oxytocin induced myometrial contractions, but decreased the frequency of spontaneous contractions. At each doses of oxytocin, $MgSO_4$ has no significant effect on the frequency of contraction in a pregnant rat model of preeclampsia induced by L-NAME.

Key words: Magnesium sulfate; L-NAME; Uterus; Preeclampsia.

Introduction

Preeclampsia is a multi-system disorder characterized by hypertension and proteinuria in the last half of pregnancy and eclampsia refers to the development of grand mal seizures in a woman with preeclampsia, in the absence of other neurologic conditions that could account for the seizure. Although most affected pregnancies deliver at term or near term with good maternal and fetal outcomes, these pregnancies are at increased risk for maternal and/or fetal mortality or serious morbidity [1,2]. Preeclampsia occurs in up to 7.5 percent of pregnancies worldwide [3,4].

The pathophysiology of preeclampsia likely involves both maternal and fetal/placental factors. Abnormalities in the development of placental vasculature in early pregnancy may result in relative placental underperfusion/hypoxia/ischemia, which then leads to release of antiangiogenic factors into the maternal circulation that alter maternal systemic endothelial function and cause hypertension and other manifestations of the disease [5].

The optimal management of a woman with preeclampsia depends on gestational age and disease severity. The definitive treatment of preeclampsia is delivery, either by labor induction or cesarean section to prevent development of maternal or fetal complications from disease progression. Delivery results in resolution of the disease. In preeclampsia management, magnesium sulphate ($MgSO_4$) is the first-line treatment for the prevention of eclamptic seizures [6].

However, $MgSO_4$ is known to relax smooth muscle and is widely used as a tocolytic agent for preterm labor. If the tocolytic effect is significant at doses used for preeclampsia, $MgSO_4$ administration could increase the length of labor.

Several physiological mechanisms (neuronal, hormonal, metabolic, and mechanical) play a role in the control of myometrial activity during delivery. Alteration of uterine contractions by drugs or phytochemicals is of great importance in obstetrics practice, as it could lead to disruption of normal course of parturition [7].

Therefore the objective of this study is to investigate the effects of $MgSO_4$ on the contractile activity of uterus in a pregnant rat model of preeclampsia induced by N-nitro-arginine methyl ester (L-NAME).

Revised manuscript accepted for publication March 7, 2013

Materials and Methods

Pharmacologically induced model of preeclampsia

Nitric oxide (NO) is a potent vasodilator that is synthesized from the amino acid L-arginine, by nitric oxide synthase (NOS). Acute blockade of NO synthesis in studies using rats has demonstrated a marked rise in systemic blood pressure in these animals. Furthermore, chronic inhibition of NOS using N-nitro-arginine methyl ester (L-NAME) in pregnant rats led to the development of a model characterized by hypertension, proteinuria, reduced glomerular filtration rate, glomerular sclerotic injury, thrombocytopenia, and intrauterine growth restriction which is similar to preeclampsia in humans [8].

Animals and study design

The study was performed in accordance with the National Institutes of Health (NIH) Guidelines for the welfare and use of laboratory animals. The study protocol used was approved by the Kırıkkale University Ethics Committee (17.03.2006-01/06).

Adult, 160-220 gram, three to four months old, 28 female Sprague-Dawley rats were used in this study. Rats were maintained under controlled conditions. Food and water were freely available under 12 h light / 12 h dark cycle. The stage of the oestrus cycle was determined in each rat by vaginal cytological examination. Female rats were made pregnant by overnight pairing with males (two females : one male). To confirm pregnancy, the vaginal smears were checked twice daily early in the morning and evening for the presence of spermatozoa. The female rats were considered as pregnant after the determination of spermatozoa. After confirming the pregnancy (day 0 = spermatozoa positive), 28 pregnant rats were randomly allocated to four groups. First group was control group (n = 7) and allowed access to tap water. Second group, called L-NAME group (n = 7), received 50 mg/kg bw/day L-NAME hydrochloride, third group, called $MgSO_4$ group (n = 7), received 600 mg/kg bw /day $MgSO_4$, and fourth group, called L-NAME + $MgSO4_4$ (n = 7), received 50 mg/kg bw/day L-NAME + 600 mg/kg bw /day $MgSO_4$ in drinking water from 11^{th} to 19^{th} day of pregnancy. The systolic and diastolic blood pressure of pregnant rats was measured on day 12^{th} and 19^{th} by tail cuff device. Blood pressure was obtained from three consecutive measurements and average pressure value was recorded as the blood pressure of the rat at each time point. Test strips were used to detect the level of protein in urine. Systolic and diastolic blood pressures were increased in L-NAME given group when compared to control group on days 12 and 19. On day 19th proteinuria was seen in L-NAME given group. On day 19 of pregnancy, rats were anesthetized with sodium thiopental (Pentothal Sodium, Abbott, Turkey, 50 mg/kg). The abdomen was opened and two horns of the uterus were separated and freed from fat. Fetal tissues and placenta were separated. The number and the weight of fetuses were determined.

Isolated rat uterine strips

The uterine strips from each horn were mounted in Dale Solution (mM): NaCl: 154; KCl: 5,4; MgCl2: 0,024; glucose: 2,77; CaCl2: 1,63 and NaHCO3: 5,95. Uterine tissues were then sliced into four thin strips of approximately ten mm long from one pregnant rat. One end of the uterine strips, where the ovarium was located, were attached to force transducers and the other end was attached to a glass holder, under a resting tension of 500 mg in four-channel (ten ml) tissue baths. The tissue medium used was maintained at pH of 7.4, temperature of 37oC and gassed with carbogen (95% O_2 and 5% CO_2).

Table 1. — *Fetal parameters at day 19 of pregnancy.*

Parameters	Control (n = 7)	L-NAME (n = 7)	$MgSO_4$ (n = 7)	L-NAME +$MgSO_4$ (n = 7)
Weight of pregnant rats	222.57 ± 10.20	223.14 ± 6.29	221.71 ± 7.54	217.14 ±5.89
Number of fetuses	8.71 ± 0.81	10.14 ± 0.74	9.43 ± 0.48	8.71 ± 0.52
Total litter weight	19.78 ± 3.27	17.12 ± 1.59	19.62 ± 1.64	15.33 ± 1.08

Data were statistically analyzed using One Way ANOVA. Values are means ± SE; n = number of rats.

Experimental protocol

The uterine strips were washed at 15-minute intervals and left to equilibrate in bathing medium for one hour and the spontaneous contractions were observed. Following equilibration the viability of the strips were assessed by stimulating the uterus with ten mIU/ml oxytocin. The tissues were washed in five-minute intervals and observed for the recovery. Zero, 0.1, 0.2, 0.4, 0.8, and 2.5 mIU/ml oxytocin was applied to the tissue bath non-cumulatively. The contact time for each concentration was ten min. After each concentration the tissues were washed again at five-minute intervals and observed for recovery. Contractions were measured with a force displacement transducer and recorded.

Data analysis

The magnitude of uterine contractile responses to each concentration was expressed as mg tension, frequency as number of uterine contractions in ten minutes. The weights of the fetuses were expressed as grams.

Statistical analysis

Data processing was performed with the SPSS 15.0 package. The normality of all data was assessed by Shapiro-Wilk test. The frequency of the uterine contractions were distributed non-parametrically therefore tested using Kruskal Wallis test followed by the Mann- Whitney U test with Bonferroni adjustment to determine which of the four groups differed from each other. In case of fetal parameters of the pregnant rats and peak amplitude of the uterine myometrial contractions, one way ANOVA test was used. Differences were considered significant when $p < 0.05$ in One Way ANOVA and Kruskal Wallis test and $p < 0.0083$ in Mann Whitney U test with Bonferroni adjustment.

Results

Fetal parameters

Rats have multiple gestations so the total weight and total number of fetuses were considered to be the indicator of fetal growth. There were no statistically significant ($p > 0.05$) differences among the fetal parameters of trial groups (Table 1).

Myometrial strip contractions

In L-NAME group at 0 and 0.1 mIU / ml oxytocin concentrations, the frequencies of the contractions in ten-min period were statistically lower than the control group ($Z = -2.850$, $p = 0.004$; $Z = -2.902$, $p = 0.004$, respectively). In $MgSO_4$ group only at zero mIU/ml oxytocin concen-

Table 2. — *The effect of L-NAME, $MgSO_4$ and L-NAME + $MgSO_4$ on the frequencies of pregnant rat myometrial strips stimulated with oxytocin concentrations for 10 minutes.*

Oxytocin Concentrations	Control (n = 28)	L-NAME (n = 28)	$MgSO_4$ (n = 28)	L-NAME + $MgSO_4$ (n = 28)	*p*
0 mIU/ml	77.89[a]	51.80[b]	51.21[b]	45.09[b]	**
0.1 mIU/ml	74.41[ab]	48.89[cd]	56.52[bd]	46.18[d]	**
0.2 mIU/ml	72.38[a]	52.75[ab]	55.52[ab]	45.36[b]	*
0.4 mIU/ml	67.64	51.98	58.66	47.71	NS
0.8 mIU/ml	65.64	52.41	61.84	46.11	NS
2.5 mIU/ml	66.29	49.18	62.95	47.59	NS

Data were statistically analyzed using Kruskal Wallis test. Data that showed significant differences in Kruskal Wallis test * = $p < 0.05$, ** = $p < 0.01$ were then analyzed by Mann-Whitney U test to check differences between couples. Differences were considered significant when $p < 0.0083$ in Mann Whitney U test. The values were given as Mean rank. a, b, c, d: Mean Rank with in row with different superscript was significantly different according to Mann Whitney U test. NS = not significant. n = number of uterine strips.

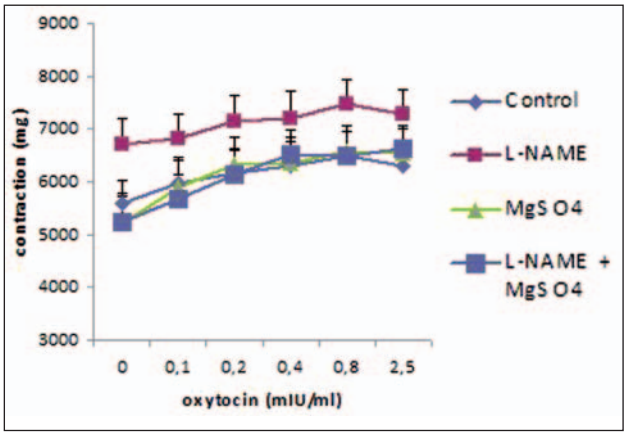

Figure 1. — The effect of L-NAME, $MgSO_4$ and L-NAME + $MgSO_4$ on the peak amplitudes of pregnant rat myometrial strips stimulated with oxytocin concentrations. Data were statistically analyzed by one way ANOVA and the values are given as mean ± standart error.

tration the frequencies of the contractions in ten-min period were statistically lower than the control group (Z = -2.973, $p = 0.003$). In L-NAME + $MgSO_4$ given group at 0, 0.1 and 0.2 mIU/ml oxytocin concentrations the frequencies of the contractions in ten-min period were statistically lower than the control group (Z = -4.018, p = 0.000; Z = -3.237, $p = 0.001$; Z = -2.902, $p = 0.004$, respectively). The effect of L-NAME, $MgSO_4$ and L-NAME+ $MgSO_4$ on the frequencies of pregnant rat myometrial strips stimulated with oxytocin concentrations for ten minutes were presented at Table 2. In L-NAME + $MgSO_4$ given group at each oxytocin concentrations, the frequencies of the contractions in ten-min period were lower but not statistically significant than the L-NAME group.

The peak amplitudes are presented in Figure 1. The peak amplitudes of the pregnant rat myometrial strips stimulated with oxytocin concentrations did not change among the trial groups.

Discussion

In the present study, there were no significant differences among the fetal parameters of trial groups. In contrast, in the study by Yallampalli and Garfield, 50 mg/kg bw/ day L-NAME decreased the weight of pups. They indicated that the mechanism for the low fetal weight in pregnant rats given L-NAME was not clear [9], but they believed that reduction of blood flow due to increased vasoconstriction of vessels by inhibition of the release of nitric oxide to the placental circulation caused this. In addition Pandhi et al. found a decrease in the weight of pups, and detected no difference on the number of the pups per rat [10].

This study showed that in inducted preeclampsia of pregnant rats the frequencies of uterus contractions were decreased compared to the control, but no difference was observed in the peak amplitude of the contractions. The inhibitory effect of preeclampsia on the frequency of uterine contraction was especially evident at low doses of oxytocin (0, 0.1 mIU/ ml). At higher doses this inhibitory effect somehow disappeared. In real clinical situations Szal *et al.* showed that in term nulliparous women, preeclampsia did not affect labor duration [11].

Magnesium sulfate is the first-line treatment for the prevention of primary and recurrent eclamptic seizures and prophylactic treatment with magnesium sulfate is indicated in all patients with severe preeclampsia [6]. Its mechanism of action is likely multi-factorial, encompassing both vascular and neurological mechanisms. Being a calcium antagonist, its effect on vascular smooth muscle is to promote relaxation and vasodilation and this may have a role in lowering total peripheral vascular resistance. In addition, $MgSO_4$ may have an effect directly on the cerebral endothelium by limiting vasogenic edema through decreasing stress fiber contraction and paracellular permeability via calcium-dependent second messenger systems such as myosin light chain kinase. Lastly, $MgSO_4$ may also act centrally to inhibit N-methyl-D-aspartate (NMDA) receptors, providing anticonvulsant activity by increasing the seizure threshold [12]

Magnesium sulfate is also widely used as the primary tocolytic agent. The ability of $MgSO_4$ to inhibit uterine contractility both in vivo and in vitro has been appreciated [13]. It also has neuroprotective properties and free radical reducing effects [14].

The mechanisms and time required for pharmacologic concentrations of $MgSO_4$ to inhibit myometrial contractility remains in question. Magnesium inhibits extracellular calcium entry, intracellular calcium release, cytosolic calcium oscillations, and phasic contractions of myometrial smooth muscle [15, 16].

In the present study, $MgSO_4$ did not change the spontaneous and oxytocin-induced myometrial amplitude of contraction, but without oxytocin, the presence of $MgSO_4$ alone depressed the frequency of contractions. Therefore in vitro, frequencies of uterine contractions are decreased but amplitudes are maintained, so possible tocolytic effect of $MgSO_4$ is by inhibition of spontaneous myometrial contractility through decreased frequency of contraction. In accordance, Kantas *et al.* showed $MgSO_4$ reduced the frequency of spontaneous contractions without affecting the amplitude in isolated myometrial strips of pregnant human and rat [17].

Uterine contractile activity is determined by the increase in intracellular free Ca^{2+} concentration in the myometrial cells [18], and oxytocin stimulates uterine contractions by two receptor mediated mechanisms, a second messenger system involving phospholipase C, which results in release of calcium from intracellular stores and the opening of calcium channels with the resultant calcium influx [19]. In an in-vitro study made by Tica *et al.* $MgSO_4$ temporarily reduced spontaneous myometrial contractions in a dose-dependent manner, with efficient regimens at 2.0-2.5 mM oxytocin-induced contractions were reduced by 30% - 40% at eight mM and decreased further at 9-10 mM [20]. Induced contractions were reduced, in a dose-dependent and time-dependent manner (maximum effect at 20 min), at higher Mg^{2+} concentrations and with non-significant proportional differences between pregnant and non-pregnant myometrium. As a conclusion, the authors decided that $MgSO_4$ acts in the inhibition of spontaneous myometrial contractility, but not of uterine-induced hyperactivity [20].

In the present study the authors showed that, in L-NAME induced preeclampsia of rats in vitro changed the frequency of the spontaneous uterine smooth muscle contractions but did not affect the maximum oxytocin contractility responses. The inhibitory effect on the frequency of uterine contraction was especially seen at low doses of oxytocin (0, 0.1 mIU/ml) in a pregnant rat model of preeclampsia induced by L-NAME. At higher doses this inhibitory effect somehow disappeared. This inhibitory effect was seen in $MgSO_4$ given group only on spontaneous contractions and in L-NAME+ $MgSO_4$ group at doses 0, 0.1 and 0.2 mIU/ml.

The most important finding in this study is that, at each doses of oxytocin, $MgSO_4$ has no significant effect on the frequency of contraction in a pregnant rat model of preeclampsia induced by L-NAME. In clinical situations also, there is no evidence that $MgSO_4$ therapy prolongs the duration of normal labor [11, 21, 22]. Witlin *et al.* showed that the use of magnesium sulfate during labor in women with mild preeclampsia at term does not affect any component of labor but did necessitate a higher dose of oxytocin [23].

In conclusion, $MgSO_4$ has no significant effect on the amplitude of spontaneous or oxytocin induced myometrial contractions, but decreased the frequency of spontaneous contractions. At each dose of oxytocin, $MgSO_4$ has no significant effect on the frequency of contraction in a pregnant rat model of preeclampsia induced by L-NAME.

Acknowledgements

This study was supported by the Kırıkkale University Research Fund Project No. 2006/20.

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