In vitro effects of ritodrine, magnesium sulfate and their combination on spontaneous contractions of myometrial strips of pregnant rat uteri

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Summary

Objective: To investigate in vitro effects of ritodrine, magnesium sulfate and their combination on spontaneous contractions of myometrial strips obtained from pregnant rat uteri.

Method: A total of 13 pregnant Sprague Dawley rats with weights between 180-200 g were used in this study. Three strips from each rat were kept in an organ bath containing 20 ml Krebs-Henseleit solution (pH: 7.4 and 37°C). 10^{-8}, 10^{-6} and 10^{-4} M concentrations of ritodrine, magnesium sulfate and the combination was applied over myometrial strips in Groups I (n: 10), II (n: 10) and III (n: 8), respectively. Amplitude and frequency of spontaneous myometrial contractions, which were recorded at the beginning of each experiment, were considered as reference values. Amplitude and frequency changes in spontaneous myometrial contractions were calculated at approximately ten-minute intervals right after the application of drugs as the percentage of difference at the first reference response.

Results: Magnesium sulfate application did not lead to any significant difference on the amplitude and frequency of contractions at any of its concentrations. 10^{-6} and 10^{-4} M concentrations of ritodrine caused a significant decrease in the amplitude of contractions. It was also found that ritodrine significantly decreased the frequency values at all concentrations. A significant decrease in amplitude was observed at 10^{-8} and 10^{-6} M concentrations in the combination group. No significant decrease in frequency values was found at any concentration in the combination group.

Conclusion: The tocolytic effect of ritodrine is superior to that of magnesium sulfate.

Key words: Ritodrine; Magnesium sulfate; Myometrium; Rat.

Introduction

Preterm birth is one of the most important causes of infant mortality and morbidity. The etiology of myometrial contractions, which arise before the full term has been completed, remains unclear. Prevention of preterm birth is still an important problem for the obstetrician. Although tocolytic treatment can be beneficial in the short-term, prolonged treatment has some drawbacks. Whatever the reason for action is, delaying the labor by using tocolytic agents saves time for steroid treatment, which is used in fetal lung maturation [1] and occasionally, it completely eliminates the threat of preterm labor. Delayed labor is also used in the transfer of mothers to a tertiary care center [2].

Pharmacological inhibition of uterine contractions, which is referred to as tocolysis, is still one of the most important treatments in preterm labor. The most effective agents for tocolysis are beta-agonists (ritodrine, terbutaline), magnesium sulfate, calcium channel blockers (nifedipine) and atosiban, an oxytocin antagonist. Agents, which provide Cox-2 enzyme inhibition and decrease prostaglandin release, also have a tocolytic effect. Specificity of these agents on the uterus is low and they may lead to severe adverse maternal and fetal effects [3]. More effective tocolytic agents with less adverse effects are necessary in the practice of obstetrics.

Idiopathic preterm labor is seen in 5-10% of all pregnancies and is responsible for 30% of preterm birth cases [4, 5]. Sixty-six percent of infant death incidents occur during the first 4-week period, and preterm birth has been considered to be responsible for approximately two-thirds of early infant deaths.

The rate of premature born infants has increased in developed countries during the last 20 years [6]. For instance, the rate of births occurring in 36 weeks or earlier in Canada between 1981 and 1992 increased from 6.3% to 6.8%. Increase in the number of multiparity, intensive obstetric interventions, developments in early diagnosis of preterm labor and widespread use of ultrasound imaging for determination of gestational age are being considered as a cause for this increase in preterm labor [6].

The purpose of this study was to investigate the in vitro effects of ritodrine, magnesium sulfate and the combination on spontaneous contractions of myometrial specimens obtained from pregnant rat uteri.

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Materials and Method

Experimental animals

A total of 13 pregnant Sprague Dawley rats with weights between 180-200 g were used in this study. Three of them were used in preliminary studies. All rats were obtained from Ege University, School of Medicine, Animal Breeding Center for Experimental Surgery. The tests were carried out in laboratories of Ege University, School of Medicine, Department of Physiology. The rats were kept with unlimited feed and water in 20°C room temperature. The study was approved by the local ethics committee of Ege University Hospital and was conducted in the Animal Research Laboratory of Ege University Hospital. All procedures were in accordance with the recommendations of the Declaration of Helsinki on the care and use of animals.

Preparation of myometrial strips

Pregnant rats, that were not in labor, were sacrificed by decapitation at 19-21 gestational days (term: 22 days). A midline abdominal incision was made. The uterus was distinguished anatomically from other tissues, the uterine cornus was rapidly excised and the surrounding connective tissues were carefully cleaned. Then the uterus was opened longitudinally along the mesenteric border. Fetuses were removed and non-uterine tissues were eliminated through dissection. Longitudinal full-thickness muscular strips (~6x2x2 mm) were cut out from the anti-mesenteric area. Three strips were obtained from each rat. The study on these three strips took approximately six hours. The strips were previously kept in a Krebs-Henseleit solution at pH 7.4 and 37°C, with a constant 5% CO_2 and 95% O_2 supply, and were used within six hours.

Preparation of the drugs used

The experimental study was carried out in three groups with myometrial strips. Three strips were obtained from each pregnant rat and ritodrine HCl (Prepar® 50 mg/5 ml ampoule, Eczacıbasi) and magnesium sulfate (Magnezyum Sülfat amp® %10 1.5 g ampoule, Biofarma) and the combination of these drugs was tested on these strips. Various concentrations of ritodrine (10^{-8}, 10^{-6} and 10^{-4} M), magnesium sulfate (10^{-8}, 10^{-6} and 10^{-4} M) a combination of ritodrine and magnesium sulfate (10^{-8}, 10^{-6} and 10^{-4} M) were applied in study groups I, II and III, respectively.

The effect of these drugs was assessed on spontaneous contractions of myometrial strips of pregnant rats. Ritodrine was used in Group I (n = 10), magnesium sulfate in Group II (n = 10) and a combination of ritodrine and magnesium sulfate was used in Group III (n = 8). At the end of each experiment, spontaneous contractions were re-written after the myometrial strip was washed off. Results in which spontaneous contractions could not be re-obtained after washing were excluded from the study.

All drugs were dissolved in distilled water and added into 20 ml organ baths. Initially, 10^{-2}, 10^{-4} and 10^{-6} M concentrations of MgSO4 and ritodrine were prepared; 0.2 ml of these concentrations were added into organ baths containing 20 ml Krebs solution. Thus, each drug was diluted 100 times and 10^{-4}, 10^{-6} and 10^{-8} M concentrations were obtained.

In vitro organ bath

Obtained strips were kept in an organ bath containing 20 ml Krebs-Henseleit solution (pH: 7.4 and 37°C). The organ bath was constantly supplied with 95% O_2 and 5% CO_2. In order to provide metabolic requirements of the muscular tissue at the optimal level, Krebs solution was refreshed at 15-minute intervals. The power transducer was on-line calibrated at the beginning of each test with a 5 g weight. A constant voltage was applied until regular spontaneous contractions occurred on myometrial strips and the muscular tissue was expected to reach a balance. After the regular spontaneous contractions occurred, 10^{-8} M, 10^{-6} and 10^{-4} M concentrations of one drug group (both drugs separately and their combination) was sequentially applied on each strip. A recording for at least ten minutes was made at each drug concentration. Spontaneous contractions of each strip were re-recorded after drug application. Krebs-Henseleit solution was prepared with 125 mM NaCl, 2.4 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 23.9 mM $NaHCO_3$ and 11.0 mM glucose. Krebs solution was recalibrated to pH 7.4 by using a pH meter and the temperature of the organ bath was adjusted to 37°C before each experiment. Isometric tensions were recorded by using a power transducer (BioPacV Model MP30, COMMAT Ltd., Istanbul) connected to an on-line PC.

Statistical analysis

Amplitude and frequency of spontaneous myometrial contractions, which were recorded in the beginning of each experiment, were considered as reference values. Amplitude and frequency changes in spontaneous myometrial contractions were calculated as the percentage of difference at the first reference response. Frequency and mean amplitude values of contractions were analyzed at approximately ten-minute intervals right after the application of drugs.

All data were statistically analyzed by using SPSS version 10.0 software. One-way ANOVA and post hoc tests were used, while multi-variate ANOVA was used for repeating data.

Results

A multiple-way ANOVA test was applied to all amplitude values in this study. When the base level was considered as 10^{-8}, 10^{-6}, 10^{-4} M, the rinsing period as factor 1 and each application as a separate group, factor 1 was found to be significant at $F_{(2,22)} = 22,215$ for amplitude of myometrial contractions, $p < 0.001$. Molarity differences and rinsing procedures were observed to be significantly different from each other, $p < 0.01$.

When all data were analyzed, a significant difference among groups was observed at the $F_{(2,22)} = 4,767$, $p < 0.05$. An interaction between applications and groups was found at the $F_{(2,22)} = 3,764$ level according to Greenhouse-Geisser. The reason for this interaction could be attributed to the fact that no change in response towards molarity difference was recorded in some groups. When the Bonferroni post hoc test was applied to the same data, a significant difference between the magnesium sulfate and ritodrine groups were found, $p < 0.05$.

The effects of magnesium sulfate and ritodrine on amplitude of myometrial contractions were found to be significantly different in both 10^{-4} M ($F_{(2,17)} = 6,706$) and 10^{-6} M ($F_{(2,21)} = 7,264$) concentrations ($p < 0.01$) and a significant difference was also found even after rinsing ($F_{(2,15)} = 6,351$), $p < 0.05$. However, the combination group was not found to be different either from the magnesium sulfate or ritodrine group.

The application of magnesium sulfate did not make any significant difference on contraction amplitude in any of the concentrations.

The data for the effect of ritodrine on amplitude of myometrial contractions in various concentrations were found to be different at $F_{(4,39)} = 4,299$, $p < 0.01$. According to the results of Duncan's post hoc test, 10^{-6} and 10^{-4} M concentrations were found to be significantly different from the baseline level, $p < 0.05$. With reference to these statistical analysis results, ritodrine at the 10^{-8} M concentration did not affect the myometrial contraction amplitude, while 10^{-4} M and 10^{-6} M concentrations caused a significant decrease (Figure 1).

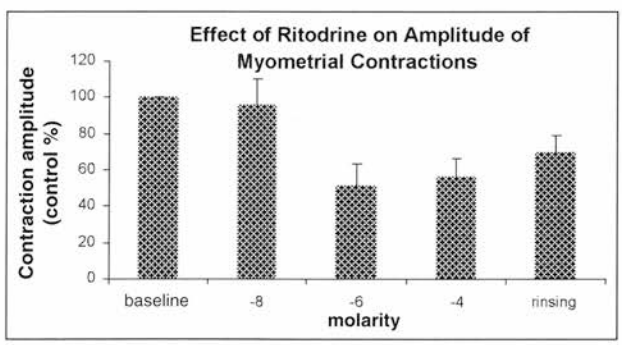

Figure 1. — Effect of ritodrine on amplitude of myometrial contractions. *Shows difference from baseline, $p < 0.05$.

A difference at the $F_{(4,28)} = 5,647$ level ($p < 0.01$) was observed when magnesium sulfate and ritodrine were applied in combination. According to the results of Duncan's post hoc test, 10^{-8} and 10^{-6} M concentrations were found to be different from the baseline level, $p < 0.05$. Interestingly, a significant amplitude decrease was observed at 10^{-8} M and 10^{-6} M concentrations of the combination therapy, while no such decrease was observed at a higher concentration of 10^{-4} M (Figure 2).

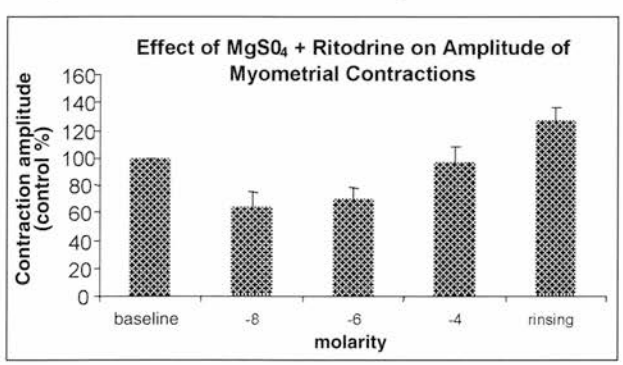

Figure 2. — Effect of combined application on amplitude of myometrial contractions.

As significant differences among groups were detected at baseline levels during the analysis of contraction frequencies per minute by using SPSS software, a new statistical analysis was performed by calculating the percentage of difference in frequency against base level.

When multiple variable ANOVA for consecutive data – where concentrations and rinsing were considered as factor 1 – was applied for percentage values, factor 1 (applications) was found to be significant at $F_{(2,44)} = 7,716$; $p < 0.001$. According to the results of the Bonferroni post hoc test, significant differences among groups at the 10^{-6} M concentration were observed between the magnesium sulfate and ritodrine groups, $p < 0.05$. Similar to the results obtained with respect to their amplitude, the magnesium sulfate and ritodrine groups were differently affected in contraction frequencies by the applications, and the results obtained in the combination group was not significantly different from the other groups. When the applications performed on the magnesium sulfate group were analyzed by using a one-way ANOVA test, no significant differences were observed.

When the applications performed on the ritodrine group were analyzed using a one-way ANOVA, significant differences were observed at $F_{(4,4)} = 9,746$, $p < 0.001$, (Figure 3). According to the results of the Bonferroni post hoc test, values obtained at 10^{-6} M and 10^{-4} M concentrations were significantly different from baseline, $p < 0.001$. Additionally, values obtained at 10^{-6} M and 10^{-4} M concentrations were significantly different from those at 10^{-8} M, $p < 0.005$. When the applications performed on the magnesium sulfate and ritodrine group were analyzed by using one way ANOVA test, no significant difference was observed.

The tocolytic effect of ritodrine on spontaneous contractions of the myometrial strip of rat uteri is shown in Figure 4.

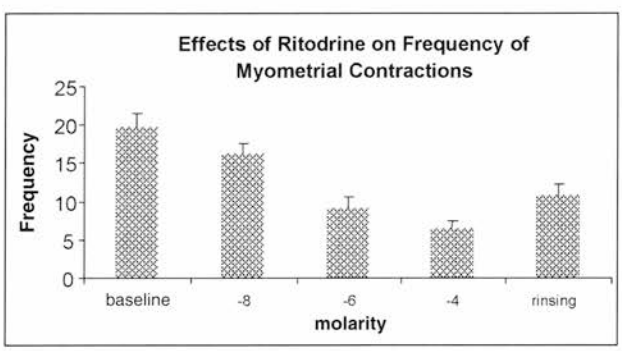

Figure 3. — Effect of ritodrine on frequency of myometrial contractions. *Shows difference from baseline level, $p < 0.001$. + Shows difference from 10^{-8} M, $p < 0.05$.

Discussion

Basically, preterm labor could be described as an escape from mechanisms which maintain uterine calmness, silence and/or stimulation of systems, which increase the activity of uterine contractions [7]. Tocolytic drugs, which are recently being used in the treatment of preterm labor, have not significantly decreased preterm birth rates or unfavorable neonatal results [8-10]. The biochemical mechanism in the etiology of preterm labor is still unclear. This is another factor partially responsible for the absence of an effective treatment option.

Ritodrine HCL, which is used for the treatment of preterm labor, leads to an intracellular increase of cyclic AMP by activating adenilate cyclase through stimulation

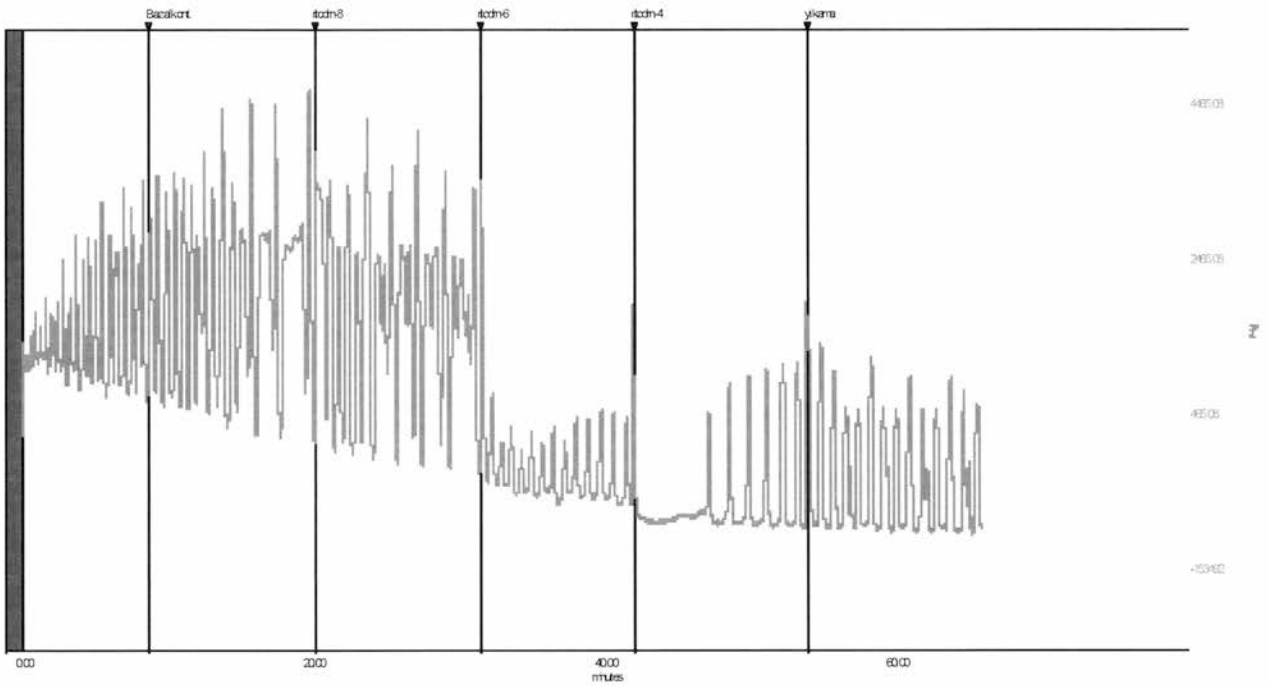

Figure 4. — Tocolytic effect of ritodrine.

of β_2 adrenergic receptors located on the outer surface of myometrial cells. Consequently, activation of kinase protein, which is dependent on cyclic AMP, leads to a decrease in intracellular calcium ion concentration and inhibition of myometrial contractions.

In accordance with the concentration of the drug, ritodrine was observed to inhibit both the frequency and amplitude of myometrial contractions in pregnant rats to a statistically significant degree. These findings were in accordance with the results of previous studies about ritodrine effects on myometrial contractions [11, 12].

In a study by Saade *et al.* [13] single and paired combinations of ritodrine, nifedipine, magnesium sulfate and terbutaline were compared in vitro on human myometrium. As a result, nifedipine was reported to be the most effective tocolytic agent. Ritodrine was observed to exhibit similar effects to that of nifedipine and both agents were reported to be more effective tocolytic agents, when compared with magnesium and terbutaline.

In a study by Zhu *et al.* [14] ritodrine and magnesium sulfate were compared in subjects with a potential for pre-term labor. Ritodrine was stated to be superior to magnesium sulfate with respect to the tocolytic effect.

In an in vitro study by Hamada *et al.* [15] ritodrine was applied with different doses (10^{-10} - 10^{-7} g/ml) at different gestational periods over spontaneous contractions on rat myometrial strips. As a result, it was reported that ritodrine showed different effects at different gestational periods, it inhibited the contractions at concentrations starting from 10^{-8} g/ml, it had a temporary α stimulatory effect, rebound contractions were observed, and spontaneous contractions recurred on long-term applications.

The effect of ritodrine on pregnant rat myometrium with respect to electrical and mechanical activity was compared with isoprenaline, in a study by Izumi *et al.* [12]. It was reported that the effect mechanism of ritodrine was similar to that of isoprenaline, and ritodrine was approximately 100 times less effective when compared with isoprenaline.

All three stages of muscle contractions can be affected by magnesium ions: excitation, excitation-contraction coupling, and the contraction apparatus itself. With a high probability, the extracellular and membrane magnesium ion affects myometrial contractions in skeletal muscle cells by modulating uptake, attachment and distribution of the calcium ion. Increased concentrations of the magnesium ion compete for attachment areas of the calcium ion and block calcium ion transition into the cell. Nevertheless, the magnesium ion activates adenilate cyclase and increases cyclic AMP. This causes an increase in calcium ion uptake by sarcoplasmic reticulum, decreases intracellular calcium concentration and inhibits uterine contractions [11, 16-19].

It was suggested that while the magnesium ion appeared to decrease depolarization frequency of skeletal muscle cells, it competes with the calcium ion to enter into the sarcoplasmic reticulum [20]. The external magnesium ion was suggested to be able to affect at least two functions taking part in effects of α adrenoreceptors, which are receptor-agonist interaction and inhibition of cyclic AMP-dependent membrane excitability [21].

Results of this study show that frequency and amplitude of spontaneous contractions in pregnant rat myometrium are not affected by magnesium sulfate.

In a study carried out on human myometrium,

Kawarabayashi *et al.* [22] investigated how electrical and mechanical activity of the oxytocin-stimulated muscle changed with magnesium. It was reported that magnesium could primarily increase the stimulatory effect of oxytocin with respect to electrical and mechanical activity on superficial areas of cell membranes and it had some intracellular effects.

In an in vitro study by Lechner *et al.* [23] magnesium sulfate was applied to a total of 29 human myometrial strips which were obtained during cesarean operations and it was observed at 10, 20 and 60 minutes that magnesium sulfate effectively inhibited contractions.

In an in vitro study by Kantas *et al.* [24] effects of ritodrine, magnesium sulfate and isradipine on human and rat myometrial strips were comparatively evaluated. Ritodrine was observed to inhibit the frequency and amplitude of spontaneous contractions on myometrial strips, depending on its concentration (10^{-8} - 10^{-5} M). Tachyflaxis developed against ritodrine at the 10^{-4} M concentration and the contractions recurred. Magnesium sulfate (10^{-7} - 10^{-4} M) inhibited the frequency of spontaneous contractions, yet did not change their amplitude. Isradipine (10^{-7} - 10^{-4} M) showed an inhibitor effect on both frequency and amplitude of spontaneous contractions, depending on the concentration. It was reported that the effects of magnesium sulfate, isradipine and ritodrine on pregnant human and rat myometrial strips were rather similar.

In conclusion, the tocolytic effect of ritodrine (on both frequency and amplitude of uterine contractions) is superior to that of magnesium sulfate. It was observed in this study that magnesium sulfate did not lead to any significant change on either amplitude or frequency of contractions at any of its concentrations.

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