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Research Article

Comparison of the Tocolytic Effects of Ritodrine and Ca^{++} Channel Blockers on Serum Oestradiol and Progesterone Levels

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Abstract: Experimental pregnancy was induced with gonadal steroids in in vivo ovariectomized rats. Conditions for premature delivery were established by giving a salt-rich diet and administering oxytocin to induce high-risk pregnancy. In order to avoid early delivery, a betamimetic ritodrine and the Ca^{++} channel blockers nitrendipine and nicardipine were administered to three separate groups. The effects of these three tocolytic agents were examined by determining serum oestradiol and progesterone levels. Additionally, systolic blood pressure was measured and tachycardia was observed.

After the administration of tocolytic agents, it was observed that serum oestradiol levels were increased while there was a decrease in serum progesterone levels. These three drugs cause no difference in hormone levels and can be used as tocolytic agents; however, it was observed that ritodrine carries the risk of tachycardia. Findings in the high-risk pregnancy group (hypertensive), to which no tocolytic agent was given, showed that the normal pregnancy period had not been completed.

Key Words: Ritodrine, Nicardipine, Nitrendipine, Oestradiol, Progesterone, Pregnancy

Ritodrin ile Ca^{++} Kanal Blokerlerinin Tokolitik Etkilerinin Serum Estradiol ve Progesteron Düzeyleri Üzerinden Karşılaştırılması

Özet: Bu çalışmada in vivo ovariektomi yapılan sıçanlara gonodal steroid uygulanarak deneysel gebelik oluşturuldu. Tuz diyeti ve oksitosin uygulanarak riskli gebelik tablosu oluşturmak suretiyle erken doğum ortamı hazırlandı. Erken doğumu önlemek için bir beta-mimetik olan ritodrin, Ca^{++} kanal blokerleri olan nitrendipin ve nikardipin üç ayrı gruba verildi. Bu üç tokolitik ajanın etkileri serum estradiol ve progesteron düzeyleri tayin edilerek incelendi. Ayrıca sistolik basınç ve taşikardi de değerlendirildi.

Tokolitik ilaç kullanan her üç grupta da estradiol düzeylerinde artış, progesteron düzeylerinde düşüş saptandı. Bu üç ilacın tokolitik etkili olarak kullanılabileceği, fakat ritodrinin taşikardi riski taşıdığı gözlendi. Tokolitik ajan verilmemiş riskli gebelik grubunda (hipertansif) saptanan bulgular bu grupta normal gebelik süresinin tamamlanmadığını gösterdi.

Anahtar Sözcükler: Ritodrin, Nikardipin, Nitrendipin, Estradiol, Progesteron, Gebelik

Introduction

In clinical trials tocolytic agents are used to prevent premature delivery and control the pregnancy (1-9). Several therapeutic attempts have been made to inhibit uterine contractions, such as with alcohol, magnesium sulphate, prostaglandin synthase inhibitors and beta-adrenergic agonists (4-6,10,11). The use of beta-adrenergic agonists, especially ritodrine, is associated with several side effects, limiting their use (12,13). Side effects such as hypertension, tachycardia and tremor have stimulated investigators to search for new drugs (14). Among the calcium channel blockers, verapamil and nifedipine were used for the first time in the treatment of premature labour (7). Nicardipine and nitrendipine, which are newly synthesized calcium channel blockers, have been seldom used as tocolytic agents. A few publications report on their use in the management of preterm labour. The tocolytic efficacy of these calcium antagonists or other tocolytic agents depends on hormonal status, especially concentrations of oestradiol and progesterone in the extracellular fluid (15). In this study, experimental pregnancy was induced by applying gonadal steroids in normotensive and hypertensive rats. Tocolytic agents were used to prolong pregnancy except in the hypertensive group. Blood samples were collected and serum concentrations of oestradiol and progesterone,

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systolic pressures and pulse rates were measured. The aim of this study was to compare the tocolytic efficacy of calcium channel blockers with less side effects and ritodrine.

Materials and Methods

Ovariectomy and Experimental Pregnancy

Thirty adult female Wistar albino rats weighing 180-230 g were used. Ovariectomy was performed on nonpregnant rats under ether anaesthesia. Intraperitonal injections of 200 µg/kg oestradiol benzoate from days 9 to 12 and a combination of 200 µg/kg oestradiol benzoate and 20 mg/kg progesterone from the days 13 to 16 following ovariectomy were administered to the rats to induce pregnancy. In addition, 2.5 IU oxytocin was administered intraperitoneally to the rats from the days 13 to 16 following ovariectomy. The rats were fed a salt-rich diet for 20 days before ovariectomy. The purpose of this process was to induce premature labour associated with hypertension.

The Groups and the Administration of Tocolytic Agents

The rats were divided into five equal groups.

Group 1: Rats were fed a normal diet. They were ovariectomized. No tocolytic agent or oxytocin was administered. Experimental pregnancy was induced. Blood samples were collected on days 16, 18, 20 and 22 following ovariectomy. Serum samples were analysed for concentrations of oestradiol and progesterone.

Group 2: Rats were fed a salt-rich diet. They were ovariectomized. Experimental pregnancy was induced. Intraperitoneal injections of oxytocin were administered for 4 days starting from day 13 following ovariectomy. Ritodrine was also administered for 7 days starting from day 13 following ovariectomy. Blood samples were collected to find out the tocolytic effect of ritodrine on days 16, 18, 20 and 22 following ovariectomy. Levels of oestradiol and progesterone were analysed in the serum samples.

Group 3: Rats were fed a salt-rich diet. They were ovariectomized. Experimental pregnancy was induced. Intraperitoneal injections of oxytocin were administered from days 13 to 16 following ovariectomy. Nitrendipine was also administered intraperitoneally from days 13 to 19 after ovariectomy. Blood samples were collected to find out the tocolytic effect of nitrendipine on days 16, 18, 20 and 22 after ovariectomy. Serum samples were analysed for the levels of oestradiol and progesterone.

Group 4: Rats were fed a salt-rich diet. They were ovariectomized. Experimental pregnancy was induced. Intraperitoneal injections of oxytocin were administered from days 13 to 16 following ovariectomy. Nicardipine was also administered intraperitonally for 7 days starting from day 13 following ovariectomy. Blood samples were collected to find out the tocolytic effect of nicardipine on days 16, 18, 20 and 22 following ovariectomy. Serum samples were analysed for the levels of oestradiol and progesterone.

Group 5: Rats were fed a salt-rich diet. They were ovariectomized. Experimental pregnancy was induced. Intraperitoneal injections of oxytocin were administered for 4 days starting from day 13 following ovariectomy. No tocolytic agent was administered. Blood samples were collected on days 16, 18, 20 and 22 following ovariectomy. Serum samples were analysed for the levels of oestradiol and progesterone.

Measurement of Systolic Pressure

Hypertension was evaluated by attaching a tail-cuff to the tail root of the rats fed a salt-rich diet. This process was performed to measure the systolic pressure on days 1, 4, 7, 10, 13, 16, 19 and 22 following ovariectomy.

Measurement of Tachycardia

Tachycardia was evaluated by measuring pulse/min with an EKG in ovariectomized rats on days 16, 20 and 22 following ovariectomy.

Collection of Blood Serum Samples

Blood samples (1.3 ml) were collected into tubes by applying a tail-cut procedure on days 16, 18, 20 and 22 following ovariectomy. The serum was separated and kept at –20 °C until all samples were collected.

Measurement of Oestradiol and Progesterone Levels

Serum was assayed for the levels of oestradiol using an oestradiol radioimmunoassay kit (ICN-No. 07231102, Oregon, New York, USA) and for the levels of progesterone using a progesterone radioimmunoassay kit (Amerlex-M No. 1657675, Amersham, UK). A Packard

Multiprias 1 Model C.5301 gamma counter was used for counting the radioimmunoassay samples.

The protocol of this experimental study is depicted in Table 1.

Statistical Procedure

SPSS for Windows Version 7.5 was used for analysis of data. The results are given as mean and standard error of the mean. Comparison of the results:

a. Paired t-test was used to compare differences between groups.

b. Mann-Whitney U test was used for non-parametric analyses.

p values less than 0.05 were considered statistically significant.

Results

The groups in the study were as follows:

Group 1: Ovariectomized, pregnant. (control group)

Group 2: Ovariectomized, pregnant + oxytocin + ritodrine + salt. (ritodrine group)

Group 3: Ovariectomized, pregnant + oxytocin + nitrendipine + salt. (nitrendipine group)

Group 4: Ovariectomized, pregnant + oxytocin + nicardipine + salt. (nicardipine group)

Group 5: Ovariectomized, pregnant + oxytocin + salt. (hypertensive group)

The means and standard errors of the means of oestradiol levels (E2) (pg/ml) and progesterone levels (P)

Table 1. The protocol of this experimental study.

	DAYS																					
Procedure	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Ovariectomy	1 2,3, 4,5																					
Oxytocin 25 I.U. I.P.													2,3, 4,5	2,3, 4,5	2,3, 4,5	2,3, 4,5						
Ritodrine 2 mg/kg I.P.													2	2	2	2	2	2	2			
Nitrendipine 2 mg/kg I.P.													3	3	3	3	3	3	3			
Nicardipine 2 mg/kg I.P.													4	4	4	4	4	4	4			
Oestradiol benzoate 200 mg/kg I.P.									1, 2,3, 4,5	1, 2,3, 4,5	1, 2,3, 4,5	1, 2,3, 4,5	1, 2,3, 4,5	1, 2,3, 4,5	1, 2,3, 4,5	1, 2,3, 4,5						
Progesterone 20 mg/kg I.P.													1, 2,3, 4,5	1, 2,3, 4,5	1, 2,3, 4,5	1, 2,3, 4,5						
Blood collection																1, 2,3, 4,5		1, 2,3, 4,5		1, 2,3, 4,5		1, 2,3, 4,5
Systolic pressure	2,3, 4,5			2,3, 4,5			2,3, 4,5			2,3, 4,5			2,3, 4,5			2,3, 4,5			2,3, 4,5			2,3, 4,5
Tachycardia																2,3, 4,5				2,3, 4,5		2,3, 4,5

1, 2, 3, 4 and 5 indicate the study groups .

(ng/ml) of all groups are given in Tables 2 and 3. The means and standard errors of the means of systolic pressure values (mm/Hg) of the test animals are given in Table 4. The means and standard errors of the means of the test animals' pulse rate on days 16, 20 and 22 are given in Table 5.

The differences between the groups daily and between the levels of a group on different days were statistically analysed.

Discussion

Numerous studies have assessed the relationship between the tocolytic activities of calcium channel blockers and hormonal changes. Several reports have suggested that tocolysis with nifedipine was more pronounced in the pregnant uterus than in the nonpregnant uterus in the management of uterine contraction (16-18). However, the tocolytic effect of nifedipine was not different in uterus of rats treated with oestradiol only or with a combination of oestradiol and progesterone (9,16).

The average gestational period in rats is 21 days. An elevated concentration of oestradiol and a decreased concentration of progesterone in the late period of gestation cause an increase in the effect of oxytocin on the uterus. Moreover, altered hormone levels indicate the beginning of lactation before birth (19-23).

In this study, pregnancy was completed in 18 days in the hypertensive group, but in the other groups pregnancy was completed in 22 days. This was in accordance with the published findings about serum E2 and P levels in normal physiological pregnancies. Serum E2 and P levels in the control group were in agreement with the levels in the ritodrine, nitrendipine and nicardipine groups. These findings confirm the effects of ritodrine, nitrendipine and nicardipine as known tocolytic agents on pregnancy. When a comparison was made statistically between the control group and the test groups, insignificant differences were found (Table 2). In these groups, E2 levels were the same as normal physiological pregnancy values (45-60 pg/ml). The significant difference between the control group and the hypertensive group may indicate that increased E2 levels

Table 2. Mean E2 levels on days 16, 18, 20 and 22 in all groups.

Groups	n	Day 16 X ± S.E.	Day 18 X ± S.E.	Day 20 X ± S.E.	Day 22 X ± S.E.
1	6	20.11 ± 0.51	30.45 ± 0.99	43.40 ± 0.60	49.00 ± 0.74
2	6	22.12 ± .0.72	33.41 ± 0.44[a]	42.51 ± 0.36[a]	50.24 ± 0.47
3	6	24.92 ± 0.41[a,b]	35.87 ± 0.17[a,b]	43.75 ± 1.13	51.26 ± 0.75
4	6	24.73 ± 0.70[a,b]	30.01 ± 0.96[b,c]	44.72 ± 0.75[a]	48.34 ± 0.69[b,c]
5	6	33.64 ± 0.19[a,b,c,d]	47.50 ± 1.64[a,b,c,d]	69.96 ± 0.96[a,b,c,d]	81.82 ± 0.48[a,b,c,d]

Significant difference ($p < 0.05$), [a] compared with Group 1, [b] compared with Group 2, [c] compared with Group 3 and [d] compared with Group 4.

Table 3. Mean P levels on days 16, 18, 20 and 22 in all groups.

Groups	n	Day 16 X ± S.E.	Day 18 X ± S.E.	Day 20 X ± S.E.	Day 22 X ± S.E.
1	6	80.50 ± 0.37	78.10 ± 0.85	67.30 ± 1.18	52.40 ± 0.37
2	6	71.50 ± .0.37[a]	67.30 ± 1.37[a]	60.90 ± 0.74[a]	52.50 ± 1.19
3	6	73.40 ± 0.49[a,b]	70.50 ± 0.90[a]	62.70 ± 0.71[a]	49.04 ± 0.77[a,b]
4	6	69.50 ± 0.88[a,b,c]	70.30 ± 0.52[a]	65.50 ± 0.42[b,c]	50.10 ± 1.20[a,b]
5	6	60.00 ± 0.77[a,b,c,d]	52.20 ± 0.89[a,b,c,d]	41.00 ± 1.23[a,b,c,d]	32.40 ± 0.25[a,b,c,d]

Significant difference ($p < 0.05$), [a] compared with Group 1, [b] compared with Group 2, [c] compared with Group 3 and [d] compared with Group 4.

Table 4. Mean systolic pressures (mm/Hg) in the experimental animals from days 1 to 20.

Groups	n	Day 16 X ± S.E.	Day 18 X ± S.E.	Day 20 X ± S.E.	Day 22 X ± S.E.
2	6	110.1 ± 1.84	166.0 ± 2.36	140.3 ± 0.73	127.3 ± 0.87
3	6	111.6 ± 1.50	170.6 ± 0.69[a]	130.0 ± 2.87[a]	122.1 ± 0.72[a]
4	6	100.0 ± 1.17[a,b]	156.3 ± 1.09[a,b]	141.1 ± 1.34[b]	123.0 ± 0.70[a,b]
5	6	110.3 ± 1.17c	166.3 ± 0.87[a,b,c]	174.3 ± 1.87[a,b,c]	186.5 ± 1.30[a,b,c]
Groups	n	Day 16 X ± S.E.	Day 18 X ± S.E.	Day 20 X ± S.E.	Day 22 X ± S.E.
2	6	110.3 ± 1.83	121.0 ± 1.02	130.3 ± 1.17	130.3 ± 0.93
3	6	122.5 ± 0.84	130.0 ± 1.33	131.0 ± 0.97	127.0 ± 1.17
4	6	117.5 ± 0.20	134.6 ± 0.50	130.0 ± 1.26	131.1 ± 1.34
5	6	110.3 ± 1.17	119.6 ± 1.19	135.3 ± 1.74	184.6 ± 1.32

Significant difference ($p < 0.05$), [a] compared with Group 2, [b] compared with Group 3, [c] compared with Group 4.

Table 5. The mean levels of pulse rate on days 16, 20 and 22 in the experimental groups.

Groups	n	Day 16 X ± S.E.	Day 20 X ± S.E.	Day 22 X ± S.E.
2	6	227.5 ± 0.51	280.5 ± 1.46	280.0 ± 1.20
3	6	237.5 ± 0.73[a]	224.5 ± 1.21[a]	241.0 ± 1.39[a]
4	6	230.5 ± 1.42[a,b]	226.0 ± 0.94	241.5 ± 1.19
5	6	207.1 ± 1.32[a,b,c]	226.8 ± 2.37[a]	244.5 ± 0.99[a,b,c]

Significant difference ($p < 0.05$), [a] compared with Group 2, [b] compared with Group 3, [c] compared with Group 4.

in the hypertensive group were due to the lactation after the pregnancy ending on day 18. There was no difference between the control group and the ritodrine group in terms of progesterone levels. There was a statistical difference between the control group and the nitrendipine and nicardipine groups (Table 3), but the values obtained were the same as the normal physiological pregnancy values.

The results of the statistical comparison of E2 and P levels in the groups according to the days confirmed the pregnancy ($p < 0.001$). In all groups E2 levels increased gradually from days 16 to 22 , while there was a gradual decrease in P levels on the same days (Tables 2, 3). In the hypertensive group on day 16 the E2 level (X = 33.64) was higher and the P level (X = 60.00) was lower than in the other groups. This situation seems to be normal, because the control group and the groups given the tocolytic agent had no risk during pregnancy.

Tocolytic agents were administered to the groups from days 13 to 19, except for the hypertensive and control groups, to prevent preterm labour. After the administration the results obtained showed that the control group, which represents the normal pregnancy model, was in agreement with the ritodrine, nitrendipine and nicardipine groups, but was different from the hypertensive group. These data indicated that tocolytic agents prolong the pregnancy. The results from the hypertensive group were different from those of the other groups, as expected, because this group has a high risk during pregnancy.

In the groups fed a salt-rich diet, systolic pressure values were measured on days 1, 4, 7, 10, 13, 16, 19 and 20. In all groups, systolic pressure values increased until day 13. After day 13, in the ritodrine, nicardipine

and nitrendipine groups systolic pressure was controlled due to the administration of the tocolytic agent (Table 4). However, in the hypertensive group the elevation of systolic pressure continued until day 20, showing that there was hypertension in this group. The normal pressure value of rats is approximately 110 mm/Hg (24).

The pulses per minute were measured in order to determine tachycardia on days 16, 20 and 22 (Table 5). The pulse rate per minute in rats was 220 (25). In the nitrendipine group and the nicardipine group given tocolytic drugs, pulse rates on day 20 confirmed that there was no tachycardia. Pulse rates in the groups on day 22 (X = 241.0 in the nitrendipine group, X = 241.5 in the nicardipine group) were slightly higher, but these values were lower than those in the ritodrine group (X = 280.0) on day 22. The high pulse rates determined on day 20 in the group given ritodrine continued until day 22. This result shows that one of the side effects of ritodrine is tachycardia.

In this study, in vivo pregnancy was induced by the administration of oestradiol and progesterone in ovariectomized rats. The use of calcium channel blockers and ritodrine as a tocolytic agent prolonged pregnancy in animals fed a salt-rich diet. Finally, the administration of calcium channel blockers and ritodrine appears to be the treatment of choice in cases of premature labour and hypertension.

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