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The *in vitro* effect of dual combinations of ritodrine, nicardipine and atosiban on contractility of pregnant rat myometrium

Muriel Doret[a,*], Georges Mellier[a], Pascal Gaucherand[a], Georges R. Saade[b], Mehdi Benchaib[c], Jean Frutoso[d], Jean Charles Pasquier[a]

Objective To compare the tocolytic potency of ritodrine, nicardipine and atosiban, used alone and in dual combinations, to see whether combinations of these drugs, which act via different pathways, could improve inhibition of uterine contractility.

Design Study on myometrial contractility *in vitro*.

Setting Laboratory of physiology, Lyon, France.

Sample Longitudinal myometrial strips from non-labouring timed pregnant Wistar rats (18 gestational days).

Methods Strips were simultaneously exposed to EC_{25}, EC_{50} or EC_{75} of dual combinations of either ritodrine and nicardipine, ritodrine and atosiban or nicardipine and atosiban ($n = 10$/group). Basal contractile activity and contractile activity after addition of each combination was measured using the 10 min integral of activity. Changes were expressed as percentage from the basal 10 min integral activity. The observed percentage inhibition of activity was compared with the expected percentage inhibition in an additive pharmacological model. When no significant difference occurred, the combination was deemed simply to have an additive tocolytic effect. When inhibition of activity was significantly greater compared with the expected percentage inhibition, the combination was deemed to have a synergistic effect.

Main outcome measure Changes in integral contractile activity in response to tocolytic combinations.

Results Ritodrine and atosiban inhibited integral activity to a greater extent than expected [e.g. using EC_{50}: observed inhibition 88.9% (13.8%) *vs* expected inhibition 75%; $P < 0.015$]. Actual inhibition by nicardipine/ritodrine [78.55% (20.4%) *vs* 75%; $P =$ n.s.] and nicardipine/atosiban [78.94% (17.8%) *vs* 75%; $P =$ n.s.] was not significantly different from expected.

Conclusions A combination of ritodrine plus atosiban exhibits a synergistic inhibition for myometrial activity, thus allowing the use of lower concentrations of each drug to achieve the same effect compared with each drug used alone. Combination of nicardipine plus ritodrine and nicardipine plus atosiban achieves only an additive effect. The potential for decreasing side effects (beta-mimetics) and costs (atosiban) when using a combination in clinical practice needs to be evaluated.

INTRODUCTION

Management of preterm labour remains the chief challenge for obstetricians today. Idiopathic preterm labour complicates 5% to 10% of all pregnancies and is responsible for 30% of preterm births (before 37 weeks of gestation)[1,2]. Currently, the drugs commonly used to inhibit preterm labour include beta-2-mimetics, calcium channel blockers and the recently introduced oxytocin antagonist atosiban. Calcium channel blockers are slightly more effective than beta-2-mimetics in delaying delivery for 48 hours, whereas beta-2-mimetics and atosiban are equivalent[3–9]. This 48 hour period is essential for the transfer of the mother and fetus to a centre with a neonatal intensive care unit, and for achieving the full benefit of corticosteroid therapy on fetal lung maturation[10,11]. However, tocolytics have not decreased preterm delivery rates. Moreover, neonatal morbidity and mortality have not been reduced by these drugs[1,3–9]. In addition, dose-dependent fetal and maternal side effects limit the use of effective treatment concentrations[6,7,12].

It is worthwhile to note that the three classes of drugs mentioned above act through different pathways to inhibit uterine contractile activity. Beta-2-mimetics induce relaxation through intracellular accumulation of cyclic adenosine monophosphate (cAMP)[13,14]. Calcium channel blockers

[a]*Department of Obstetrics and Gynaecology, Edouard Herriot Hospital, Lyon, France*
[b]*Department of Obstetrics and Gynecology, UTMB, Galveston, Texas, USA*
[c]*Department of Reproductive Biology, Edouard Herriot Hospital, Lyon, France*
[d]*Laboratory of Physiology, University of Medicine Rockefeller, Lyon, France*

* **Correspondence**: Dr M. Doret, Department of Obstetrics and Gynaecology, Hopital Edouard Herriot, 1 Place d'Arsonval, 69008 Lyon, France.

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directly prevent influx of calcium ions responsible for smooth muscle cell contraction, whereas oxytocin receptor antagonists act indirectly to prevent the rise of intracellular calcium concentration in response to the oxytocin-induced increase in inositol 1,4,5-triphosphate production (IP_3)[14–16]. Simultaneous blockage of these different pathways could result in a synergistic effect capable of potentiating the uterine relaxation induced by each single drug. Most importantly, a synergistic effect could also allow a reduction of the therapeutic concentrations needed for each single drugs, thereby leading to a decrease in maternal and fetal side effects. The aim of this *in vitro* study was to assess the relaxant effects of dual combinations of ritodrine (beta-2-mimetics), nicardipine (calcium channel blockers, dihydropyridine class) and atosiban (oxytocin antagonist) on the contractile activity of pregnant rats' myometrium.

METHODS

Myometrial longitudinal strips were obtained from a total of 25 non-labouring timed pregnant Wistar rats on day 18 of gestation (term: day 22). Strips were mounted in glass organ chambers filled with Krebs–Henseleit solution bubbled with 5% CO_2 and 95% O_2 (37°C, pH ~7.4), and isometric tension was recorded using a force transducer connected to an online computer. Myometrial strips were then allowed to equilibrate until regular spontaneous contractile activity stabilised.

Basal activity was then calculated as the integral of uterine activity over 10 min. Changes in the 10 min integral 10 min after application of each concentration of each single drug or combinations were expressed as percentage of changes from the basal 10 min integral. The concentration–response curves of each single drug were previously determined and used to calculate the effective concentrations that inhibited 25% (EC_{25}), 50% (EC_{50}) and 75% (EC_{75}) of basal activity (Table 1)[17].

Strips were then exposed to dual combinations of the EC_{25}, EC_{50} or EC_{75} of ritodrine, nicardipine and atosiban: ritodrine plus nicardipine, ritodrine plus atosiban and nicardipine plus atosiban ($n = 10$ per group; strips were removed from at least eight different animals to reduce individual variation effect). Control strips were removed from the same rats and studied at the same time.

Table 1. Mean molar concentrations of ritodrine, nicardipine and atosiban previously determined to inhibit 25% (EC_{25}), 50% (EC_{50}) and 75% (EC_{75}) of the basal contractile activity. These concentrations were used in the dual combinations tested.

Drugs	EC_{25}	EC_{50}	EC_{75}
Ritodrine	8.1×10^{-8}	3.1×10^{-7}	1.2×10^{-6}
Nicardipine	1.3×10^{-9}	5.8×10^{-9}	2.6×10^{-8}
Atosiban	2.4×10^{-8}	1×10^{-7}	4.4×10^{-7}

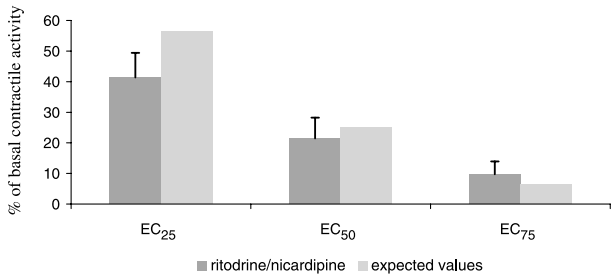

Fig. 1. Contractile activity observed after addition of the combinations of EC_{25}, EC_{50} and EC_{75} of ritodrine and nicardipine. Data are expressed as mean (SEM).

To investigate the relaxant effect of each combination, the pharmacological interaction was defined as additive, synergistic or antagonistic interaction. An additive effect is defined by an expected percent inhibition of integral activity equivalent to the sum of inhibition produced by the concentration used for each drug. Hence, when EC_{50} of each drug are used, one drug is inhibiting 50% of the basal contractile activity, and the second drug is inhibiting 50% of the remaining contractile activity, that means 25%. Thus, using EC_{50}, the final contractrile activity inhibition in an additive model is 75%. In the same way, when EC_{25} and EC_{75} of each drug are used in combination, the expected inhibitions in an additive model are 43.75% and 93.75%, respectively.

We compared experimentally observed inhibition with expected inhibition using a paired Student's t test; $P < 0.05$ was considered as statistically significant. When no significant difference occurred between the observed and the expected percentage inhibition in activity, the combination of the two drugs was deemed additive. When inhibition of activity was significantly greater or lower compared with the expected percentage inhibition in activity, the dual combination was characterised as synergistic or antagonistic, respectively.

The composition of the Krebs–Hensleit solution was (in mM): glucose 11.2, magnesium sulphate 1.2, monobasic potassium phosphate 1.2, potassium chloride 4.7, sodium chloride 118.3, bihydrate calcium chloride 2.5 and sodium bicarbonate 25. Atosiban was provided by Ferring Pharmaceutical Industries (Copenhagen, Denmark). Nicardipine

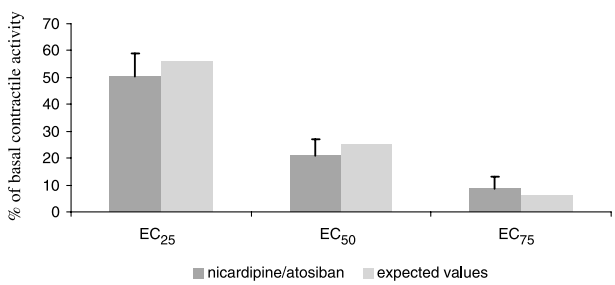

Fig. 2. Contractile activity observed after addition of combinations of EC_{25}, EC_{50} and EC_{75} of nicardipine and atosiban. Data are expressed as mean (SEM).

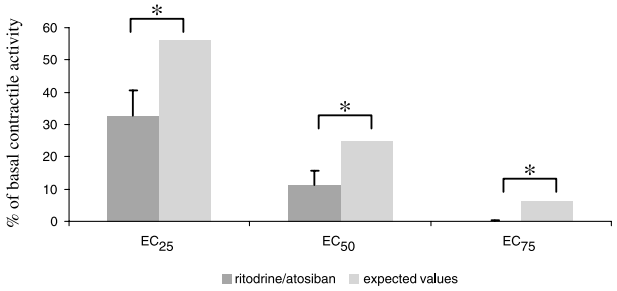

Fig. 3. Contractile activity observed after addition of the combinations of EC_{25}, EC_{50} and EC_{75} of ritodrine and atosiban. Data are expressed as mean (SEM). $*P < 0.05$.

was purchased from Novartis (Basel, Switzerland) and ritodrine from Solvay Pharmaceutical Industries (Brussels, Belgium).

RESULTS

All three combinations (ritodrine/nicardipine, ritodrine/atosiban and nicardipine/atosiban) inhibited myometrial contractile activity, whereas the control group activity remained unchanged. The inhibitions with the combinations of ritodrine/nicardipine and atosiban/nicardipine were not significantly different from the expected inhibition in activity at any of the three ECs, indicating an additive effect of these drugs (Figs 1 and 2). The combination of ritodrine/atosiban resulted in an inhibition in activity that was significantly higher than the expected inhibition at all ECs tested [e.g. when EC_{50} was used: observed inhibition was 88.9% (13.8%) *vs* expected inhibition of 75%; $P < 0.015$; Fig. 3]. Using the EC_{50} of both drugs produced about 90% inhibition of basal contractile activity. Using EC_{75}, contractile activity was completely inhibited in 9 of the 10 strips. Thus, a combination of ritodrine and atosiban resulted in a synergistic inhibition of myometrial contractility.

DISCUSSION

The currently available treatments for preterm labour have not significantly reduced the rate of preterm delivery or adverse neonatal outcomes[1,3–9]. The biochemical mechanisms involved in the aetiology of preterm labour remain unknown, a factor that is partly responsible for the lack of effective therapy. In a simplistic way, preterm labour can be described as an escape from the mechanisms maintaining uterine quiescence and/or induction of the systems augmenting uterine contractile activity[18]. Therefore, the currently used treatments can be divided into two groups: (1) drugs promoting relaxant pathways through activation of adenylate cyclase, accumulation of cAMP and hence activation of protein kinase A (such as ritodrine), and (2) drugs preventing the rise in intracellular calcium concentration, the central event leading to smooth muscle contraction, either directly (such as calcium channel blockers) or indirectly through inhibition of IP_3 production (such as oxytocin antagonists and inhibitors of prostaglandin synthesis)[13–16,19].

Each of the tocolytic agents used in our study acts through a different pathway to achieve uterine relaxation. We hypothesised that combinations of tocolytics may prove to have a synergistic effect. First, our study demonstrated that the combinations nicardipine/ritodrine and nicardipine/atosiban lead to an additive effect. Therefore, at a given concentration, the combination is twice as potent as when each drug was used alone. These results are consistent with the findings of two previously published *in vitro* studies on the tocolytic effect of nifedipine, another dihydropyridine type calcium channel blocker, used in combination with ritodrine, on the myometrial contractile activity in non-pregnant rats and uterine tissue from non-labouring pregnant women[20,21].

However, we found that the combination of ritodrine and atosiban induced a synergistic tocolytic effect. Therefore, this combination potentiates the relaxant effect induced by each single drug, and allows the use of lower effective concentrations to achieve the same inhibitory effect on uterine contractility. This synergistic inhibitory effect may be explained by the involvement of two mainly independent pathways leading to tocolysis. Indeed, ritodrine/atosiban is the only combination where a drug stimulating the cAMP relaxant pathway and a drug preventing the calcium release from intracellular storage sites and calcium influx across plasma membranes through inhibition of IP_3 production are used simultaneously[13,14]. In a recently published study, we found that rofecoxib, a specific inhibitor of the inducible isoform of cyclooxygenase (COX-2), a drug that also prevents IP_3 production induced by the production of prostaglandins, exhibited a synergistic effect on the inhibition of uterine contractile activity when used in combination with ritodrine[17]. Therefore, targeting these two pathways appears to produce an improved tocolytic effect.

The synergistic tocolytic effect of ritodrine used in combination with atosiban may provide the opportunity to improve the maternal and fetal side effect profile. Treatment with beta-2-mimetics is associated with major side effects in about 80% of the patients[12]. These side effects include fetal tachycardia as well as maternal tachycardia, nausea, palpitations, tremor and headache. Major side effects such as pulmonary oedema, cardiac arrhythmia, myocardial ischemia and maternal death have also been described[3,8,12]. In about 14% of patients, tocolysis with beta-2-mimetics has to be discontinued[12]. These dose-dependent side effects limit ritodrine maximal therapeutic dose to 350 μg/hour. However, the synergistic effect exhibited by the combination with atosiban should allow the use of lower effective concentrations of beta-2-mimetics, hence leading to a reduction in side effects. Despite similar efficacy and a dramatically lower side effects rate compared with ritodrine

(4–8%), the clinical use of atosiban remains limited by the high costs of this treatment[4–7]. Therefore, the combination of atosiban with ritodrine may also have the added advantage of decreasing the costs of tocolysis. However, we have to consider that the side effects common to both drugs (such as maternal cardiovascular side effects) may also be enhanced by this combination. Indeed, atosiban is not only an oxytocin receptor antagonist, but also binds to the vasopressin V1a receptor which is involved in vasomotor tone regulation[22,23]. Therefore, vasodilatation induced by beta-2-mimetics resulting in reactive tachycardia may possibly be augmented. However, the combination of atosiban and beta-2-mimetics is not expected to increase the risk of water retention and pulmonary oedema, as (1) Chou *et al.*[24] demonstrated that modulation of the antidiuretic action of vasopressin is predominantly mediated by V2 receptors, and (2) Windle *et al.*[25] showed that atosiban does not affect the antidiuretic and natriuretic properties of vasopressin.

No studies have been performed so far to assess the effectiveness of these three combinations of tocolytic agents *in vivo*. Therefore, animal and clinical studies are needed to establish the optimal therapeutic doses to be used in combination, and to assess the efficacy, potency and safety of these combinations in the management of preterm labour.

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References

1. Norwitz ER, Robinson JN. A systematic approach to the management of preterm labor. *Semin Perinatol* 2001;**25**:233–235.
2. Keirse MJNC. New perspectives for the effective treatment of preterm labor. *Am J Obstet Gynecol* 1995;**173**:618–628.
3. Gyetvai K, Hannah ME, Hodnett ED, Ohlsoon A. Tocolytics for preterm labor: a systematic review. *Obstet Gynecol* 1999;**94**:869–877.
4. Moutquin JM, Sherman D, Cohen H, et al. Double-blind, randomized, controlled trial of atosiban and ritodrine in the treatment of preterm labor: a multicenter effectiveness and safety study. *Am J Obstet Gynecol* 2000;**182**:1191–1199.
5. Papatsonis DNM, Kok JH, Van Geijn HP, Bleker QP, Ader HJ, Dekker GA. Neonatal effects of nifedipine and ritodrine for preterm labor. *Obstet Gynecol* 2000;**95**:477–481.
6. Worldwide Atosiban versus Beta-agonists Study Group. Effectiveness and safety of the oxytocin antagonist atosiban versus beta-adrenergic agonists in the treatment of preterm labour. *Br J Obstet Gynaecol* 2001;**108**:133–142.
7. French/Australian Atosiban Investigators Group. Treatment of preterm labor with the oxytocin antagonist atosiban: a double-blind, randomized, controlled comparison with salbutamol. *Eur J Obstet Gynecol Reprod Biol* 2001;**98**(2):177–185.
8. Tsatsaris V, Papatsonis D, Goffinet F, Dekker G, Carbonne B. Tocolysis with nifedipine or beta-adrenergics agonists: a meta-analysis. *Obstet Gynecol* 2001;**97**:840–847.
9. King JF, Flenady VJ, Papatsonis DNM, Dekker GA, Carbonne B. Calcium channel blockers for inhibiting preterm labor. *Cochrane Database Syst Rev* 2002;(2):CD002255.
10. Crowley P. Prophylactic corticosteroids for preterm birth. *Cochrane Database Syst Rev* 2000;(2):CD000065.
11. Chien LY, Whyte R, Aziz K, Thiessen P, Matthew D, Lee SK. Improved outcome of preterm infants when delivered in tertiary care centers. *Obstet Gynecol* 2001;**98**:247–252.
12. The Canadian Preterm Labor Investigators Group. Treatment of preterm labor with the beta-adrenergic agonist ritodrine. *N Engl J Med* 1992;**327**:308–312.
13. Scheid CR, Honeyman TW, Fay FS. Mechanism of β-adrenergic relaxation of smooth muscle. *Nature* 1979;**277**:32–36.
14. Monga M, Creasy RK. Pharmacologic management of preterm labor. *Semin Perinatol* 1995;**19**:84–96.
15. Sanborn BM. Ion channels and the control of myometrial electrical activity. *Semin Perinatol* 1995;**19**:31–40.
16. Fuchs AR. Plasma membrane receptors regulating myometrial contractility and their hormonal modulation. *Semin Perinatol* 1995; **19**:15–30.
17. Doret M, Mellier G, Benchaib M, Piacenza JM, Gharib C, Pasquier JC. In vitro study of tocolytic effect of rofecoxib, a specific cyclo-oxygenase 2 inhibitor. Comparison and combination with other tocolytic agents. *Br J Obstet Gynaecol* 2002;**109**:983–988.
18. Lopez Bernal A, Rivera J, Europe-Finner N, Phaneuf S, Asboth G. Parturition: activation of stimulatory pathways or loss of uterine quiescence? *Adv Exp Med Biol* 1995;**395**:435–451.
19. Childress CH, Katz VL. Nifedipine and its indications in obstetrics and gynecology. *Obstet Gynecol* 1994;**83**:616–624.
20. Saade GR, Taskin O, Belfort MA, Erturan B, Moise KJ. In vitro comparison of four tocolytic agents, alone and in combination. *Obstet Gynecol* 1994;**84**:374–378.
21. Gallagher LA, Gautieri RF. Influence of a combination of ritodrine and nifedipine on the isolated rat uterus. *Res Commun Chem Pathol Pharmacol* 1992;**75**:3–18.
22. Maggi M, Fantoni G, Baldi E, et al. Antagonists for the human oxytocin receptor: an in vitro study. *J Reprod Fertil* 1994;**101**:345–352.
23. Manning M, Stoev S, Cheng LL, Wo NC, Chan WY. Design of oxytocin antagonists, which are more selective than atosiban. *J Pept Sci* 2001;**7**:449–465.
24. Chou CL, DiGiovanni SR, Luther A, Lolait SJ, Knepper MA. Oxytocin as an antidiuretic hormone. II: Role of V2 vasopressin receptor. *Am J Physiol* 1995;**269**:F78–F85.
25. Windle RJ, Judah JM, Forsling ML. Effect of oxytocin receptor antagonists on the renal actions of oxytocin and vasopressin in the rat. *J Endocrinol* 1997;**152**:257–264.

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