Ability of nifedipine to prolong parturition in rats

T. S. Tracy* and C. D. Black†

Department of Pharmacy Practice, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47906, USA

Summary. Rats were randomly assigned to treatments: (i) no surgery control; (ii) saline control; (iii) 0.25, 0.5, 1.0 or 2.0 μg nifedipine $\text{kg}^{-1} \text{min}^{-1}$; or (iv) 5.0 μg ritodrine $\text{kg}^{-1} \text{min}^{-1}$. All drug treatments increased the interval between pup deliveries compared with the no surgery and saline controls. Apparent complete tocolysis was observed in 20, 60, 80 and 80% of the animals receiving 0.5, 1.0 or 2.0 μg nifedipine $\text{kg}^{-1} \text{min}^{-1}$ or 5.0 μg ritodrine $\text{kg}^{-1} \text{min}^{-1}$, respectively. A positive pharmacodynamic relationship was observed for the nifedipine doses. Analysis of pup viability showed no statistically significant difference among treatments. Treatment with 2.0 μg nifedipine $\text{kg}^{-1} \text{min}^{-1}$ gave a delay in pup delivery comparable to that with ritodrine.

Keywords: nifedipine; ritodrine; preterm labour; tocolysis; rat

Introduction

Preterm labour is associated with a high risk of morbidity and mortality to mother and fetus, and has significant medical costs. Only ritodrine has been approved by the US Food and Drug Administration for the treatment of premature labour, but it has undesirable side-effects (Benedetti, 1983), which have prompted researchers to explore the use of other agents for the treatment of preterm labour.

One agent currently under investigation is nifedipine, which is effective in delaying fetal delivery in humans (Ulmsten *et al.*, 1984; Kaul *et al.*, 1985; Read & Wellby, 1986; Ferguson *et al.*, 1989). Animal studies have been conducted using various models to study the effects of nifedipine on uterine contractions and the delivery process (Hahn *et al.*, 1984; Golichowski *et al.*, 1985; Abel & Hollingsworth, 1985a,b, 1986). In all cases, nifedipine caused a significant reduction in the frequency or strength of uterine contractions or delayed delivery. Pharmacodynamic and preliminary toxicity profiles for nifedipine were not established in these studies. In preliminary studies, (Ferguson *et al.*, 1989) sublingual and oral administration of nifedipine as tocolytic therapy delayed delivery in 11 of 13 human patients, wide variability in plasma concentrations being observed. Nifedipine was detected in five of 11 neonates studied. However, pharmacodynamic analyses comparing plasma concentration with degree of tocolysis have not been performed.

An animal model is therefore needed to establish pharmacodynamic and preliminary toxicity profiles for nifedipine and to compare these results with those for ritodrine. This study, using a rat model, evaluated the ability of nifedipine to delay delivery of pups and assessed associated drug plasma concentrations and pup survival rate.

Materials and Methods

Model

The study was based on a model developed by Hahn *et al.* (1984), which assessed the delay in delivery of a second pup after administration of nifedipine by an oral gavage technique. Modifications to this model were sought because

*Present address and reprint requests: Division of Clinical Pharmacology, Indiana University School of Medicine, 1001 W. 10th Street, Indianapolis, IN 46202, USA.

†Present address: Department of Pharmacy Practice, University of Toledo, Toledo, OH 43606, USA.

of concerns that oral gavage may interfere with normal delivery and confound data interpretation. The Hahn *et al.* (1984) model did not provide for evaluating subsequent pup deliveries and so an intravenous system using a bolus dose followed by continuous infusion was chosen for drug administration to avoid bioavailability deviations and to attain steady-state nifedipine plasma concentrations immediately.

Animals and mating

All procedures were performed according to protocol established and approved by the Purdue University Animal Care and Use Committee. Wistar rats (300–350 g) were housed in group cages and allowed food and water *ad libitum*. The room was maintained at 22°C and 44–55% relative humidity with lights on at 07.00 h and off at 19.00 h. During mating, one male was placed in a cage with three females and they remained together for 4 days. Female rats were weighed daily and those exhibiting significant (>50 g) and continuous weight gain 2 weeks after the cohabitation period were assumed pregnant and entered into the study protocol. The day of expected delivery was based on the assumption of a 23-day gestation period for rats.

Treatment

Three to five days before expected delivery, an intravenous catheter was inserted, following a procedure modified from Enrione *et al.* (1987): Intramedic® tubing was inserted into the left jugular vein and exited on the subscapular area of the back. The catheter tubing was attached to a Button Infuser® (Markwell Medical; normally intended for multiple insulin dosing in man) with an attached 27-gauge needle to allow a Luer-lock connection to the intravenous tubing. This catheter system was flushed twice a day with 0.1–0.2 ml heparin (100 units ml⁻¹) to maintain patency.

Nifedipine and ritodrine solutions for intravenous administration were prepared on the day of expected delivery to minimize drug decomposition. Nifedipine powder (10 mg) (Pfizer Laboratories, New York, USA) was dissolved in ethanol and glycerin and diluted to the proper concentration (to allow infusion at 1.5 ml h⁻¹) with a sufficient volume of sterile water for injection. The final concentrations of ethanol and glycerin (both added to enhance nifedipine solubility) in all nifedipine infusion solutions were 3 and 10%, respectively. Ritodrine HCl (10 mg ml⁻¹) (Astra Pharmaceutical, Westboro, MA, USA) was diluted to the desired concentration using 0.9% NaCl injection.

Five pregnant female rats were randomly assigned to each group and treated with 0.25, 0.5, 1.0 or 2.0 µg nifedipine kg⁻¹ min⁻¹; 5.0 µg ritodrine kg⁻¹ min⁻¹ (the maximum dose approved by the US Food and Drug Administration) (appropriately adjusted for body weight), 0.9% NaCl (control) or no treatment control. On day 23 after initiation of mating, the pregnant animals were observed continuously for signs of labour (overtly noticeable pup movement within the abdomen and/or vaginal bleeding). Immediately after delivery of the first pup, a bolus infusion was given to the nifedipine-treatment animals. Nifedipine kinetic parameters in rats (Testa *et al.*, 1979) were used to predict steady-state concentrations and to calculate the bolus doses. The bolus infusion was administered over 1 min, followed by the appropriate continuous infusion at 1.5 ml h⁻¹ using a syringe pump. Ritodrine and 0.9% NaCl were also infused at 1.5 ml h⁻¹, but no bolus dose was given for ritodrine infusions as this is not done clinically in humans and, therefore, no bolus was given for the saline-treated animals. Infusions were continued until all pups had been delivered or apparent complete tocolysis was suspected. In animals exhibiting a delay, but not a cessation, of deliveries, all pups were assumed to have been delivered if normal maternal activity had resumed and no pups had been delivered for 2 h. During pilot studies, the validity of this was established and thus, allowing for variability, 2 h was chosen as the time for discontinuing therapy. The infusion was allowed to continue for 8 h in animals exhibiting immediate or near-immediate complete tocolysis (zero to three pups delivered after therapy initiation). In the case of complete tocolysis, it was considered that, if delivery had been delayed for 8 h, complete tocolysis had occurred and that normal delivery should be allowed to resume as it had been a considerable period since the maternal membranes had ruptured. Reversibility of effect was measured as ability of the rat to re-initiate pup delivery following apparent complete tocolysis and cessation of drug therapy for 8 h.

The interval between each subsequent pup delivery following the first pup was measured for each animal. Plasma samples for nifedipine were obtained from the ophthalmic branch of the carotid artery via a capillary tube temporarily inserted into the periorbital space at 15 min after initiation of infusion and at the end of all deliveries (or after 8 h in animals exhibiting complete tocolysis) and frozen immediately at -15°C until analysis. Pup viability was assessed as the number of pups remaining alive 6 h after delivery.

Assay

Nifedipine plasma concentrations were analysed using a procedure modified slightly from Kleinbloesem *et al.* (1984). This assay had a limit of sensitivity of 10 ng ml⁻¹ and an intra-assay coefficient of variance of 4.58%. All procedures were performed under controlled sodium lighting because nifedipine can photodecompose.

Statistical analysis

Data were analysed by one-way ANOVA followed by Student–Newman–Keuls multiple comparison of means.

Results

A statistically significant difference in interval between pup deliveries was observed between drug-treated and control rats (Table 1). Although these mean data did not appear to show a progressive dose-response curve, upon examination of the total data a graded response was seen comparing all nifedipine treatments and ritodrine treatments with the no-surgery and saline controls: 20, 60, 80 and 80% of animals, treated with 0.5, 1.0 or 2.0 $\mu\text{g nifedipine kg}^{-1} \text{ min}^{-1}$ or 5.0 $\mu\text{g ritodrine kg}^{-1} \text{ min}^{-1}$, respectively, exhibited apparent complete tocolysis (defined as absence of pup delivery for 8 h following almost immediate cessation of deliveries after drug administration) whereas the animals in the other treatment groups did not show this effect. Because of the averaging procedure, the mean values for interval between pup delivery therefore became skewed, as this parameter was essentially without value in animals in whom tocolysis was exhibited. This resulted in the doses of 1.0 and 2.0 $\mu\text{g nifedipine kg}^{-1} \text{ min}^{-1}$ and 5.0 $\mu\text{g ritodrine kg}^{-1} \text{ min}^{-1}$ appearing to be less effective than 0.5 $\mu\text{g nifedipine kg}^{-1} \text{ min}^{-1}$, when, in fact, they caused an immediate or nearly immediate cessation of pup delivery in three, four and four animals, respectively.

Table 1. Mean interval between pup deliveries and number of pups delivered during drug treatment in rats

Treatment	<i>n</i>	Interval between pups (min)	Number delivered
No surgery	5	4.4 $\pm$ 0.4	13.0 $\pm$ 3.5
Saline	5	6.7 $\pm$ 1.3	12.6 $\pm$ 4.9
Nifedipine ($\mu\text{g kg}^{-1} \text{ min}^{-1}$)			
0.25	5	27.4 $\pm$ 21.9*	8.4 $\pm$ 3.0
0.5	5	52.9 $\pm$ 2.5*	5.4 $\pm$ 2.5*
1.0	5	22.4 $\pm$ 10.6*	4.8 $\pm$ 4.5*
2.0	5	44.4 $\pm$ 37.3*	2.4 $\pm$ 2.1*†
Ritodrine ($\mu\text{g kg}^{-1} \text{ min}^{-1}$)			
5.0	5	32.3 $\pm$ 22.2*	2.2 $\pm$ 3.9*†

Data are means $\pm$ SD.

*Significantly different from controls ($P < 0.05$).

†In these treatments, one to three pups were delivered before complete tocolysis occurred.

This 'false skewing' of the data can be partially resolved by comparing the mean number of pups delivered during drug treatment (Table 1). For 2 $\mu\text{g nifedipine kg}^{-1} \text{ min}^{-1}$ and 5.0 $\mu\text{g ritodrine kg}^{-1} \text{ min}^{-1}$, the values include cases where one to three pups were born before complete tocolysis occurred. All drug treatments, except 0.25 $\mu\text{g nifedipine kg}^{-1} \text{ min}^{-1}$, produced a statistically significant decrease in the mean number of pups delivered during drug treatment. There was a consistent decrease in the number of pups delivered during drug treatment (i.e. the remainder were delivered after 8 h of apparent drug-induced tocolysis with increasing doses of nifedipine or with ritodrine). This suggests a progressive effect in delaying and/or stopping pup delivery with an increase in drug dose. Theoretically, the randomization process should have balanced any differences in the total number of delivered pups and thus have no effect on the percentage of pups delivered during drug treatment. This was verified by there being no statistical difference in the total number of pups delivered in any of the treatment groups.

Plasma samples were obtained for the nifedipine treatments at 15 min after initiation of treatment and at the end of all deliveries or 8 h after apparent complete tocolysis. The best-fit line for the

mean (of 15 min and end of all deliveries) plasma concentrations of nifedipine and their relationship with the corresponding overall mean interval between pup deliveries (Fig. 1) exhibits a correlation coefficient of 0.6721, indicating a statistically significant positive pharmacodynamic correlation between the paired data sets. The mean nifedipine plasma concentration in animals exhibiting apparent complete tocolysis was 363.1 ± 187.1 ng ml⁻¹, consistent with the pharmacodynamic relationship suggested in Fig. 1.

Fig. 1. Linear regression fit of pharmacodynamic properties of nifedipine in delaying pup delivery to rats: mean plasma concentration versus average interval between deliveries.

No distinction was made between pups stillborn and those dying after delivery, because it was impossible to distinguish between those stillborn due to natural causes and those stillborn due to drug treatment. Controls were treated in a similar manner to reduce any bias. Drug treatments produced a slightly lower percentage of viable pups delivered (Table 2) than did the controls, but these values were not statistically significant ($P > 0.25$) after analysis by one-way ANOVA. In some cases, not all pups had been delivered (because of complete tocolysis) and the percentage viability for pups delivered during drug treatment and after drug treatment was therefore also measured (Table 2). In cases where this occurred, a χ^2 analysis was performed to determine whether any differences existed: it showed overall differences in the proportions of viable pups delivered across treatment groups ($P < 0.01$). However, when each of these cases was analysed individually, using Student's *t* test, no statistically significant difference was determined specific to any one of the treatments.

Table 2. Total percentage of viable pups delivered to rats

Treatment	Total viable	During treatment	After treatment
No surgery	92.6 ± 5.7	92.6 ± 5.7	n/a
Saline	86.9 ± 21.1	86.9 ± 21.1	n/a
Nifedipine (µg kg ⁻¹ min ⁻¹)			
0.25	87.4 ± 14.1	87.4 ± 14.1	n/a
0.5	74.5 ± 13.0	75.4 ± 23.6	72.8 ± 28.54*
1.0	72.8 ± 17.0	67.8 ± 21.0	77.0 ± 24.04*
2.0	74.1 ± 17.7	91.7 ± 16.7	71.7 ± 23.04*
Ritodrine (µg kg ⁻¹ min ⁻¹)			
5.0	77.4 ± 18.0	94.5 ± 7.8	66.8 ± 31.14*

Data are means ± SD.
*Not statistically significantly different compared with percentages during treatment.
n/a: not applicable.

Discussion

This model for pregnant rats appears valid for evaluating the tocolytic response of nifedipine therapy compared with the clinical standard ritodrine and controls. Previous models have not been able to measure such factors as attenuation of effect, reversibility of effect and fetal viability, as was possible using this model. Hahn *et al.* (1984) developed a model measuring the time delay after drug administration by oral gavage until delivery of the second pup. Some uncertainty concerning the applicability of this model arises in that the mean interval between pups in the present study was 4.4 ± 0.4 min in no-surgery controls, whereas Hahn *et al.* measured a mean of approximately 16 min between pup deliveries in animals receiving vehicle (control treatment) via the oral gavage technique. The gavage technique may itself cause a physiological response that affects the interval between pups.

Abel & Hollingsworth (1985a, b, 1986) used a model wherein rats received prophylactic tocolytic therapy after artificially induced labour. This model used ovariectomy with subsequent oestrogen therapy to induce uterine contractions and labour and then measured the number of pups remaining *in utero* (after killing the mother) after 3 days of treatment, thus giving no indication whether labour already in progress could be stopped or whether labour is reinitiated after ceasing tocolytic therapy.

The model for pregnant ewes used by Golichowski *et al.* (1985) does allow for the reinitiation of labour as well as haemodynamic monitoring, but again uses artificial induction of labour. Although such induction is more convenient for an investigator, in that the onset of labour can be controlled more accurately, it is uncertain whether this mimics the natural process of labour initiation. Thus, the model is not applicable to the clinical situation.

The pharmacodynamic activity of nifedipine appeared to provide a positively correlated response in delaying pup delivery relative to plasma concentration. The doses of nifedipine chosen for this research approximated the range ($\mu\text{g kg}^{-1}$) currently administered to humans for approved use in alleviating angina pectoris (Nifedipine package insert, 1986). It therefore appears that tocolysis is an extension of the pharmacological activity of nifedipine in doses ($\mu\text{g kg}^{-1}$) paralleling those used for the treatment of heart disease. Owing to the significant variability in the apparent pharmacokinetic traits among animals, as well as the onset of tocolysis, from these data a specific dose of nifedipine cannot be used as a target to achieve a particular tocolytic effect. However, the plasma concentration and corresponding tocolytic effect achieved with a given dose can be assessed with the expectation that an increase in dose will produce a graded, positive increase in effect. This type of pharmacodynamic relationship has been demonstrated for nifedipine in the treatment of angina pectoris, the effective plasma concentration of nifedipine varying from 20 to 150 ng ml⁻¹ in patients (Chaitman *et al.*, 1984). Ferguson *et al.* (1989) reported significant pharmacokinetic variability among 11 patients exhibiting delay of delivery after administration of nifedipine.

The complete tocolytic response to nifedipine was reversible and normal delivery could be reinitiated by the animal after discontinuation of nifedipine infusion, corroborating the findings of Ferguson *et al.* (1989). However, with continued infusion, the activity of nifedipine in delaying pup delivery appeared to attenuate when less than complete tocolysis was achieved. This attenuation of effect has been shown to occur also during ritodrine therapy (Johansson & Andersson, 1981).

Pup viability was not statistically different across treatment groups. When complete tocolysis was achieved, pup viability was not statistically significantly different upon reinitiation of labour compared with that of pups delivered during drug administration before complete tocolysis. Care must be exercised in interpreting these viability data because of the small samples. However, Ahokas *et al.* (1988) recently showed that nifedipine does not adversely affect uteroplacental blood flow in hypertensive term-pregnant rats and Ferguson *et al.* (1989) reported no adverse effects on human neonates, despite detectable nifedipine concentrations in some neonates, adding support to the hypothesis that nifedipine does not cause fetal toxicity.

The results of this study confirm the tocolytic properties of nifedipine and partly delineate its pharmacodynamic and toxicity profiles.

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