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Comparison of effects of cyclooxygenase inhibitors on myometrial contraction and constriction of ductus arteriosus in rats

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Abstract

The aim of this study was to compare the tocolytic effect of a selective cylooxygenase-2 inhibitor, DFU (5,5-dimethyl-3(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5H)-furanone), indomethacin and nimesulide on myometrial strips isolated from rats in both lipopolysaccharide-induced preterm labour and term labour. We also compared the constrictor effects of DFU and indomethacin on the fetal ductus arteriosus. Myometrial strips were obtained from preterm and term labour Wistar albino rats and were mounted in organ baths for the recording of isometric tension. DFU, nimesulide and indomethacin significantly inhibited KCl-, oxytocin-, prostaglandin E_2- and prostaglandin $F_{2\alpha}$-stimulated contractions of myometrial strips isolated from rats in preterm and term labour. The E_{max} value of indomethacin was significantly lower than those for DFU and nimesulide ($P<0.05$), with no change-log (10) EC_{50} values. There was no significant difference between in -log (10) EC_{50} and E_{max} values of DFU and nimesulide for any of the tissues ($P>0.05$). In addition, there was no significant difference between $-\log$ (10) EC_{50} and E_{max} values for each of these three agents in myometrial tissues isolated from rats in preterm and term labour ($P>0.05$). Fetal ductus arteriosus was significantly constricted by DFU (10 or 100 mg/kg) in preterm and term rats, although DFU (10 or 100 mg/kg)-induced constriction ratios were significantly lower than those for indomethacin ($P<0.05$). These data demonstrate that DFU, a specific cyclooxygenase-2 inhibitor, could be considered as a new therapeutic agent for preterm labour. However, careful attention should be given to constriction of the fetal ductus arteriosus.

Keywords: DFU; Nimesulide; Indomethacin; Myometrium; Ductus arteriosus

1. Introduction

Prevention of preterm labour is an important subject for the obstetrician. It complicates 10% of pregnancies all over the world and is responsible for 75% to 80% of the perinatal mortality in infants without major malformations (Black et al., 1996).

Pharmacological inhibition of uterine contractions remains one of the cornerstone treatments for preterm labour. Inhibitors of smooth muscle contraction, like β_2-agonists, magnesium sulphate, and Ca^{2+} channel blockers, are the most important agents used to prevent preterm delivery but their efficacy and safety are questionable (Keirse, 1995). New drugs which allow more effective treatment of preterm labour with lesser side-effects are needed.

Although the mechanisms that lead to initiation of preterm or term uterine contractions are not entirely understood, it is clear that prostaglandins play a key role in the initiation of myometrial activity (Olson et al., 1995). Cyclooxygenase enzymes are major components of the prostaglandin synthesis pathway. Two isoforms of cyclooxygenase have been identified: cyclooxygenase-1 and cyclooxygenase-2 (Smith and Dewitt, 1996). Cyclooxygenase-1 is constitutively expressed in tissues with constant prostaglandin synthesis, while cyclooxygenase-2 is inducible and is associated with inflammation (Funk, 1993). In humans, cyclooxygenase-2 up-regulation is more strongly associated with the onset of preterm and term labour (Zuo et al., 1994; Gibb, 1998). Thus the effective inhibition of cyclooxyge-

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nase-2 may have a central role in the attenuation of preterm labour due to inflammation in humans.

Nonsteroidal anti-inflammatory drugs (NSAID) inhibit both isoform activities. They are potent tocolytic agents that prolong pregnancy for over 7 days (Higby et al., 1993; Gyetvai et al., 1999). Indomethacin, a nonselective cyclooxygenase inhibitor, is used to prevent preterm deliveries (Slater et al., 1999; Slattery et al., 2001; Morales and Madhav, 1993). Nevertheless, the use of indomethacin as a tocolytic agent is limited by several important pregnancy-specific adverse effects (Norton et al., 1993). Some reports suggest that the adverse effects of nonselective cyclooxygenase inhibitors may be attributable to the inhibition of cyclooxygenase-1 activity (Slater et al., 1995; Norton et al., 1993). Nimesulide (*N*-(4-nitro-2-phenoxyphenyl)-methanesulphonilamide) shows 30- to 100-fold selectivity for inhibition of cyclooxygenase-2 compared to cyclooxygenase-1 (Taniguchi et al., 1995; Miralpeix et al., 1997). It was recently shown that nimesulide inhibited in vitro spontaneous contractions of myometrial strips taken from pregnant women at term (Sawdy et al., 1998). In the human, neonatal end-stage renal failure associated with maternal ingestion of the nimesulide has been reported (Peruzzi et al., 1999). DFU (5,5-dimethyl-3(3-fluorophenyl)-4-(4-methylsulphonyl)-phenyl-2(5H)-furanone was identified as a novel highly selective cyclooxygenase-2 inhibitor. It shows 1000-fold selectivity for inhibition of cyclooxygenase-2 compared to cyclooxygenase-1 (Riendeau et al., 1997). However, there are no reports about the tocolytic effect and fetal side-effects of DFU in the literature.

We therefore compared the effects of DFU, nimesulide and indomethacin on KCl-, oxytocin, prostaglandin E_2- and prostaglandin $F_{2\alpha}$-stimulated contractions of myometrial strips isolated from rats in lipopolysaccharide-induced preterm and term labour. We also investigated the effects of DFU and indomethacin on constriction of the fetal ductus arteriosus to assess the fetal side-effects of the drugs.

2. Materials and methods

2.1. Animals

Term ($n = 40$; 25 to measure the constrictor effect on fetal ductus arteriosus, 15 for in vitro contractility studies) and preterm ($n = 72$; 20 to determine the incidence of preterm delivery, 25 to measure the constrictor effect on fetal ductus arteriosus, 15 for contractility studies, 12 to measure the prostaglandin $F_{2á}$ levels on myometrial strips) pregnant Wistar albino rats, weighing 180–220 g, were used throughout the study. All procedures were performed under the guidelines of the Animal Care and Use Committee of Cumhuriyet University School of Medicine. The rats were housed in a 22 °C temperature room with water and food ad libitum. Virgin female rats were placed in separate cages with one male each, and left overnight. The day that vaginal plug and spermatozoa in the vaginal smear were detected was designated as day 0 of gestation. The rats were divided into term and preterm groups.

2.2. Lipopolysaccharide-induced preterm delivery procedure

Preterm rats were divided randomly into two groups. In the preterm-delivery group, rats ($n = 10$) were intraperitoneally treated with a lipopolysaccharide (*E. coli*, serotype 026:B6; Sigma, St. Louis, MO, USA) twice at 1400 and 1700 on the 15th day of pregnancy at a dose of 50 μg/kg. In the control group, rats ($n = 10$) were given 0.25 ml distilled water twice at 14:00 and 17:00 on the 15th day of pregnancy. The incidence of preterm (≤ 18 days of pregnancy) and term (19 or 21 days of pregnancy) deliveries was observed in the early morning and late afternoon during the experimental period.

2.3. Measurement of fetal ductus arteriosus

Indomethacin (10 and 100 mg/kg; Sigma) or DFU (10 and 100 mg/kg; Merck, New Jersey, USA) was administered through a gastric tube in a suspension of 2 ml distilled water containing 5% gum arabic on the 21st day of pregnancy ($n = 5$ for each drug dose) and on the 18th day of pregnancy ($n = 5$ for each drug dose). In the control group, preterm ($n = 5$) and term ($n = 5$) rats were given 2 ml distilled water as placebo.

Pregnant rats were killed by cervical dislocation 12 h after the administration of drugs, and placebo and thoracal region from each embryo were obtained. Tissues were fixed in 10% neutral formaldehyde for 48 h, dehydrated with increasing concentrations of ethanol and embedded in paraffin. Serial sections 5 μm thick of the thoracal region of each embryo were taken, stained with haematoxylin-eosin, and the appropriate areas were photographed.

Fetal ductus arteriosus and pulmoner artery diameters were measured using a Jenamed II photomicroscope with a drawing tube and a graticle. Magnification was calculated as $\times 100$ in $\times 10$ objective lens using a graticle with length 1 mm and a millimetric ruler. The graticle was replaced by tissue sections containing fetal ductus arteriosus and pulmonary artery fields. The inner diameters of each fetal ductus arteriosus and pulmonary artery in each section were measured in millimeters with a millimetric ruler, then each value was multiplied by 100 in order to convert the diameters to micrometers. For these measurements, five tissues chosen at random were used for each animal. The mean fetal ductus arteriosus and pulmonary artery diameters were determined in micrometers and the ratio of the narrowest part of the pulmonary artery to the fetal ductus arteriosus (fetal ductus arteriosus/pulmonary artery) was accepted as the contraction effect of drugs. The differences between groups were compared using Student's *t* test.

Table 1
Preterm and term delivery days in lipopolysaccharide (LPS)-injected (2×50 μg/kg, intraperitoneally) and distilled water (2×0.25 ml, intraperitoneally)-injected (control) rats

	n	Day of delivery					
		Preterm			Term		
		16	17	18	19	20	21
Control	10				1 (%10)	4 (%40)	5 (%50)
LPS-injected	10	7 (%70)	2 (%20)	1 (%10)			

2.4. Measurement of myometrial contractile activity

Pregnant rats were killed by cervical dislocation at 21 days of gestation (term) and 18 days of gestation (preterm). A midline abdominal incision was made. The uterine horns were rapidly excised and carefully cleaned of surrounding connective tissue, then opened longitudinally along the mesenteric border. Fetuses were removed and nonuterine tissues were dissected away. We obtained five full-thickness longitudinal muscle strips (approximately $8 \times 2 \times 2$ mm) from each animal and incubated the strips in temperature-controlled (37 °C) 10-ml organ baths containing modified Krebs' solution (NaCl 125 mM, KCl 2.4 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 0.5 mM, $NaHCO_3$ 23.9 mM and glucose 11.0 mM) aerated with 95% O_2 and 5% CO_2 (pH = 7.4).

The myometrial strips were allowed to equilibrate at 1-g tension for 60 min before the addition of the experimental drugs and were washed every 15 min. The myometrial tension was recorded isometrically with a Grass FT03 force-displacement transducer and recorded on a Grass model 79E polygraph (Grass, Quincy, MA, USA). The recorder was calibrated so that 1-g tension was represented as 1-cm vertical displacement. Paper speed was set at 2.5 mm/min. Myometrial contractions started within 10 min after the strips were mounted in the organ bath and stabilized in 60 min. Preliminary time-control experiments with no further drug additions showed that strips exhibit stable uterine activity for at least 4 h after preparation in this manner.

Five sets of experiments were performed with myometrial strips obtained from preterm ($n = 15$) and term ($n = 15$) pregnant rats. We used the five myometrial strips isolated from each rat. We tested the effects of dimethyl sulfoxide (DMSO), the solvent vehicle for DFU, nimesulide and indomethacin on one of the strips in the first set. In the second, third, fourth and fifth sets, we evaluated the effects of DFU (10^{-9}–10^{-4} M), nimesulide (10^{-9}–10^{-4} M) and

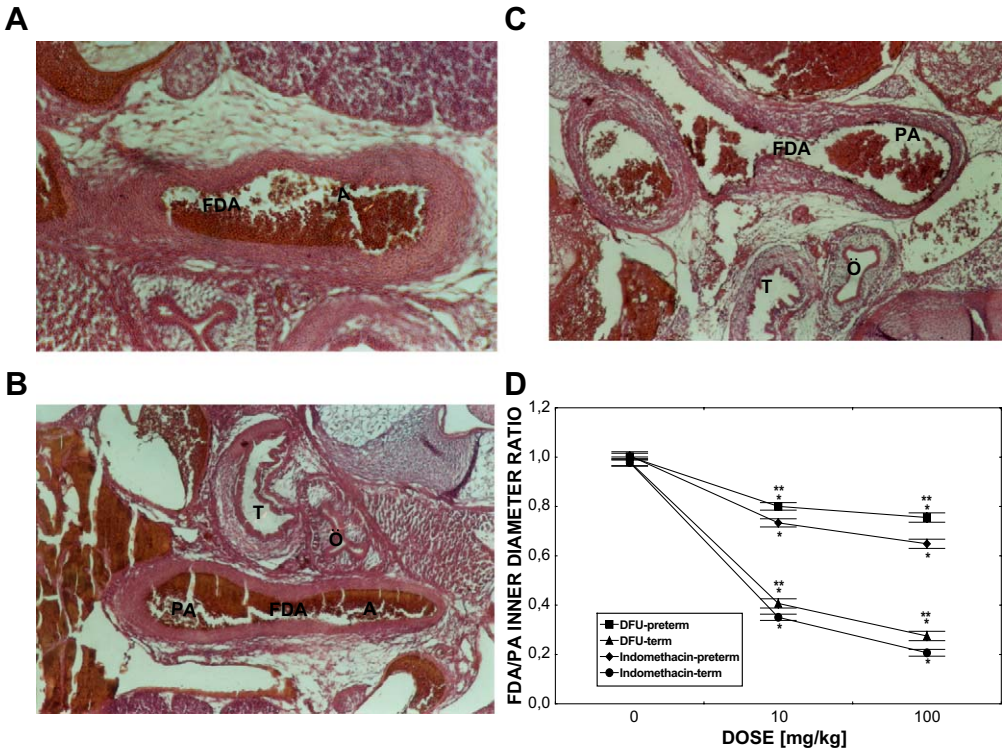

Fig. 1. Sagital cross sections of the main pulmonary artery, ductus, and descending aorta (A, B, and C). These three cross sections of hearts in preterm rats were recorded at the same magnification. The fetal ductus arteriosus was well patent in the control heart (A). Four hours after administration of DFU at a dose of 10 mg/kg (B) or 100 mg/kg (C), the fetal ductus arteriosus was significantly constricted. The ductus had a constriction at the distal end with a small central opening. The dose–effects of DFU and indomethacin for fetal ductus arteriosus/pulmonary artery inner diameter ratio were studied 4 h after maternal administration of DFU and indomethacin. Fetal ductus arteriosus/pulmonary artery ratios (mean ± S.E.M.) after administration of DFU and after administration of indomethacin are plotted (D). FDA, fetal ductus arteriosus; PA, pulmonary artery; A, aorta; T, trachea; O, esophagus. *$P < 0.05$ vs. controls of DFU and indomethacin, **$P < 0.05$ vs. indomethacin of DFU. For colour see online version.

indomethacin (10^{-9}–10^{-4} M), respectively, on KCl (80 mM)-, oxytocin (10 mU/ml)-, prostaglandin E_2 (10^{-7} M) and prostaglandin $F_{2\alpha}$ (10^{-7} M)-stimulated myometrial contractions. KCl, oxytocin, prostaglandin E_2 and prostaglandin $F_{2á}$ were added to the organ baths 15 min before from DFU, nimesulide and indomethacin. The effects of cumulative concentrations of DFU, nimesulide and indomethacin on KCl-, oxytocin-, prostaglandin E_2 and prostaglandin $F_{2\alpha}$-stimulated myometrial contractions were measured, and the values for $-\log(10)\ EC_{50}$ and mean maximal inhibition (E_{max}) were compared. Maximal inhibitor effects were calculated for each concentration–response curve. The EC_{50} value of each drug, which represents 50% of the maximal inhibitor effect, was used as a means of comparing potency. EC_{50} values were calculated by linear regression of the probit of response versus $\log_{10}$ molar concentration for each of the cyclooxygenase inhibitors.

The chemicals used in the in vitro experiments were nimesulide, indomethacin, oxytocin, prostaglandin E_2 and prostaglandin $F_{2\alpha}$ from Sigma, DFU from Merck. All chemicals were dissolved in distilled water, except DFU, nimesulide and indomethacin, which were dissolved in

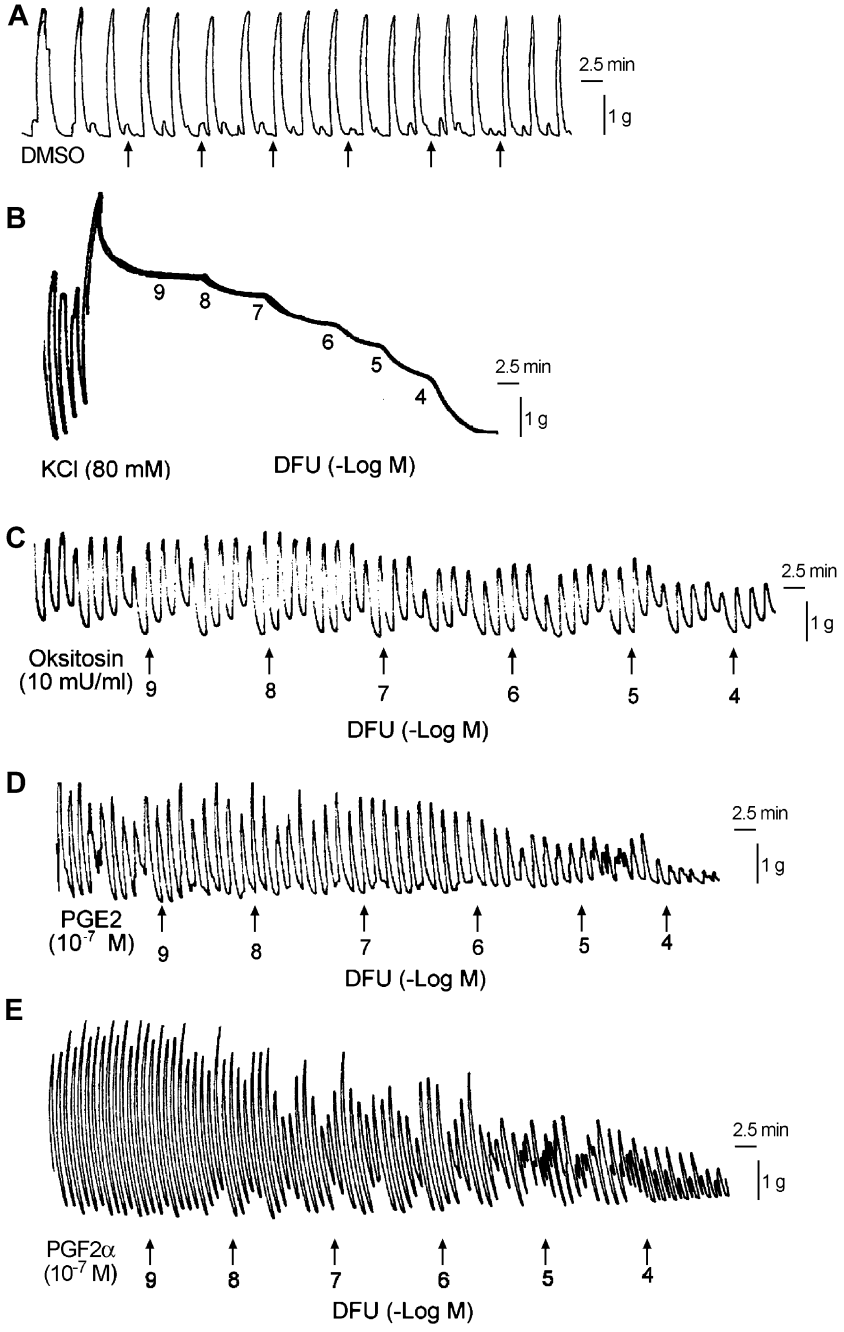

Fig. 2. Representative records of effects of DMSO (A) and 10^{-9}–10^{-4} M DFU on KCl (B)-, oxytocin (C)-, prostaglandin E_2 (D)- and prostaglandin $F_{2\alpha}$ (E)-stimulated contractions of preterm rat myometrium.

DMSO and then diluted with distilled water for preparation of decreasing concentrations of these drugs. Drug solutions added to the organ bath never exceeded 1% of the total volume. All drug solutions were freshly prepared on the day of the experiments.

At the start of each experiment, the first 15 min with KCl (80 mM)-, oxytocin (10 mU/ml)-, prostaglandin E_2 (10^{-7} M)- and prostaglandin $F_{2\alpha}$ (10^{-7} M)-stimulated myometrial contractions was considered as a reference response. The effect of each cyclooxygenase inhibitor was assessed by expressing the percentage change of contraction amplitude (gram) from the initial reference response for the 15-min period after the addition of each drug concentration. Data were presented as means ± S.E. and analyzed by repeated measures of analysis of variance (ANOVA) with the Newman–Keuls test, and a *t* test when appropriate. A *P* value of <0.05 was considered significant. All statistical analyses were performed using Statistica for Windows 6.0. (Statsoft, Tusla, USA).

2.5. Measurement of prostaglandin $F_{2\alpha}$ in myometrial strips

Myometrial strips taken from lipopolysaccharide-injected preterm rats were stimulated with KCl and oxytocin and incubated with 10^{-5} M DFU for 15 min in the organ bath. Afterwards, myometrial strips were homogenized in buffer (0.1 M phosphate, pH 7.4, containing 1 mM EDTA and 10 μM indomethacin). The homogenate was then centrifuged for 10 min, and the supernatant was collected in a clean tube. Samples were applied to a C-18 SPE cartridge (Waters, Milford, MA, USA) preconditioned by washing with 5 ml methanol and then with 5 ml UltraPure water. The SPE cartridge was washed with 5 ml UltraPure water followed by 5 ml of high-performance liquid chromatography (HPLC) grade hexane. Prostaglandin $F_{2\alpha}$ was eluted with 5 ml ethyl acetate containing 1% methanol. The ethyl acetate eluate was evaporated with a stream of nitrogen gas. The residue was dissolved in 450 μl of diluted enzyme immunoassay (EIA) buffer. Prostaglandin $F_{2\alpha}$ in myometrial strip extracts was assayed with EIA kits (Cayman Chemical, Ann Arbor, MI, USA). Sampling data were divided by the weight of the myometrial strip, and the results were expressed in pg/mg myometrium. Assays were performed in duplicate and the intra-assay coefficient of variation of prostaglandin $F_{2\alpha}$ was 8%.

3. Results

The incidence of preterm delivery in lipopolysaccharide-injected (2×50 μg/kg, intraperitoneally) rats ($n=10$) was 100% on days 16–18 of pregnancy. Distilled water (2×0.25 ml, intraperitoneally)-injected rats ($n=10$) had no preterm delivery (Table 1).

The results of DFU treatment on the ductus arteriosus in preterm (on the 18th day of pregnancy) rats are shown in Fig. 1B and C. Sagittal cross sections of the fetal heart showed increased constriction of the fetal ductus arteriosus after administration of both DFU and indomethacin compared with the control (fetus without medication) (Fig. 1D). The fetal ductus arteriosus was significantly constricted by the administration of 100 and 10 mg/kg DFU (contraction ratios were 25 ± 8% and 20 ± 6%, respectively) compared with the control in preterm rats. The fetal ductus arteriosus was constricted significantly by 100 and 10 mg/kg indomethacin (contraction ratios were 36 ± 7% and 27 ± 6%, respectively) compared with the control in preterm rats. In the study with term (on the 21st day of pregnancy) rats, the fetal ductus arteriosus was significantly constricted after administration of both DFU and indomethacin. However, the contraction rates were very high, compared with those in the preterm fetus (Fig. 1D).

Fig. 2 demonstrates representative recordings of the effects of DFU (10^{-9}–10^{-4} M) on KCl (80 mM)-, oxytocin (10 mU/ml), prostaglandin E_2 (10^{-7} M) and prostaglandin $F_{2\alpha}$ (10^{-7} M)-stimulated contractions of myometrial strips isolated from preterm pregnant rats. DMSO (used as solvent vehicle of DFU, nimesulide and indomethacin) had no effect on myometrial contractions (Fig. 2). Table 2 presents the values for $-\log(10)\ EC_{50}$ and E_{max} of DFU, nimesulide and indomethacin on KCl-, oxytocin-, prostaglandin E_2- and prostaglandin $F_{2\alpha}$-stimulated contractions of preterm ($n=15$) and term ($n=15$) pregnant rats myometrium. The E_{max} values for DFU and nimesulide (selective cyclooxygenase-2 inhibitors) were significantly greater than those calculated for indomethacin (nonselective cyclooxy-

Table 2
$-\log 10\ EC_{50}$ and E_{max} (%) values for the inhibitor effects of DFU, nimesulide and indomethacin on KCl-, oxytocin-, prostaglandin E_2 and prostaglandin F_2-stimulated contractions of preterm and term pregnant rat myometrium

	$-\log 10\ EC_{50}$		E_{max} (%)	
	Preterm	Term	Preterm	Term
KCl				
Indomethacin	6.16 ± 0.07	6.30 ± 0.05	69.60 ± 7.09	66.34 ± 5.74
Nimesulide	6.18 ± 0.08	6.36 ± 0.05	97.68 ± 5.20*	92.62 ± 4.88*
DFU	6.52 ± 010	6.48 ± 0.06	98.75 ± 2.79*	95.70 ± 4.87*
Oxytocin				
Indomethacin	6.06 ± 0.08	6.28 ± 0.06	57.28 ± 11.71	57.72 ± 8.87
Nimesulide	6.25 ± 0.08	6.32 ± 0.07	86.60 ± 9.77*	88.13 ± 5.35*
DFU	6.30 ± 0.10	6.46 ± 0.09	90.98 ± 11.06*	91.20 ± 6.20*
Prostaglandin E_2				
Indomethacin	6.12 ± 0.07	6.20 ± 0.05	42.20 ± 6.26	44.80 ± 8.16
Nimesulide	6.18 ± 0.08	6.36 ± 0.05	67.37 ± 6.77*	80.14 ± 5.60*
DFU	6.52 ± 0.10	6.48 ± 0.06	71.78 ± 6.35*	83.24 ± 5.52*
Prostaglandin $F_{2\alpha}$				
Indomethacin	6.18 ± 0.05	6.16 ± 0.03	50.20 ± 7.52	47.67 ± 6.09
Nimesulide	6.18 ± 0.08	6.36 ± 0.05	81.55 ± 4.57*	76.97 ± 5.42*
DFU	6.52 ± 010	6.48 ± 0.06	85.23 ± 5.62*	80.92 ± 6.57*

Values are means ± S.E.
* $P<0.05$ vs. indomethacin.

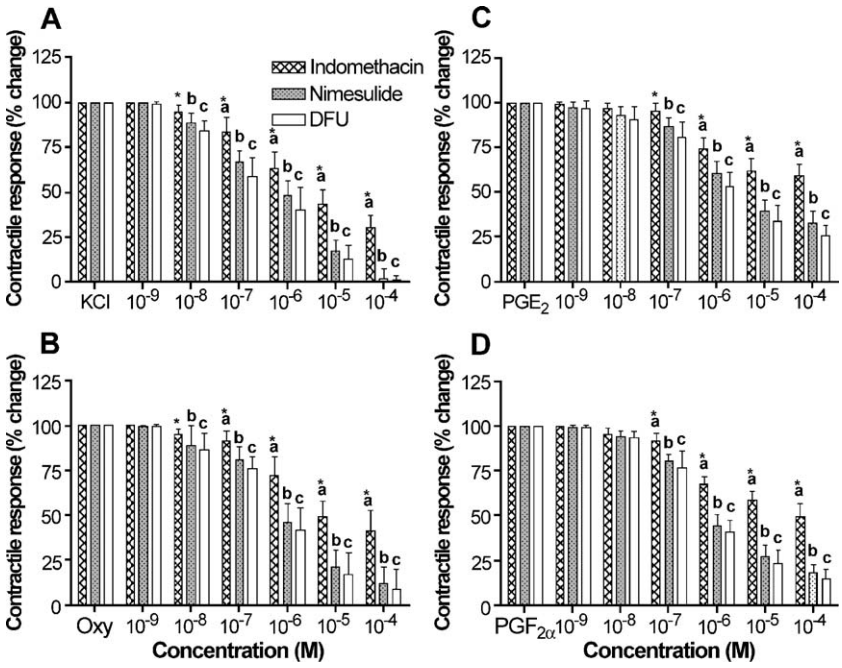

Fig. 3. Effect of DFU (10^{-9}–10^{-4} M), nimesulide (10^{-9}–10^{-4} M) and indomethacin (10^{-9}–10^{-4} M) on the amplitude of KCl (A)-, oxytocin (B)-, prostaglandin E_2 (C)- and prostaglandin $F_{2\alpha}$ (D)-stimulated contractions of myometrial strips isolated from preterm rats ($n=15$). Data are expressed relative to the control (precontractile response) for each drug. Data represent the mean ± S.E. of 15 experiments. *$P<0.05$ vs. controls of DFU, nimesulide and indomethacin, **$P<0.05$ vs. indomethacin of DFU and nimesulide.

genase inhibitor) ($P<0.05$), with no change of the $-\log$ (10) EC_{50} values ($P>0.05$), in myometrial strips isolated from rats in preterm and term labour. The effects of DFU and nimesulide were similar in all myometrial tissues. In addition, there were no significant difference between $-\log$ (10) EC_{50} and E_{max} values for each of these three agents between myometrial tissues isolated from rats in preterm and in term labour ($P>0.05$).

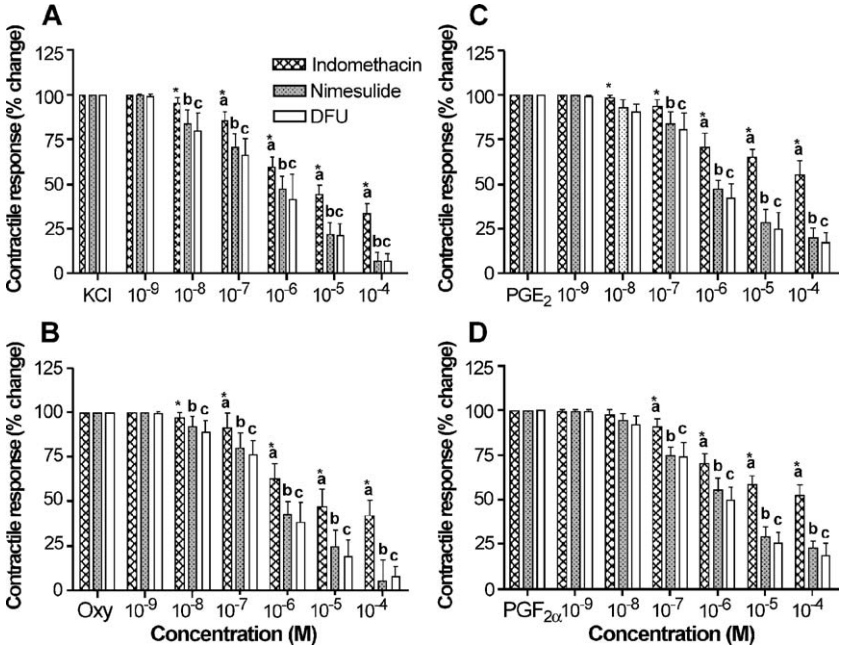

Fig. 4. Effect of DFU (10^{-9}–10^{-4} M), nimesulide (10^{-9}–10^{-4} M) and indomethacin (10^{-9}–10^{-4} M) on the amplitude of KCl (A)-, oxytocin (B)-, prostaglandin E_2 (C)- and prostaglandin $F_{2\alpha}$ (D)-stimulated contractions of myometrial strips isolated from term rats ($n=15$). Data are expressed relative to the control (precontractile response) for each drug. Data represent the mean ± S.E. of 15 experiments. [a]$P<0.05$ vs. controls of DFU. [b]$P<0.05$ vs. controls for nimesulide. [c]$P<0.05$ vs. controls for indomethacin. *$P<0.05$ vs. indomethacin, DFU and nimesulide.

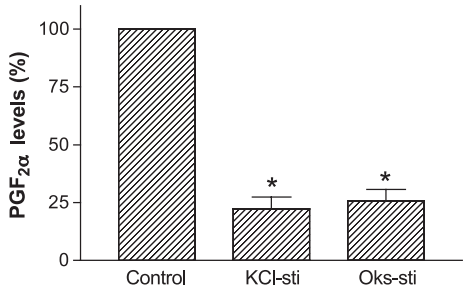

Fig. 5. Decrease (% change) of prostaglandin $F_{2\alpha}$ levels after administration of 10^{-5} M DFU in KCl- and oxytocin-stimulated myometrial strips taken from lipopolysaccharide-injected rats compared with the controls. *$P<0.05$ vs. controls.

Figs. 3 and 4 summarise the data for the effect of increasing concentrations of DFU, nimesulide and indomethacin on KCl-, oxytocin-, prostaglandin E_2- and prostaglandin $F_{2\alpha}$-stimulated contractions of myometrial strips isolated from preterm and term pregnant rats, respectively. DFU (10^{-9}–10^{-4} M), nimesulide (10^{-9}–10^{-4} M) and indomethacin (10^{-9}–10^{-4} M) concentration dependently inhibited the myometrial contractions in all tissues. The inhibitor effect of DFU and nimesulide was similar, but the inhibitor effect of indomethacin was significantly lower than that of DFU and nimesulide in all myometrial tissues.

The levels of prostaglandin $F_{2\alpha}$ after administration of 10^{-5} M DFU on KCl- and oxytocin-stimulated myometrial strips taken from lipopolysaccharide-injected rats are shown in Fig. 5. The decrease in concentration of prostaglandin $F_{2\alpha}$ after administration of 10^{-5} M DFU in KCl-stimulated myometrial strips ($n=4$) was $77.74 \pm 5.20\%$ compared with that of DFU nontreated myometrial strips (control) ($n=4$) ($P<0.05$). The decrease in concentration of prostaglandin $F_{2á}$ after administration of 10^{-5} M DFU in oxytocin-stimulated myometrial strips ($n=4$) was $74.22 \pm 4.90\%$ compared with the control ($n=4$) ($P<0.05$).

4. Discussion

The results of this study clearly demonstrate that DFU, a specific cyclooxygenase-2 inhibitor, is a potent uterine relaxant agent in preterm and term pregnant rat myometrium. In our experiments, we compared the inhibitory effect of DFU with that of indomethacin and nimesulide on KCl-, oxytocin-, prostaglandin E_2- and prostaglandin $F_{2\alpha}$-stimulated contractions of myometrial strips isolated from preterm and term pregnant rats. DFU, nimesulide and indomethacin concentration dependently inhibited the KCl-, oxytocin-, prostaglandin E_2- and prostaglandin $F_{2\alpha}$-stimulated myometrial contractions in all tissues. The E_{max} values for DFU and nimesulide were significantly greater than those calculated for indomethacin in both preterm and term rats. There was no significant difference between the $-\log_{10} EC_{50}$ values of DFU, nimesulide and indomethacin in all tissues. This indicates that the efficacy of DFU and nimesulide is significantly greater than that of indomethacin, but the potency for the three drugs was not significantly different in the preterm and term pregnant rats.

Intraamniotic infection and chorioamnionitis account for as much as 20% of all cases of preterm labour (Romero et al., 1989). Elevated concentrations of interleukin-1, interleukin-6, interleukin-8, tumor necrosis factor alpha, and prostaglandins E_2 and $F_{2\alpha}$ have been found in patients with intra-amniotic infection and preterm labour (Dudley and Trautman, 1994). Additional evidence suggesting a role for proinflammatory cytokines and prostaglandins as mediators of preterm labour comes from animal and in vitro studies. In pregnant mice, systemic administration of recombinant interleukin 1β induced preterm labour that could be prevented by pretreatment with interleukin-1 receptor antagonist (Romero et al., 1992). Rauk and Chiao (2000) demonstrated interleukin-1 induced increases in prostaglandin production by human myometrial cells through increased cyclooxygenase-2 expression. Zuo et al. (1994) observed increases in myometrial cyclooxygenase-2 throughout pregnancy and prior to labour at term. The increases in cyclooxygenase-2 mRNA and protein correlated with immunostaining for prostaglandin E_2 and prostaglandin $F_{2\alpha}$. Uterine tissues express both forms of cyclooxygenase; however, the relative contribution of each to prostaglandin production changes, in both preterm and term labour (Teixeira et al., 1994; Hirst et al., 1998). Several studies have demonstrated a dramatic increase in the inducible form of cyclooxygenase-2 in both preterm and term labour (Hirst et al., 1998; Slater et al., 1998; Skinner and Challis, 1985) These studies suggest that with the onset of preterm and term labour, prostaglandins are produced predominantly by the action of cyclooxygenase-2 in fetal membranes. Rat myometrium may also be a source of cytokine-induced prostaglandins in the setting of preterm labour with infection and term labour.

The expression of cyclooxygenase-2 rather than cyclooxygenase-1 was enhanced in myometrium from women in labour, both preterm and at term (Slater et al., 1999; Zuo et al., 1994) and cyclooxygenase-2 selective inhibitors reduce the production of prostaglandins in myometrium (Poore et al., 1999). It was suggested that the use of selective cyclooxygenase-2 inhibitors to prevent preterm labour may result in effective tocolysis (Slattery et al., 2001; Poore et al., 1999) and may be associated with fewer adverse effects than are nonselective cyclooxygenase inhibitors (Takahashi et al., 2000). Nonselective inhibitors of both cyclooxygenase-1 and -2 such as indomethacin have been used as tocolytic agents since the mid-1970s. However, the adverse side effects of these agents, particularly their effects on the fetal ductus arteriosus and fetal renal function, have limited their use for preterm labour (Besinger et al., 1991). Recently, selective cyclooxygenase-2 inhibitors have been used in the management of preterm labour in the hope of avoiding fetal complications. Celecoxib, the selective cyclooxygenase-2 inhibitor,

is reported to increase pressure and resistance of fetal lamb ductus arteriosus (Takahashi et al., 2000), and a large amount of Celecoxib (600 mg/kg) induces contraction of murine ductus arteriosus at near term (Reese et al., 2000). On the other hand, the selective cyclooxygenase-2 inhibitor, 5-bromo-2-(4-fluorophenyl)-3-(4-methylsulfonyl)thiophene (DuP697), did not affect ductal patency in the newborn pig (Guerguerian et al., 1998). DFU was identified as a novel orally active and highly selective cyclooxygenase-2 inhibitor (Riendeau et al., 1997). There are no data in the literature related to the constrictor effect on the fetal ductus arteriosus and the in vitro tocolytic effect of DFU on preterm and term pregnant rat myometrium. Therefore, in another part of our study, we compared the constrictor effects of DFU and indomethacin on the rat ductus arteriosus.

The risk of indomethacin to the fetal ductus arteriosus is reported to increase with gestational age (Moise, 1993). In our study, the fetal ductus arteriosus was significantly constricted on administration of 10 and 100 mg/kg DFU and indomethacin compared with the control in preterm rats. In term fetus, the constriction after administration of either DFU or indomethacin was very high compared with that observed in the preterm fetus. The constrictor effect of indomethacin was significantly greater than those calculated for DFU in preterm and term fetuses, suggesting that DFU might be safer for the fetal ductus arteriosus than indomethacin. Therefore, careful attention seems to be necessary when giving DFU to preterm labour patients.

To evaluate the mechanism by which DFU prevents preterm labour in rats, we measured the concentration of prostaglandin $F_{2\alpha}$ in myometrial tissue, since prostaglandin $F_{2\alpha}$ has previously been shown to be a major prostaglandin in myometrial tissue during labour (Romero et al.,1989). In this study, the concentration of prostaglandin $F_{2\alpha}$ on administration of DFU (10^{-5} M) was significantly decreased in KCl- and oxytocin-stimulated myometrial strips obtained from lipopolysaccharide-injected preterm rats. The reduction in prostaglandin $F_{2\alpha}$ concentration with DFU on KCl- and oxytocin-stimulated myometrial strips was $77.74 \pm 5.20\%$ and $74.22 \pm 4.90\%$, respectively, compared to the control. From these results, we speculate that the mechanism by which DFU prevents lipopolysaccharide-induced preterm delivery in rats could be due to, at least in part, inhibition of the production of prostaglandin $F_{2\alpha}$ in myometrial tissue. The earlier finding that prostaglandin $F_{2\alpha}$ opposed the inhibitory effect of indomethacin (Garrioch, 1978) supports a role for exogenous prostaglandins in myometrial contraction, but not necessarily for prostaglandins produced by the myometrium itself. Prostaglandin $F_{2\alpha}$ causes the release of Ca^{2+} into the cell cytoplasm through activation of the FP receptors (Griffin et al., 1998), so it seems likely that this effect of prostaglandin $F_{2\alpha}$ offsets the inhibition of cellulary membrane Ca^{2+} channels by DFU.

Prostaglandins are potent uterotonic agents, but it is possible that cyclooxygenase-1 and cyclooxygenase-2 inhibitors exert their uterine relaxant effect by mechanisms other than inhibition of prostaglandin synthesis. In addition to suppressing the arachidonic cascade, indomethacin could inhibit uterine contractility through other mechanisms, although for most other effects, the median effective dose is generally much higher than that for cyclooxygenase inhibition (Beatty et al., 1976). Chief among these potential effects is a potent inhibitory effect on myometrial cyclic adenosine 3,5-monophosphate phosphodiesterase, thus increasing tissue cyclic adenosine monophosphate levels (Beatty et al., 1976) and direct inhibitory effects on voltage-gated Ca^{2+} channel currents in human myometrial cells (Sawdy et al., 1998). Previous reports suggest that nimesulide may have another mechanism of action. Sawdy et al. (1998) have demonstrated that both nimesulide and indomethacin reduced the Ca^{2+} channel current in human myometrial myocytes at concentrations that inhibited contractility. It was suggested that Ca^{2+} antagonism may be one of several mechanisms of action for cyclooxygenase-1 and cyclooxygenase-2 inhibitors, including the inhibition of prostaglandin synthesis.

An increase in the intracellular Ca^{2+} concentration plays an essential role in the generation of smooth muscle contractions. An influx of extracellular Ca^{2+} into the intracellular space via voltage-dependent Ca^{2+} channels and the subsequent release of Ca^{2+} from intracellular sites may both be involved in this process. Activation of voltage-dependent Ca^{2+} channels is thought to be mainly responsible for the generation of both spontaneous and KCl-induced myometrial contractions. On the other hand, in myometrial tissues, the action of oxytocin is due to the release of Ca^{2+} from storage sites by the production of inositol triphosphate (IP_3) or by the activation of receptor-operated Ca^{2+} channels (Kawarabayashi et al., 1997; Marc et al., 1986). Thus, KCl-induced contractions are assumed to be due to the voltage-dependent Ca^{2+} influx, and oxytocin-induced contractions are thought to be related to the pharmaco-mechanical coupling, which consists of activation of the receptor/phosphoinositide–phospholipase C (PI-PLC) pathways and Ca^{2+} release from storage sites by IP_3. In contrast, prostaglandin E_2 and prostaglandin $F_{2\alpha}$ produce myometrial contractions primarily by stimulating intracellular sites to release Ca^{2+} (Davis et al., 1987; Yang et al., 1997). In our study, DFU, nimesulide and indomethacin inhibited KCl-, oxytocin-, prostaglandin E_2, prostaglandin $F_{2\alpha}$-induced myometrial contractions. Inhibition of exogenous prostaglandin E_2- and prostaglandin $F_{2\alpha}$-induced myometrial contractions by DFU, nimesulide and indomethacin suggests that their effects could be independent of cyclooxygenase inhibition and may be due to a decreasing intracellular Ca^{2+} concentration.

In conclusion, our findings suggest that DFU, a potential tocolytic agent, may have a dual mechanism of action on inhibiting uterine contractility; through Ca^{2+} channel blocking and/or through inhibition of prostaglandin synthesis. However, careful attention should be paid

to the fetal ductus arteriosus when using DFU preterm labour therapy. The exact mechanisms of relaxation of cyclooxygenase-2 inhibitors will therefore be the subject of further research.

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