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The Effect of Danofloxacine on *in vitro* Rat Myometrium

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ABSTRACT

The aim of this study was to determine the effect of danofloxacine on *in vitro* rat myometrium. The myometrium (n=60) obtained from adult female rats. After myometrium showed spontaneous contractions, the contractions were regulated by injecting 0.1mM oxytocin. Then, 5, 10, 20, 40 and 80 µmol danofloxacine was added to isolated organ bath in 10 min intervals, and frequency, peak amplitude and peak area calculations were recorded. With oxytocin supplementation, frequency, peak amplitude and peak area calculations were determined as 6.7±1.0, 1874±107, 1749±68, respectively. With danofloxacine supplementation, frequency and peak amplitude first increased then slowed down in relation to the dose and the peak area decreased in relation to the dose ($P<0.05$). In conclusion, by adding danofloxacine to the media after contractions caused by an oxytocin treatment, we observed biphasic activity. The effects of danofloxacine high doses (40 and 80 µmol) may inhibit both K^+ channels and the intracellular-induced mechanism.

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INTRODUCTION

Danofloxacine is a synthetic fluoroquinolone antimicrobial drug. Fluoroquinolones are synthetic antibiotics which are commonly used in human and veterinary medicine (Shen *et al*., 2004). It is known that fluoroquinolones are effective on both Gram-negative and Gram-positive bacteria in animals as well as mycoplasmas (McGuirk *et al*., 1992). These are bactericidal, have broad spectrum of activity and act by concentration-dependent killing mechanism (Sarasola *et al*., 2002; AliAbadi and Lees, 2003). This group of antibiotics is widely used especially in urogenital system, respiratory and digestive system infections in cattle, sheep and poultry animals (Huebschle *et al*., 2006). Danofloxacine exhibits its pharmacologic influence by preventing the effect of DNA gyrase (topoisomerase II) enzyme in bacteria. It exists in the form of metanosulphonate salt and dissolves quite well in water. In terms of its general characteristics, it shares the common characteristics of fluoroquinolone (Tagaya *et al*., 1995; Becker *et al*., 2001).

Fluoroquinolone has a number of side effects; amongst these important are neuro and cardiotoxicity. For the neurotoxicity (epileptogenic effect) one of the proposed mechanism is related to the antagonism of GABA-A receptor, with the consequent decrease in chloride conductance, although quinolones binding to its receptor is weak and cannot properly explain their epileptogenic properties (Akahane *et al.,* 1989). Anderson *et al*. (1989) reported that effect of fluoroquinolone is related to the blockade of the cardiac potassium channel HERG (Anderson *et al.,* 2001). It is known that fluoroquinolone group of drugs act as GABA antagonist in the organism which blocks K channels connected to ATP (Ito *et al.,* 1993; Becker *et al.,* 2001). A second mechanism related to the activation of the NMDA receptor by abolishing the Mg_2^+ block in the ion channel has been proposed (Akahane *et al*., 1989).

The effects of fluoroquinolone on smooth muscles in various animals were analyzed (Tagaya *et al.,* 1995; Di Nucci *et al*., 1998). Di Nucci *et al*. (1998) studied the effect of norfloxacin and enrofloxacin on isolated rat ileums and reported that it had a biphasic effect. It was observed that in dog respiratory tracts, the smooth muscles first contracted and later the contractions decreased with the injection of ciprofloxacin in relation to the dose. The purpose of this study was to determine the effect of danofloxacine on rat uterus contractions in *in vitro* conditions.

MATERIALS AND METHODS

The experimental protocol used was approved by the Department of Animal Care and Use Committee of the Turkish Ministry of Agriculture and adhered to the European Community Guiding Principles for the Care and Use of Animals. In the present study, 30 Wistar (200-220 g) non-pregnant female rats were used. The rats were procured from the Animal House of Faculty of Medicine, Firat University. The rats were killed with cervical dislocation and their uteri were removed. Myometrium layers were removed using Krebs solution (NaCl 118 mM, KCl 4.69 mM, $MgSO_4$ 0.6 mM, $NaHCO_3$ 25 mM, $CaCl_2$ 2.5 mM, Glucose 11.1 mM), and 2 muscle samples were taken from each uterus. The isolated organs bathed in isometric transducer (Harward Apparatus, Limited, Harward, England).

The serosa and endometrium layers of uterine cornua taken from non-pregnant rats and these layers were separated. Cross sections of 10 x 2 x 2 mm were taken from the myometrium muscle layer and treated. The treatments were carried out in Krebs solution which was prepared afresh daily. The uteri that had any risk of infection were excluded from the experiment. Muscle cross sections were connected to the hook at the bottom of the isolated organ bath with 20 ml-capacity, containing Krebs solution which was continuously supplied with CO_2 (5%) + O_2 (95%). Its upper part was fixed on a micrometric player and the other part was connected to a transducer which was connected to a computer. After the muscle was hung, a 1 g voltage was applied and it was calibrated in the calculations. This continued until the muscle adapted to this contraction (60-120min). During this waiting period, the muscle was washed with a fresh solution every 20min. At the end of the waiting period, after spontaneous contractions and contractions caused by oxytocin (0.01mM; Oksitosin 5IU/ml, Vetas, Turkey), danofloxacine @ 5, 10, 20, 40 and 80µmol were added. Oxytocin and danofloxacine effects on contrac- tions were analyzed. Activity changes in myometrium muscles were evaluated using a computer program during the experiments. The values before the application were accepted as control values. This study was performed according to the methods described by Wray (1993) and Ayar (2007).

Measurements in all experiments were made at 10 min intervals. The period before and after the application were compared in terms of peak amplitude and frequency. The distance between the basal line in the amplitude calculation and peak point of contraction was measured in mm units and taking the calibration used in that experiment into account, its gram unit value was calculated.

Statistics analysis

Statistical analysis was carried out by using the SPSS for Windows, ver.10 (SPSS Inc., Chicago, IL, USA). Differences between means were established by analysis of variance, (ANOVA) with $P<0.05$ considered significant (SPSS, 10.0, 2000).

RESULTS

Frequency number of rat myometrium which was stimulated with oxytocin was 6.7 after adding 5 µmol danofloxacine, no difference was seen; but as the dose was increased, the frequency number also significantly increased ($P<0.05$). With 80 µmol dose, the frequency number significantly decreased to 4.7 ($P<0.05$, Table 1). The peak amplitude in oxytocin-treated myometrium, 5 and 20µmol danofloxacine treatment, was not significantly different to 1874, 1897 and 1823g, respectively. 10 µmol danofloxacine treatment, it was significantly increased to 2139g ($P<0.05$), 40 and 80µmol danofloxacine was significantly decreased to 850 and 337, respectively ($P<0.05$). The peak area was 1749 in oxytocin injected samples, and it was significantly decreased ($P<0.05$) in relation to the increasing dose of danofloxacine (Table 1).

DISCUSSION

In mammals the uterus is relatively quiescent during 95% of pregnancy. Local, maternal, mechanical, or fetal stimuli cause transition of the myometrium from a quiescent to an active state at term, and generate synchronous high-frequency, high-amplitude contractions resulting in parturition. By this process the fetus is expelled from the uterus to the extra uterine environment, which also involves coordinated cervix dilation in a manner that allows passage of the fetus through the birth canal (Wray, 1993; Lye *et al*., 1998). Pharmacological control of myometrial contractions is of importance not only for understanding and modulation of normal parturition but also for interventions to cause preterm delivery and to hasten post partum involution of the uterus. The process of normal uterine involution, usually completed within 4–5 weeks postpartum, involves the expulsion of lochia and the reduction of uterine size. If uterine involution is delayed, this can result in bacteria and fluids persisting in the lumen of uterus. Ideally, the postpartum period is a non-infectious event. But, uterine infection, contamination of the uterus with pathogenic organisms, is a major problem in reproductive management that reduces the reproductive efficiency of cows and the profit potential of dairy farms (Ocal *et al*., 2004). Uterine infections often occur after obstetric aid and retained fetal membranes in the cow. Postpartum metritis, a severe and occasionally life-threatening bacterial infection of the uterus, is a common disease of dairy cows that usually occurs within 7 days postpartum. It has been associated with serious delays of uterine involution, leading to a delay in the resumption of ovarian activity. As a consequence, the fertility of the cow is adversely affected (Mateus *et al*., 2002) and an intervention is required. Antibiotics are still commonly used as a therapy in puerperal metritis, pyometra and endometritis, fetal membrane retention and in repeat breeders. The broad-spectrum of fluoroquinolone has been widely used in treatment of infectious diseases including uterine infections in dairy animals (McGuirk *et al*., 1992).

Although some important pharmacological features of fluoroquinolone such as intrusion to tissues in vivo

Table 1: The effect of different doses of danofloxacine on frequency, peak amplitude and peak area on oxytocin-induced *in vitro* rat myometrium

Drug/Dose	Frequency (number/10min)	Peak amplitude (g)	Peak area (mm^2)
Oxytocin (0.1 mM)	6.7 ± 1.0	1874 ± 107	1749 ± 68
Danofloxacine (μmol)			
5	6.8 ± 1.2[a]	1897 ± 80[a]	1685 ± 70[a]
10	7.7 ± 1.5[b]	2139 ± 119[b]	1505 ± 102[b]
20	10.1 ± 1.6[c]	1823 ± 145[a]	948 ± 115[c]
40	12.1 ± 1.7[c]	850 ± 164[c]	451 ± 84[d]
80	4.7 ± 1.0[d]	337 ± 115[d]	230 ± 104[e]

Values with different letters in a column differ significantly (P<0.05).

conditions and remaining levels are adequately studied, there is limited information on their adverse effects on uterus myometrium especially in *in vitro* conditions. It was shown that uterus contractions occur spontaneously or with a stimulant (oxytocin, prostaglandins etc.) in an *in vitro* environment (Downing *et al*., 1988). It was reported that spontaneous contractions occur depending on calcium and by a pacemaker like formation seen in the heart muscle of which anatomic location is not exactly known (Wray 1993). In voltage induced contractions, however, it was determined that the intrusion of Ca in to cells was effective in the formation of depolarization (Zünkler and Wos, 2003), whereas K channels were mediating repolarization (Wray, 1993). Uterus contractions in our study in *in vitro* conditions are in line with the contraction types reported by Wray (1993).

It was reported that the fluoroquinolone group of antibiotics block DNA gyrase enzymes and show antibacterial effects; and as a GABA antagonist in the organism, they block ATP-dependant potassium channels (Ito *et al*., 1993). Zünkler and Wos reported that among fluoroquinolone, lomefloxacin and norfloxacin blocked ATP- sensitive K+channels in B cells in the pancreas. Additionally, that fluoroquinolone caused dose-dependant blockage of K+ channels and increased insulin secretion (Saraya *et al*., 2004)

The effects of fluoroquinolone on different smooth muscles in *in vitro* conditions were analyzed. Tagaya *et al.* (1995), investigated that by adding ciprofloxacin (2 x 10^{-3} M) in a dog's respiratory system smooth muscles in an *in vitro* environment increased myometrium contractions, and this may be due to repolarization/ hyperpolarization. Ito *et al*. (1993) reported that in isolated dog and rabbit artery smooth muscles ciprofloxacin and ofloxacin completely repressed the contractions caused by phenylephrine and norepinephrine. Di Nucci *et al.* (1998) investigated the effects of norfloxacin and enrofloxacin on isolated rat ileums. They reported that fluoroquinolone injection resulted in a biphasic effect in the contractions in ileums followed by relaxation. It's inhibitory or stimulant effects on smooth muscles of rat intestine may be caused by the presence of GABA, prostaglandin and ATP in the environment (Di Nucci *et al*., 1998).

In our study, by adding danofloxacin to the media after contractions caused by an oxytocin treatment, we observed biphasic activity. It was observed that danofloxacin significantly increased (P<0.05) the frequency and peak amplitude of the contractions at low doses. It was also noted that the peak area decreased significantly in relation to the increased dose of danofloxacin. After the highest dose of 80μmol danofloxacin was applied, a blockage of hyperpolarization type occurred. As the above mentioned researchers (Ito *et al*., 1993; Di Nucci *et al*., 1998; Anderson *et al*., 2001; Saraya *et al*., 2004) concluded, it is believed that these effects caused by danofloxacin in isolated rat uterus could have been due to the inhibition of K+ channels.

In conclusion, by adding danofloxacine to the media after contractions caused by an oxytocin treatment, we observed biphasic activity. The effects of danofloxacine high doses (40 and 80μmol) may inhibit both K^+ channels and the intracellular-induced mechanism. Nevertheless, for a better explanation on the subject *in vitro* and *in vivo*, further investigations are needed.

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