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Nitrofurantoin inhibits contractions of myometrium isolated from pregnant and nonpregnant rats

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Abstract

We aimed to investigate the effects of nitrofurantoin, a commonly used antibiotic for urinary tract infections, on spontaneous contractions of rat myometrium isolated from 16-day pregnant and nonpregnant rats. Myometrial strips were suspended in a standard organ bath and after the manifestation of spontaneous contractions under 1 g of resting tension, nitrofurantoin was applied to the organ bath as 50, 250 and 500 µM doses. Amplitude and frequency of contractions were recorded for 20 minutes before and after application of the drug. The bath was washed to remove the drug from the medium after recording the effect of each dose and the contractions were enrolled again to show the reversible effect of the drug. The effects of nitrofurantoin on amplitude (milligrams) and frequency of spontaneous contractions were evaluated. Data were statistically analyzed using the Student's t test and $p<0.05$ was considered to be statistically significant. Nitrofurantoin inhibited spontaneous contractions in a dose dependent manner. Although both the frequency and amplitude of contractions were significantly inhibited at all doses, the inhibition of contractions were 100% at 500 µM dose, which corresponds to the dose used for prophylaxis in clinical practice. The results of this in vitro study indicated that nitrofurantoin inhibits spontaneous contractions of myometrium isolated from pregnant and nonpregnant rats.

Keywords: Nitrofurantoin, myometrium, contraction

Introduction

Preterm birth is defined by WHO as births occurring before 37 completed weeks or 259 days of gestation since the first day of woman's last menstrual period [1]. Despite the great advances in medicine, it is still a significant perinatal health problem all over the world, not only in terms of associated mortality but also in terms of associated short and long term morbidity resulting in enormous physical, psychological and economic costs [2,3]. Events leading to preterm birth are still not completely understood but it is thought that the etiology is multifactorial. Infections play an important role in preterm birth. Approximately 40 percent of preterm deliveries are thought to be the result of infection [4]. Although it is known that many infections including gonococcal cervicitis, bacterial vaginosis, HIV, malaria are associated with increased risk of preterm birth, urinary tract infections gather the greatest interest since they are the most common bacterial infections during pregnancy [5]. It is known that urinary tract infections are not only associated with preterm delivery but also increase the risk of low birth weight infant and perinatal mortality [6]. So specific interventions for early detection, treatment and prevention of recurrence are taken during pregnancy. Nitrofurantoin is an agent commonly used for these purposes [7].

The aim of this study was to investigate the effect of this commonly used antibiotic on myometrium isolated from pregnant and nonpregnant rats. To our knowledge, this is the first study in the literature investigating the effects of nitrofurantoin on contractility.

Material and Method

The study protocol was approved by the Ethics Committee and the study was carried out in the Laboratory for Animal Experimentation, Firat University, Elazig, Turkey. A total of 24 female Spraque Dawley rats, aged 10-12 weeks and weighing between 200-250 g were used. During the study period, rats were kept in seperate cages, each containing five rats. The rats were maintained on a 12-hour light and 12-hour dark regimen and fed at libitum with free access to food and water until the end of the study. Cytologic smears were done daily to document estrous cycle and rats in the estrus phase were kept in separate cages with a male rat. Vaginal smears were done the next morning and those rats who exhibited sperms in their smears were accepted as pregnant and that day was accepted as the first day of pregnancy. Rats in the diestrus phase constituted the non-pregnant group in the experiment. Study was done on two group of animals; Group 1 constituted 12 rats in diestrus phase and Group 2 consisted 12

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pregnant rats whose pregnancy were terminated at the 16th day. Two longitudinal uterine smooth muscle strips measuring 12x2x1 mm were prepared from each rat and 8 strips were used for each drug dose in each group. One end of the strip was tied to a fixed hook with surgical silk and the other end to an isometric force transducer. It was suspended in a standard organ bath that contained Krebs-Henseit solution (composed of 118 mM/lt NaCI, 4,7 mM/lt KCl, 1,2 mM/lt MgSO4, 2,5 mM/lt CaCl2, 1,2 mM/lt KH2PO4, 25 mM/lt NaHCO3, 11 mM/lt glucose, EDTA 0.003 mM/lt) and was maintained at 37oC and aerated with a mixture of 95% 02 and 5% CO2. Isometric contractions of the muscle strips were monitored under constant passive force of 1 g. Using physiologic power converter (FDT05, Commat Ltd, Ankara, Turkey) contractions were enrolled by MP150 WS Windows program.

The agent used in the study was nitrofurantoin (Biofarma, Istanbul, Turkey). It was dissolved in required amounts before application into the organ bath. The ingredients of Krebs-Henseit solution were obtained from Sigma (Disenhofen, Germany)

Following the stabilization of spontaneous contractions in approximately 60 minutes, contractions were recorded for 20 minutes which constituted the control values. 50 µM nitrofurantoin was added to the organ bath and contractions were again recorded for 20 minutes. The bath was washed thereafter to remove the drug from the medium and the contractions were enrolled again to show the reversible effect of the drug. This procedure was repeated for 250 and 500 µM doses and eight strips were used for each dose. The washing of the bath after each procedure prevented the formation of cumulative dose.

Results of frequency are recorded in terms of contractions per 20-min period and results for amplitude (maximum extent of contraction, measured from the position of equilibrium) are reported in terms of milligrams (mg). The effects of different doses of nitrofurantoin on frequency and amplitude were analyzed for 20-min periods for each tissue. The mean frequency and amplitude of contractions were calculated and compared.

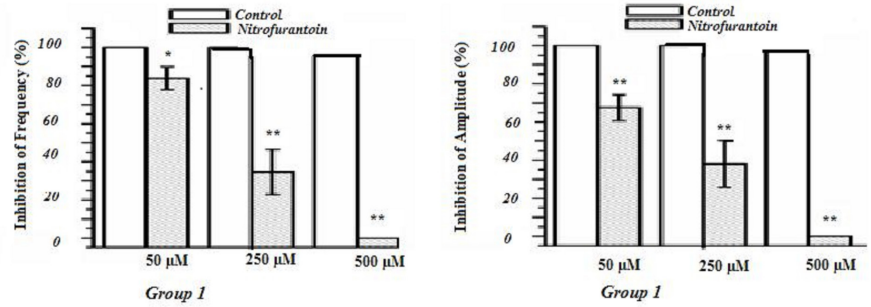
All values are expressed as means±SEM. All statistical analysis are performed using the statistical programme SPSS for Windows (version 21.0.1, SPSS, Inc. Chicago, Illinois).

Data were statistically analyzed using the Students' t -test. $P<0.05$ was considered to be significant.

Results

A total of 48 myometrial strips were used in the study. The tissue samples were embedded in the organ bath by applying 1 g. of resting tension and nearly 60 minutes after the insertion of muscle strips spontaneous contractions were regulated. Nitrofurantoin were added to the organ bath at 50, 250 and 500 µM doses after the regulation of spontaneous contractions.

In Group 1, application of nitrofurantoin at non-cumulative doses as 50, 250 and 500 µM inhibited the frequency of contractions 16.2% (p=0.030), 65.4% (p=0.001) and 100% (p=0.001) respectively. Inhibition in amplitudes of contractions were as 32.5% (p=0.02), 62% (p=0.001) and 100% (p=0.001) respectively in those doses (Figure 1).



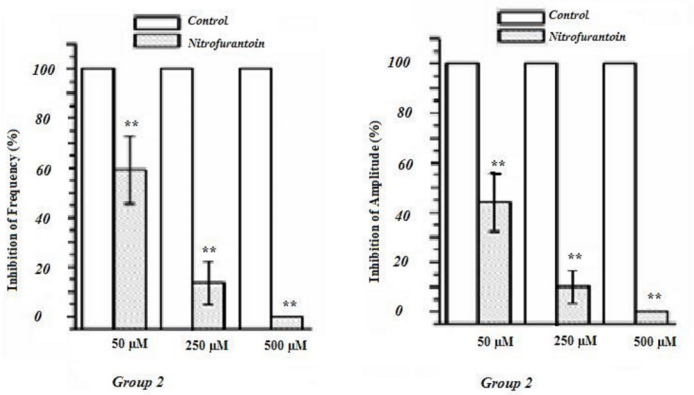
*: p<0.05; **: p<0.001

Figure 1. Dose dependent inhibition of frequency and amplitude in Group 1

In Group 2, non-cumulative application of 50, 250 and 500 µM doses of nitrofurantoin inhibited the frequency of contractions 41% (p=0.22), 86,5% (p=0.001) and 100% (p=0.001). Inhibition of the amplitudes of contractions in this group were as 56% (p=0.02), 90% (p=0.01) and 100% (p=0.001) respectively. (Figure 2)

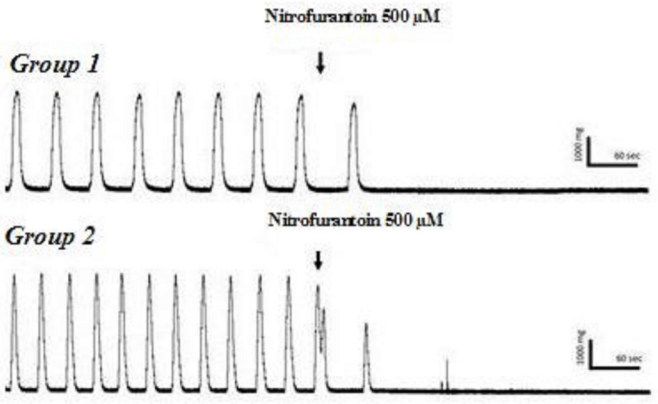
The application of nitrofurantoin at non-cumulative doses as 50, 250 and 500 µM inhibited both the frequency and amplitude of contractions in statistically significant amounts in both groups. In 500 µM doses, which corresponds to the dose used for prophylaxis in clinical practice, inhibition at frequency and amplitude were %100 in both groups. (Figure 3)

It is observed that inhibition in frequency and amplitude are more prominent in the pregnant group. Return of contractions after removal of the drug showed us the reversible character of inhibition by the drug.



***: p<0.05; **: p<0.001**

Figure 2. Dose dependent inhibition of frequency and amplitude in Group 2



*: p<0.05; **: p<0.001

Figure 3. Representative recordings demonstrating contractions before and after application of 500 µM nitrofurantoin in Group 1 and Group 2

Discussion

Urinary tract infections are the most common bacterial infections encountered during pregnancy. It is classified by the site of bacterial proliferation as asymptomatic bacteriuria, cystitis and pyelonephritis. Asymptomatic bacteriuria is commonly defined as the presence of at least 100,000 organisms in 2 consecutive urine samples in the absence of declared symptoms. Although pregnancy does not increase the incidence of asymptomatic bacteriuria which is approximately 2 to 7 percent among women, screening and treatment of it becomes necessary during pregnancy [8]. Physiological and anatomic changes causing urinary stasis in pregnancy increases the risk of clinical disease. Asymptomatic bacteriuria will go on to develop pyelonephritis in up to 40% of cases if left untreated [9]. Pyelonephritis is a serious medical condition in pregnancy and it has been estimated that as many as 20 percent of women with severe pyelonephritis develop complications that include septic shock syndrome or its variants, such as acute respiratory distress syndrome [10]. Endotoxins and fever increases uterine activity and can cause preterm birth. Fever in the first trimester can be teratogenic and can cause miscarriage [11]. Infectious Diseases Society of America recommend screening of all pregnant women for asymptomatic bacteriuria at least once in early pregnancy [12]. Because of the risk of recurrent or persistent bacteriuria after completing the treatment, urine cultures are repeated regularly until the completion of pregnancy [13]. Suppressive therapy with low dose of nitrofurantoin every night (50-100 mg.) is recommended for women who experienced pyelonephritis once in pregnancy or for whom recurrent or persistent asymptomatic bacteriuria is observed.

Nitrofurantoin is a synthetic nitrofuran and was first approved for use in the United States in 1953. It is still among the first line drugs in treatment of urinary tract infections and is the most frequently prescribed antimicrobial agent for urinary tract infection among obstetrician-gynecologists [14]. It is bactericidal against uropathogens. Antibacterial resistance to nitrofurantoin is rare, which makes it an attractive choice for long term treatment. The limitation of nitrofurantoin is its poor activity against Proteus species. Nitrofurantoin also may incite hemolytic anemia in patients who have glucose-6-phosphate dehydrogenase deficiency. It can rarely cause hepatotoxicity, interstitial pneumonitis, lupus like syndromes, peripheral neuropathy and exfoliative dermatitis.

We have searched and not found any study done about the effect of that a commonly used agent, nitrofurantoin, on uterus contraction. Our study showed that inhibitory effect of nitrofurantoin was dose dependent and inhibition was %100 at 500 µM which is the dose commonly used in clinical practice.

We also studied the effect of this drug on nonpregnant rat uterus because artificial reproductive techniques are more commonly used nowadays and it is known that for a successful embryo implantation uterine quiescence is very important [15].

Myometrial contractions are important in many aspects of obstetrics and gynecology practice so effects of many antibiotics on myometrial contractions have been studied for many years. The finding of the inhibitory effect of erythromycin on rat myometrial contractions was the starting point of many studies including ours [16]. Dose dependent inhibitory effect of claritromycin in isolated human myometrium was observed by Celik et al [17]. Not only antibiotics but also other drugs have been studied in respect to their effects on myometrial dynamics. It has been shown that human menopausal gonadotropin and highly purified urinary FSH supress the myoelectrical signals and provides uterine quiescence [18]. It was also demonstrated that estrogen, progesterone and hCG reduce the frequency of uterine contractions [19].

The current study has limitations. First of all, it is an in vitro study and these results must also be confirmed by in vivo studies. New studies must can be designed to clarify its mechanism of action. Also possible additive effect of nitrofurantoin to tocolytics may be subject to further studies.

Conclusion

The results of this study demonstrates that nitrofurantoin is an agent that inhibits spontaneous contractions in both pregnant and nonpregnant rat uterus. This is an important property since nitrofurantoin is an agent commonly used for treatment and prophylaxis of urinary tract infection. This feature may make it superior over other antibiotics used for urinary tract infections in pregnancy and in those planning pregnancy and may be important in convincing the patients for to use the drug until the end of pregnancy. The reversible character of inhibition is also important since it can be interpreted as successful labour can be possible after discontinuation of the drug.

This study was presented in the 25. European Congress of Obstetrics and Gynecology in May 2017, in Turkey.

Competing interests
The authors declare that they have no competing interest.

Financial Disclosure
The financial support for this study was provided by the investigators themselves.

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