

ORIGINAL RESEARCH

Medicine Science 2019;8(1):192-6

The effects of nitrofurantoin on rat urinary bladder contraction

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Received 18 October 2018; Accepted 11 January 2019

Available online 08.02.2019 with doi:10.5455/medscience.2018.07.8987

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Abstract

Nitrofurantoin is a drug used in the antibacterial treatment of urinary tract infections (UTIs) for over 60 years. The aim of our study was to investigate the possible effects of nitrofurantoin, which is commonly used as a urinary tract antiseptic, on bladder contractions in male rats. Bladder tissues obtained from male Sprague-Dawley rats were used in the study (n=24). After decapitation, bladder tissues were suspended in an isolated organ bath of 5 ml containing a Krebs-Ringer bicarbonate solution by applying a tension of 1.5 grams. Nitrofurantoin was administered to three groups at doses of 50, 500 and 1000 µM, respectively. The area, peak-to-peak (p-p) and frequency values of bladder contractions were analyzed before and after administration of nitrofurantoin. The data obtained from the analysis were evaluated using the Paired T-Test in the IBM SPSS Statistics Software. Nitrofurantoin was observed to have an inhibitory effect on bladder contractions at all doses. The decrease in the area and peak-to-peak values was statistically significant at all doses (P<0.05). It has been observed that nitrofurantoin inhibited bladder contractions. Based on these findings, it is thought that the fact that frequent urination, which is commonly seen in UTIs, is reduced after nitrofurantoin therapy may be due to the inhibitory effect of nitrofurantoin on bladder contractions.

Keywords: Contraction, nitrofurantoin, tissue bath, urinary bladder

Introduction

Urinary tract infections are the most common bacterial infection in all age groups [1]. While *Escherichia coli* (*E. coli*) is responsible for 80% of the UTI cases, other organisms (e.g., *Klebsiella*, *Enterobacter*, *Pseudomonas*, *enterococci*, *staphylococci*) can lead to UTIs [2]. The most common symptoms are dysuria, polyuria, pollakiuria, suprapubic discomfort, and flank pain. The most important diagnostic criterion is the presence of clinical symptoms [3]. Until urine culture results are obtained, broad spectrum antibiotics such as penicillins or beta-lactams, cephalosporins, fluoroquinolones and carbapenems can be started empirically [3]. The treatment option can be changed according to urine culture results [4]. However, nitrofurantoin is still a first-choice drug in the antibacterial treatment of urinary tract infections for over 60 years in the world [5,6].

Nitrofurantoin is a broad-spectrum bactericidal antibiotic. It is used effectively in the treatment of UTIs caused by *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Enterococcus* spp. and *Staphylococcus aureus*. It exhibits antimicrobial activity by both interfering the synthesis of proteins embedded in the cell wall and the DNA synthesis of Gram-positive and Gram-negative bacteria [7]. Moreover, another cause of recurrent UTI is urinary incontinence. Urinary incontinence develops due to anatomic disorder associated with vaginal delivery [8] and postmenopausal estrogen deficiency [9] in women and due to benign prostatic hypertrophy (which is more common among older) in men [10]. In addition, recurrent UTI in both genders is one of the major causes of urinary incontinence [11]. In other words, urinary incontinence and recurrent UTI cause each other. One of the most important reasons for this is that hyperreflexia occurs in the external urethral sphincter and the detrusor muscle during UTI. This also causes urinary incontinence. While this situation is improved with the treatment of UTI, recurrent UTI may lead to permanent hyperreflexion in the external urethral sphincter and the detrusor muscle [12]. This situation creates a serious social problem and negatively affects people's social lives.

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To cope with this situation, they have to constantly use urine pads. This also constitutes another source of infection depending on the hygiene of urine pad, as well as adversely affecting the quality of life of persons [13]. However, it has been focused on an effective antibacterial treatment in fighting with UTI for years. Bladder hyperactivity caused by UTI and pathological and psychological problems associated with this have been ignored.

The major problem in the treatment of UTI is that the multiple antibiotic resistance occurs as a result of frequent prescription of antibiotics due to recurrent UTI [14]. As antibiotic resistance develops, treatment response decreases and infection recurs [15]. This creates a severe vicious cycle in the treatment and prevention of UTIs. However, nitrofurantoin is an oral antibiotic that plays a key role in the treatment of acute uncomplicated cystitis (AUC) caused by multidrug-resistant gram-negative bacilli. This makes it superior to other antibiotics [16]. Moreover, recent studies have shown that the use of prophylactic nitrofurantoin as a single oral dose for 6-12 months in women with recurrent urinary tract infection reduced the frequency of recurrent urinary tract infection [17]. All these properties are the main reasons why nitrofurantoin is a first-line drug in the treatment and prophylaxis of UTI. However, the effect of this drug, which is so effective in UTIs, on the changes in bladder contraction due to infection is unknown [18].

The contraction of the smooth muscle of the bladder is regulated partly by the purinergic signal. It occurs when extracellular purines like ATP and UTP are released in neuromuscular junctions as neurotransmitters or as a response to environmental stress from somatic cells and when they bind to P2X (1-7) receptors [19]. In studies conducted so far, the effect of the ADP on bladder contraction has been focused on especially for the activity of P2X1 signaling pathway. However, in recent studies, it has been claimed that the P2Y signaling pathways of the ADP might also be employed; and studies have been conducted to determine which sub-type of the P2Y receptor family is effective (Figure 1) [20-22].

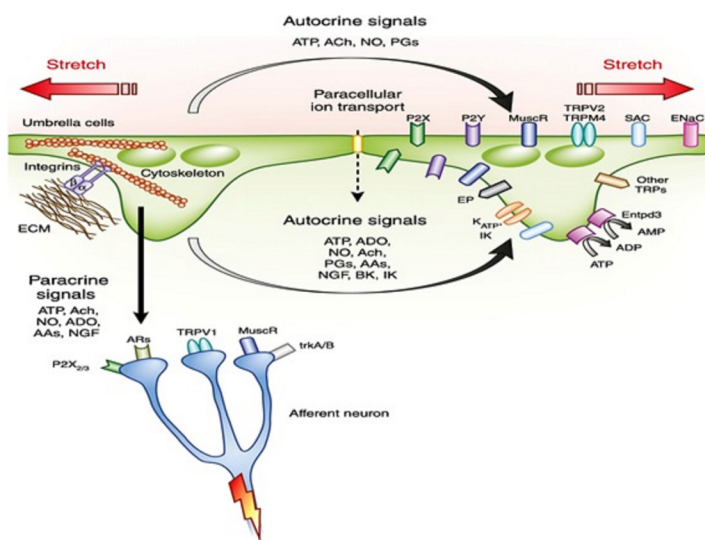


Figure 1. Mechanically induced signaling from the urothelium in response to membrane stretch. Release of a number of potent mediators occurs in response to urothelial stretch eliciting both autocrine- and paracrine-mediated effects. AA, adrenergic agonist; ADO, adenosine; AR, adrenergic receptor; BK, maxi K channel; EMC, extracellular matrix; ENaC, epithelial sodium channel; Entpd3, ectonucleoside triphosphate diphosphohydrolase 3; EP, PG receptor; NGF, nerve growth factor; P2Y, purinergic receptor Y; SAC, stretch-activated ion channel; trkA/B, tyrosine kinase receptors for nerve growth factor; TRP, transient receptor potential channel. [23]

Studies to date have focused particularly on the antibacterial activity of nitrofurantoin. There is no study investigating the efficacy of ancan bladder on the contractile activity of smooth muscle. The contractile activity of the bladder is an important result as UTI is a major cause. Therefore, in our study, we aimed to reveal the possible efficacy of nitrofurantoin on contractile activity of bladder. In previous studies, the inhibitory activity of nitrofurantoin on some smooth muscles such as the uterus is known. Based on this information, we think that nitrofurantoin has similar efficacy on bladder smooth muscle, and it can be used in cases of overactive bladder, except for its antibacterial use alone. so that UTM's most important in the treatment of overactive bladder is one of the reasons we believe we can develop a new perspective.

Material and Methods

24 male Sprague-Dawley rats weighing 200-250 g that were obtained from the Firat University Experimental Research Center were used in the study. Animals were decapitated without anesthesia in order to avoid myorelaxant effect of anesthesia. Their bladders were removed. Then, strips (each approximately 2 mm wide x 8 mm long) were prepared from bladder tissues. They were placed in an isolated organ bath of 5 ml that contained a Krebs-Henseleit solution (composition in mM: NaCl 118, KCl 4.7, MgSO₄ 1.2, CaCl₂ 1.25, KH₂PO₄ 1.2, NaHCO₃ 25, glucose 11, EDTA 0.03) and was continuously ventilated with 95% O₂/5% CO₂. Then, a tension of 1.5 gr was applied. It was waited for about 45-60 minutes for regulating spontaneous contractions. The bathtubs were washed with a Krebs-Henseleit solution for 15 min. Contractions were recorded by MP150WS for Windows (Biopac Systems Inc, CA, USA) with the help of a physiological power converter (FDT05, Commat Ltd, Ankara, Turkey). After spontaneous contractions were regulated, nitrofurantoin was intervally administered to three groups at doses of 50, 500 and 1000 µM, respectively. All data were normalized with respect to the basal area, peak-to-peak (p-p) and frequency expressed as percent of basal values of contractions were analyzed before and after administration of nitrofurantoin.

Statistical Analysis

All values were expressed as mean ± standard deviation (M±SD). All statistical analysis was performed using the SPSS for Windows Version 12.0. Statistical evaluation was performed using the Paired T-Test. A p-value of less than 0.05 was considered statistically significant. The obtained data were statistically normalized

Results

First Findings (50 µM Nitrofurantoin)

After bladder sections were placed in an isolated organ bath containing a Krebs-Henseleit solution, they were followed for about 60 minutes for regulating spontaneous contractions due to tension. During this time, organ bath wells were replaced with fresh Krebs-Henseleit solution every 15 min, and then 50 µM nitrofurantoin was administered (Figure 2). This dose was about 1/10 of the dose administered per kilogram in human. The mean area values before and after administration of 50 µM nitrofurantoin were 100±0.0 and 69±8.3, respectively. The mean peak-to-peak values before and after administration of 50 µM nitrofurantoin were 100±0.0 and 49.7±5.1, respectively. The mean frequency

values before and after administration of 50 μM nitrofurantoin were 100 ± 0.0 and 83.8 ± 5.2 , respectively. According to these results, it was observed that 50 μM nitrofurantoin led to a statistically significant reduction in the area (Figure 3), peak-to-peak (Figure 4) and frequency (Figure 5) values of bladder contractions when compared with pre-administration ($p < 0.05$).

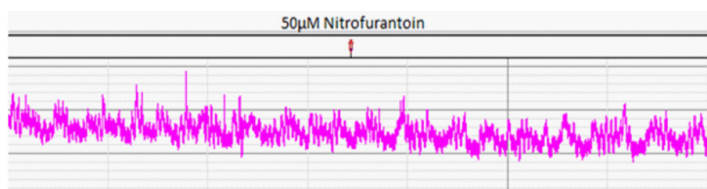


Figure 2. Administration of 50 μM nitrofurantoin

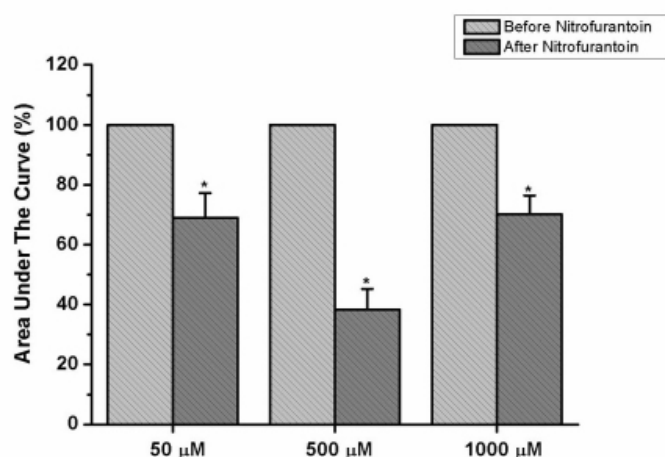


Figure 3. Area under the curve. Data are presented as the mean $\pm$ SEM for all groups. * $p < 0.05$ ($n = 7$).

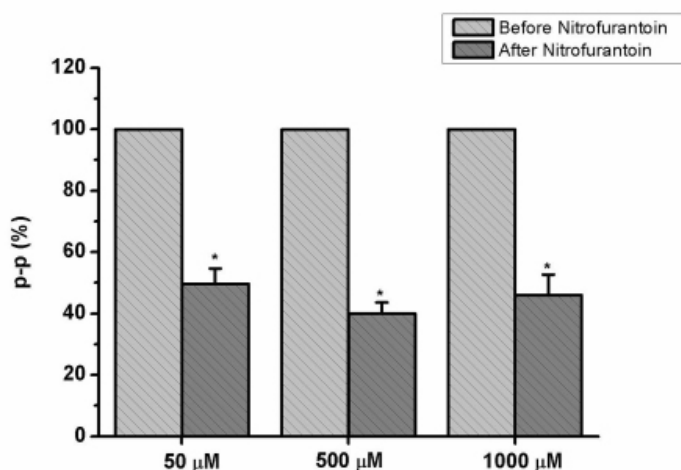


Figure 4. Peak to peak. Data are presented as the mean $\pm$ SEM for all groups. * $p < 0.05$ ($n = 7$).

Second Findings (500 μM Nitrofurantoin)

After bladder sections were placed in an isolated organ bath containing a Krebs-Henseleit solution, they were followed for about 60 minutes for regulating spontaneous contractions due to tension. During this time, organ bath wells were replaced with fresh Krebs-Henseleit solution every 15 min, and then 500 μM nitrofurantoin was administered (Figure 6). This dose was about the dose administered per kilogram in human. The mean area

values before and after administration of 500 μM nitrofurantoin were 100 ± 0.0 and 38.3 ± 6.9 , respectively. The mean peak-to-peak values before and after administration of 500 μM nitrofurantoin were 100 ± 0.0 and 40.1 ± 3.6 , respectively. The mean frequency values before and after administration of 500 μM nitrofurantoin were 100 ± 0.0 and 24.7 ± 9.6 , respectively. According to these results, it was observed that 500 μM nitrofurantoin led to a statistically significant reduction in the area (Figure 3), peak-to-peak (Figure 4) and frequency (Figure 5) values of bladder contractions when compared with pre-administration ($p < 0.001$).

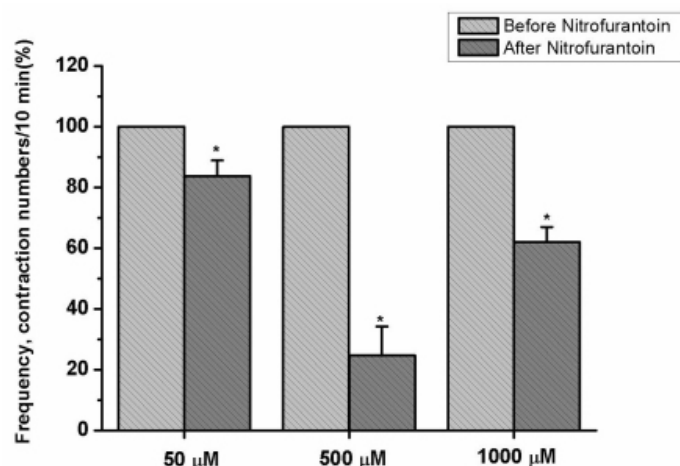


Figure 5. Frequency (counts/10 min). Data are presented as the mean $\pm$ SEM for all groups. * $p < 0.05$ ($n = 7$).

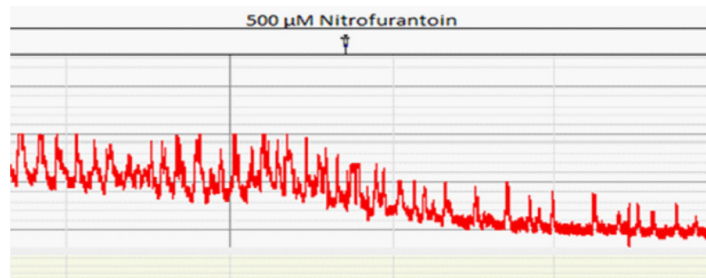


Figure 6. Administration of 500 μM Nitrofurantoin

Third Findings (1000 μM Nitrofurantoin)

After bladder sections were placed in an isolated organ bath containing a Krebs-Henseleit solution, they were followed for about 60 minutes for regulating spontaneous contractions due to tension. During this time, organ bath wells were replaced with fresh Krebs-Henseleit solution every 15 min, and then 1000 μM nitrofurantoin was administered. This dose was about twice the dose administered per kilogram in human. The mean area values before and after administration of 1000 μM nitrofurantoin were 100 ± 0.0 and 70.2 ± 6.3 , respectively. The mean peak-to-peak values before and after administration of 1000 μM nitrofurantoin were 100 ± 0.0 and 46.6 ± 6.7 , respectively. The mean frequency values before and after administration of 1000 μM nitrofurantoin were 100 ± 0.0 and 62.1 ± 4.9 , respectively. According to these results, it was observed that 1000 μM nitrofurantoin led to a statistically significant reduction in the area (Figure 3), peak-to-peak (Figure 4) and frequency (Figure 5) values of bladder contractions when compared with pre-administration ($p < 0.05$).

In the light of these findings, it was observed that nitrofurantoin led to a statistically significant inhibition on spontaneous bladder contractions at doses of 50, 500 and 1000 μM ($p < 0.05$). The most significant inhibition was observed at 500 μM nitrofurantoin ($p < 0.001$). As a result, it was shown that nitrofurantoin had an inhibitory effect on bladder contractions.

Discussion

According to the findings we obtained in this study, nitrofurantoin had an inhibitory effect on spontaneous bladder contractions. Considering the drug dose administered per kilogram in the treatment, three different doses of nitrofurantoin (50 μM =1/10 of the treatment dose, 500 μM = the treatment dose, and 1000 μM = twice the treatment dose) led to a statistically significant reduction in the area, peak-to-peak (p-p) and frequency values of bladder contractions ($p < 0.05$). However, the most significant inhibition was observed at 500 μM nitrofurantoin.

Nitrofurantoin is a broad-spectrum bactericidal antibiotic. It is used effectively in the treatment of UTIs caused by *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Enterococcus* spp. and *Staphylococcus aureus*. It exhibits antimicrobial activity by both interfering the synthesis of proteins embedded in the cell wall and the DNA synthesis of Gram-positive and Gram-negative bacteria and nitrofurantoin, a non-specific attack on bacterial ribosomal proteins, leads to the inhibition of bacterial enzymes involved in carbohydrate synthesis and high concentration of DNA, RNA and total protein synthesis [7]. Nitrofurantoin is a drug used in the antibacterial treatment of urinary tract infections for over 60 years [6]. While its use in older individuals is still a matter of debate, nitrofurantoin is still a first-choice drug in most urinary system infections in premenopausal and postmenopausal women [5]. Moreover, nitrofurantoin is an oral antibiotic that plays a key role in the treatment of AUC caused by multidrug-resistant gram negative bacteria [16]. Recent studies have shown that the use of prophylactic nitrofurantoin as a single oral dose for 6-12 months in women with recurrent urinary tract infection reduced the frequency of recurrent urinary tract infection. The effects on bladder contractions of nitrofurantoin, which is commonly used in the treatment of UTI, were not investigated. One of the most frequent causes of recurrent urinary tract infections is urinary incontinence that occurs due to changes in bladder contraction, and also recurrent urinary tract infections cause urinary incontinence [8-11]. This study that we performed in the light of these information showed that nitrofurantoin had an inhibitory effect on bladder contractions besides antibacterial activity. This finding we obtained suggests that the underlying mechanism responsible for nitrofurantoin is so effective in the treatment of recurrent UTI is not solely dependent on antibacterial activity.

Changes in bladder smooth muscle contraction are shown to be a major factor in the development of recurrent urinary tract infection and urinary incontinence. In other words, while UTI changes bladder activity, deterioration of bladder smooth muscle contraction triggers UTI [19]. Therefore, in addition to antibacterial therapy, inhibition of bladder contraction is also important. Recent studies clearly show that urinary tract infection changes bladder contraction; however, the underlying physiopathological mechanisms have not been clarified [25,26]. Furthermore, a recent study has found

that symptoms such as frequent urination, nocturia, urgency which are caused by an increase in bladder activity due to urinary tract infection have been improved by appropriate antibiotherapy. In this context, it has been determined that the use of sequential, combined antibiotic therapy (ciprofloxacin 500 mg bid, cephalexin 500 mg tds, doxycycline 100 mg bid) against Gram-positive and Gram-negative bacteria in individuals with overactive bladder significantly reduced the symptoms [27]. However, there is no study on the direct effect of these antibiotics on bladder contractile activity. As a result, nitrofurantoin inhibits bladder contractions according to our findings. Based on these findings, it is suggested that nitrofurantoin will not only be used as an antibacterial drug, but also as a pharmacological agent that can be used in the treatment of overactive bladder or urinary incontinence pathologies which is a very important cause of urinary tract infection. Further studies are needed to explain which nitrofurantoin inhibits bladder contractions using physiological mechanisms. Our study is the first study on the efficacy of nitrofurantoin on bladder contractions. There is very little study investigating the efficacy of nitrofurantoin in the literature on smooth muscle contractile activity, and in this study, we aim to explain this pathophysiological mechanism in our further study since its mechanism of action is not described.

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

It has been found that nitrofurantoin (a broad spectrum antibiotic) had an inhibitory effect on spontaneous bladder contractions. Moreover, our study has shown that not only was there a reduction in bladder activity due only to antiinflammatory effect, but also did nitrofurantoin directly inhibit bladder contractile activity. These data suggest that the only underlying mechanism responsible for the use of prophylactic nitrofurantoin in women with recurrent urinary tract infection reduces the frequency of recurrent urinary tract infection is not dependent on the bactericidal effect. We think that the findings we obtained may shed light on the underlying mechanism responsible for the use of prophylactic nitrofurantoin in recurrent urinary tract infections reduces the frequency of recurrent urinary tract infection. Further studies are needed to explain the molecular mechanism underlying the inhibitory effect of nitrofurantoin on bladder contraction. When all these data were taken into account, it has been found that nitrofurantoin, which is an important pharmacological agent in the treatment and prevention of urinary tract infections, inhibited spontaneous bladder contractions.

Competing interests

The authors declare there is no conflict of interest with regard to data presented in this article.

Financial Disclosure

All authors declare no financial support.

Ethical approval

All animal use procedures were approved by the Firat University Animal Experiments Local Ethics Committee and rats were treated in accordance with the national and international laws and policies on the care and use of laboratory animals.

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