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Inhibitory effects of mefenamic acid on rat urinary bladder contractions in vitro

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Abstract

Aim: In this study, the effect of mefenamic acid, a nonsteroidal anti-inflammatory drug, on the unconstrained contractile movement of the bladder muscle of female Spraque Dawley rats in diestrus was investigated. Since the effect of mefenamic acid on bladder smooth muscle is not known and its use for other conditions may cause possible side effects on the bladder, its effect was investigated for the possibility of using it as an active substance in the treatment of bladder problems.

Materials and Methods: In the experimental study, 1.5 cm long smooth muscle strips from seven female Sprague Dawley rats were prepared and suspended in an isolated organ bath system containing Krebs solution under 1.5 g tension. The system was gassed continuously with oxygen/carbon dioxide mixture (95%:5%) and isometric contractions were recorded. At a dose of 300 µM, the contraction/relaxation effects of mefenamic acid concentration on bladder smooth muscle were investigated. The area under the curve (AUC) and amplitude values of bladder contractions were analyzed before and after mefenamic acid administration. With the data obtained from the analysis, the effect of mefenamic acid on bladder contractions was evaluated using paired t-tests in SPSS Statistical Software.

Results: The decrease in area and amplitude values was statistically significant ($p<0.05$). Mefenamic acid had an inhibitory effect on bladder contractions.

Conclusion: The present findings demonstrate the relaxant effect of mefenamic acid on the rat bladder and if similar effects are observed in human studies, mefenamic acid may be effective in the treatment of voiding problems such as bladder incontinence.

Introduction

Mefenamic acid, an N-phenylanthranilic acid derivative, is a drug belonging to the "phenamate" family of nonsteroidal anti-inflammatory drugs (NSAIDs) with marked anti-inflammatory, antipyretic and analgesic activity [1]. The use of mefenamic acid is quite common in clinical practice due to its wide medical use [2]. Clinical uses include, menorrhagia, premenstrual syndrome, pain management (such as gynecological and obstetric pain, primary dysmenorrhea, musculoskeletal injury, toothache and osteoarthritis), urticaria, asthma, pediatric use and Alzheimer's disease [1]. Compared to many other NSAIDs, mefenamic acid shows anti-inflammatory effects by stimulating the immune system [3]. It is also a proliferation inhibitor against cancer cells (bladder, lung cancer) in humans [4].

The pharmacodynamic properties of mefenamic acid are primarily ascribed to its ability to block arachidonic acid metabolism through inhibition of cyclooxygenase action [1]. In any case, in recent years it has been suggested that NSAIDs owe their action to more than inhibition of prostaglandin synthesis [5]. A similar relationship applies to smooth muscle contraction. Although prostaglandins are associated with drug-induced compressions [6], a nonlinear relationship between drug-induced cocontraction and prostaglandin discharge has been described [7]. In literature, it is shown that prostaglandin synthesis inhibitors caused hindrance of unconstrained and drug-induced contractions within the nearness or nonappearance of anti-inflammatory activity, proposing that there's no relationship between restraint of prostaglandin synthesis and the

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spasmolytic impacts of these drugs [8, 9].

Mefenamic acid inhibits concentration-dependent contractions induced by methacholine and prostaglandin F2α [10]. However, it has been shown that this spasmolytic effect does not match with pertussis toxin-sensitive G proteins. Other spasmolytic NSAIDs tie to G proteins in an unexpected way since this toxin increments the impacts of phenylbutazone and piroxicam and diminishes the impacts of diclofenac and naproxen. Pertussis toxin may have exerted this effect by modulating the ionic channel conductance of G proteins [11] and the activity of enzymes involved in smooth muscle contraction [12]. Therefore, adenylyl cyclase and protein kinase C-dependent G proteins may be included within the impacts of NSAIDs on the rat uterus. Inactivation of adenylyl cyclase by pertussis toxin may clarify the uprooting of the relaxant impact of these drugs since cyclic adenosine monophosphate unwinds uterine smooth muscle. Results from related studies propose that a few NSAIDs tried actuate smooth muscle relaxation through instruments free of hindrance of prostaglandin synthesis but related with restraint of extracellular calcium influx through instruments related or disconnected to pertussis toxin-sensitive G proteins [10].

Using a whole-cell patch clamp, mefenamic acid has been shown to increase slow delayed rectifier current (IKs) activity in a dose-dependent manner by converting the slowly activated IKs into a nearly linear current with instantaneous onset, which can be inhibited by an IK blocker. Cell studies recommend that mefenamic acid diminishes the voltage affectability of the IK channel and causes a hyperpolarizing move within the voltage reliance of channel enactment [13]. In this study, it is aimed to reveal the effects of mefenamic acid, an NSAID used in urinary tract infections, on the bladder smooth muscle in rat.

Materials and Methods

Animals

The study was performed in the Research Laboratory of the Department of Physiology, Faculty of Medicine, Firat University. Experimental research was allowed with the decision numbered 04-01 dated 08.03.2023 of the Animal Experimental Ethics Committee of Firat University. The study was conducted by preparing 14 strips of bladder from seven Sprague-Dawley rats (200-250 g) in the diestrus phase of the estrous cycle. The rats were housed in a regularly ventilated, constant temperature (21°C) and relative stickiness environment under a 12 h light/12 h dark cycle and fed with standard rat chow and tap water (*ad libitum*). To confirm that the rats were in diestrus stage, vaginal smears were taken from each rat by the same person to avoid inconsistency in the results and the stage of the cycle was determined microscopically. Rats confirmed to be in diestrus were decapitated, a midline thoraco-abdominal incision was made and all bladder tissues were removed into a petri dish containing Krebs-Henseleit solution.

Preparation of the tissue and isometric tension recording

Isolated organ bath water system (Grass FT 03 tension transducer) and a tube containing 95% O_2 + 5% CO_2 mixture were used in the experiment. Bladder tissues from the animals were cut into 1.5x5 mm long strips. The tissues were processed at 37°C using Krebs-Henseleit solution (KHS, mM: NaCl 118, KH_2PO_4: 1.18, $NaHCO_3$: 15.8, $MgSO_4$: 1.2, $CaCl_2$: 2.4, KCl: 4.7, Glucose: 11.5, EDTA: 0.016) and continuously gassed with 95% O_2 + %5 CO_2 mixture in a 10 ml organ bath at 37°C. The preparations were regulated for 90 minutes at a resting tension of 1.5 g. During this time, the tissues were washed with Krebs solution every 15 minutes. After the recovery period, mefenamic acid at a concentration of 300 µM was applied. The responses to the applied agent were recorded by a power transducer and transferred to a recording system (BIOPAC MP100, Commat Ltd.). The effects of mefenamic acid on spontaneous bladder contractions were measured by changes in mean amplitude and area under the contraction curve (AUC). Contraction activity (mean amplitude and AUC) was taken as 100% during the control period. AUC and amplitude values of contractions before and after the application were normalized as % change.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, Version 22.0. (Armonk, NY: IBM Corp.). The number of animals to be used in the experiments; 8% deviation, type 1 error (α) 0.05 and type 2 error (β) (Power=0.80) were determined by power analysis. Descriptive statistics were mean ± standard error (mean ± SE). Data were evaluated using paired samples t-test. Differences at $p<0.05$ were considered statistically significant.

Results

The effect of mefenamic acid on female rat bladder smooth muscle was evaluated. Isometric tension changes were measured in isolated rat bladder smooth muscle. Mefenamic acid caused a statistically significant decrease in the AUC and amplitude values of spontaneous bladder contractions at 300 µM dose ($p<0.001$). After 300 µM dose administration, amplitude values of contractions were calculated as 32.1±7.5 and AUC values as 16.5±5.1 (Figure 1).

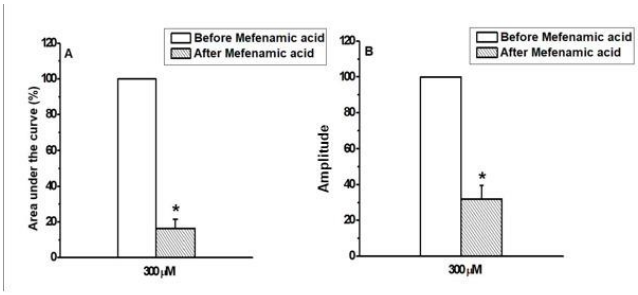

Figure 1. Inhibitory effect of mefenamic acid (300 µM) on urinary bladder contraction. Effects on A) Area Under the Curve B) Amplitude values. * $p<0.001$.

The original tracings obtained at 300 µM doses in the isolated organ bath are shown in Figure 2.

Discussion

The effect of the mefenamic acid, which is used in urinary tract infections due to its anti-inflammatory and analgesic

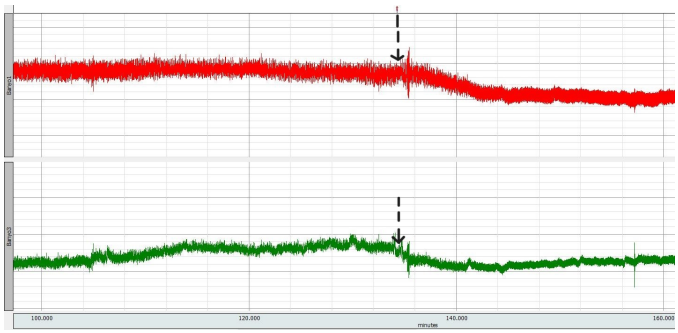

Figure 2. Original recording showing the mefenamic acid-induced concentration-dependent inhibition on spontaneous peristaltic activity of the urinary bladder strips (The application of mefenamic acid is indicated by the arrow).

effects, on bladder smooth muscle contractions was not known. Therefore, in this study, the effect of mefenamic acid was investigated in samples taken from rat bladder tissues. In the study, mefenamic acid, a widely used NSAID in the clinic, was shown to have an inhibitory effect on bladder smooth muscle contractions.

Anderson and Kohn showed that indomethacin which belongs to the NSAIDs decreased unconstrained motility in rabbit detrusor muscle, affecting the constrain as well as the frequency of contractions, and this suppression was partially antagonized by calcium or prostaglandin E2 [14]. It was also determined that, the basal tone of the detrusor muscle was not altered by indomethacin. Hills et al. documented decreased phasic spontaneous activity as well as tone of the detrusor muscle in many species after indomethacin, flufenamate and meclofenamate which are belongs to the NSAIDs [15, 16]. On the other hand, prostaglandins have also been shown to play an important role in maintaining bladder tone during distension [15, 16]. Bladder distension induced by increasing saline volumes causes an increase in endovesical pressure and a concomitant release of prostaglandins, both of which are inhibited by indomethacin [17]. In this study, the inhibitory effect of mefenamic acid was also demonstrated. Several drugs inhibiting prostaglandin synthesis have been shown to have spasmolytic effects on rat uterine contractions [18]. These effects may be related to the effect of NSAIDs on the membrane by inhibiting calcium entry. These drugs may also have an inhibitory effect on calmodulin [19]. Inhibition of calmodulin may contribute to several effects of NSAIDs such as spasmolytic or inhibition of inflammatory mediator release [20]. Mefenamic acid provides critical protection against increased levels of tumor necrosis factor-α and interleukin-1 β (IL-1β). It has already been observed that the use of mefenamic acid reduces reactive protein (CRP) levels, reflecting its significant anti-inflammatory activity [21].

In refined porcine aortic endothelial cells in the presence of mefenamic acid, the bradykinin-induced increase in intracellular calcium was attenuated and transient, as seen in Ca^{2+} -free medium. Moreover, the slow and prolonged increase in intracellular calcium elicited by cyclopiazonic acid, which was entirely subordinate on the presence of extracellular Ca^{2+} , was abolished by mefenamic acid. These findings indicate that mefenamic acid can specifically block Ca^{2+} influx induced by bradykinin and cyclopiazonic acid in endothelial cells [22].

Mefenamic acid blocks transient receptor potential melastatin 2 (TRPM2) [21] channels and reduces calcium influx, which in turn inhibits the nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammatory [23]. Mefenamic acid is a potent and selective inhibitor of NLRP3 inflammasome, acting independently of COX inhibition by blocking the volume-regulated TRPM2 and anion channel. By inhibiting NLRP3 inflammatory, Mefenamic acid minimizes the generation of levels of the proinflammatory cytokine IL-1β. The established safety and efficacy of mefenamic acid and its multiple pathways, including COX inhibition, bradykinin antagonism, reduction of uterine resting tone and contraction frequency, NLRP3 inhibition and reduction of CRP levels, make mefenamic acid an ideal choice in the treatment of dysmenorrhea [24,25]. In the present study, the relaxant effects of mefenamic acid have been demonstrated and additional studies are needed to elucidate the underlying mechanisms.

Patient satisfaction with current drugs used for urinary tract problems such as overactive bladder and urinary incontinence, significantly reducing the patient's quality of life, may be low due to side effects or insufficient efficacy. The comes about from the current study confirm the relaxant effect of mefenamic acid on the rat bladder and if similar effects are seen in human studies, mefenamic acid may be a suitable drug candidate to investigate for bladder incontinence.

Ethical approval

Ethical approval was received for this study from Fırat University Animal Experiments Ethics Committee (Approval no: 2023/ 04-01).

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