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Mefenamic acid reduces the contractility of isolated uterine smooth muscle of rat

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Abstract

Aim: Mefenamic acid is a nonsteroidal anti-inflammatory drug widely used for the treatment of pain and inflammation. The drug is indicated in patients with mild to moderate pain and inflammatory diseases, usually toothache, painful menstruation, muscle or joint pains and postpartum pain. There are not enough studies showing the effectiveness of this drug, which is significantly effective in gynecological pains, on the uterine contraction and relaxation mechanism. This study was carried out to investigate the effects of mefenamic acid on the uterine contraction and relaxation mechanism.

Materials and Methods: In the study, seven female intact Sprague-Dawley rats in the diestrus period were used. Longitudinal myometrium sections of 1.2 cm length, 2 mm width and 1 mm thickness from animals were suspended in an isolated organ bath containing crebs solution. After the regulation period, mefenamic acid was administered at a dose of 300 µM. Contractile changes were monitored using an isometric transducer. Before and after the application, the area under the curve (AUC), frequency and peak to peak (p-p) values were normalized as % change. Statistical analyzes of the data were performed with the Paired Sample T test in SPSS 22.0 program.

Results: Mefenamic acid caused a statistically significant decrease in the p-p, AUC and frequency values of spontaneous uterine contractions at 300 µM dose ($p<0.001$).

Conclusion: Mefenamic acid has an inhibitory effect on uterine contractions. This drug, which is widely used in the clinic for menstrual pain caused by abnormal uterine contractions, especially in young women, may show its analgesic effect by inhibiting uterine contractions.

Introduction

Mefenamic acid is a nonsteroidal anti-inflammatory medication (NSAID) with antipyretic, analgesic, and anti-inflammatory action. In addition to being used to relieve menstrual pain, it can be used for musculoskeletal pain (osteoarthritis, rheumatoid arthritis), sports injuries, moderate and mild painful conditions, headaches, toothaches, post-operative and postpartum pain [1,2]. Mefenamic acid is a phenamic acid derivative derived from N-aryl anthranilic acid [3,4]. Mefenamic acid, like many NSAIDs, binds to cyclooxygenase and suppresses prostaglandin formation. Mefenamic acid, one of the widely used NSAIDs in the clinic, has dual effects such as inhibiting prostaglandin synthesis and blocking prostaglandin receptors, which makes this drug unique and more effective especially in the treatment of dysmenorrhea [5]. The inhibition of prostaglandins by mefenamic acid causes the onset of uterine relaxation more rapidly, making its analgesic effect stronger [6]. One of the possible medical uses of mefenamic acid in this regard is from its effect on smooth muscle. Effects on transit time of nutrients in intestines and effects on gastric muscle, tracheal smooth muscle have been reported. It has also been shown to modulate both mesenteric vein and renal blood flow. It may decrease norepinephrine or photoactivation-induced arterial contractions [7-9]. Along with reducing pain, mefenamic acid reduced uterine tone and the frequency of uterine contractions [10]. Mefenamic acid can inhibit enhanced uterine contractility caused by prostaglandin endoperoxide analogues *in vitro* [11]. There are not enough studies showing the effectiveness of this drug, which is significantly effec-

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tive in gynecological pain and problems, on the uterine contraction and relaxation mechanism. The purpose of this study was to look into the effects of mefenamic acid on the uterine contraction and relaxation mechanisms.

Materials and Methods

Animals

In this study, G power test was applied to determine the sample size and the n value was determined as 7.In this research seven female Spraque Dawley rats weighing between 200-250 g were used. Experimental research was allowed with the decision numbered 04-01 dated 08.03.2023 of Fırat University Experimental Animals Local Ethics Committee. All animals were kept in plastic cages at constant room temperature (21±2°C) with 12 hours of light/12 hours of darkness, with standard feeding and no limitations. Uterus of animals in the diestrus period of their sexual cycles on the day of the experiment was taken between 09:00 and 10:00 in the morning. The subjects were decapitated without anesthesia and the uterine tissues were removed. Following decapitation, small longitudinal myometrium sections (1.2 cm long, 2 mm wide and 1 mm thick) taken from the uterine tissue were hung in an isolated organ bath containing Krebs solution.

Preparation of myometrium strips

After cervical dislocation, abdominal regions of rats in diestrus period were opened. Carefully dissected two uterine horns and quickly placed in a petri dish containing Krebs–Henseleit solution (KHS, mM: KCl 4.7, $MgSO_4$ 1.2, NaCl 118, KH_2PO_4 1.18, $CaCl_2$ 2.4, $NaHCO_3$ 15.8, Glucose 11.5, EDTA: 0.016). Uterine tissues were opened properly in the longitudinal direction. 1.2x2x1 cm sections, including all uterine folds, were taken from the opened uterine horns and cut into strips longitudinally.

Organ bath contractility experiments

The prepared tissue sections were placed in 10 ml glass chambers containing Krebs-Henseleit solution at 37°C and continuously ventilated with a mixture of 5% CO_2-95% O_2, by applying one g of tension. Both ends of the sections were tied with silk threads; one end was attached to the bottom of the chamber and the other end was attached to the isometric power transducer and hung vertically in the organ bath. The solution in the bath was changed every 15 minutes. The isometric contractions were recorded. After a 90-minute regulation period, the contractions of the regular self-twitch strips were recorded for 10 minutes using an isometric force transducer, and these data were used as control data. Then, mefenamic acid was administered at a dose of 300 µM.

Statistical analysis

Statistical analyzes of the data were evaluated with Paired T Test using IBM SPSS Statistics for Windows, version 22 (IBM Corp.) due to the fact that the data were based on before and after the study, the data conformed to normal distribution and the sample size. Differences at the $p<0.05$ level were considered significant.

Results

Results The effect of mefenamic acid on spontaneous uterine contractions was evaluated (Figure 1). Isometric tension changes were measured in the isolated rat uterus. The area under the curve contraction frequency, and peak to peak (p-p) values of the animals' myometrial strips before and after the application of mefenamic acid were normalized as % change. When mefenamic acid was administered at a dose of 300 µM, it was found to have a significant reduction effect on the spontaneous (uninduced) area under the curve compared to before administration arithmetic mean ± standard deviation (14.9 ± 9.6, $p<0.001$). Mefenamic acid also significantly reduced the frequency of spontaneous uterine contractions and peak-to-peak values in the order of arithmetic mean ± standard deviation (p-p 7.1± 7.1, frequency 7.3± 5.4, $p<0.001$) (Figure 1).

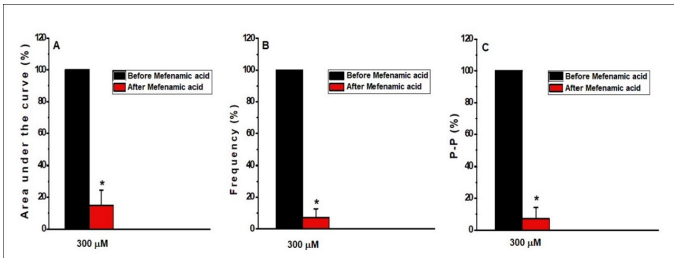

Figure 1. Effects of mefenamic acid on spontaneous uterine contractions. Effects on A) Area Under the Curve B) Frequency C) Peak to Peak values. * $p<0.001$.

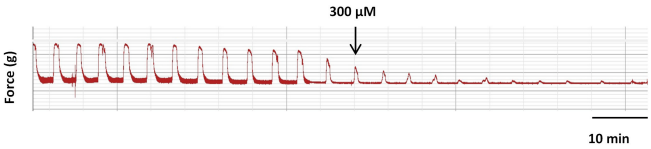

Figure 2. Original recording showing the mefenamic acid-induced concentration-dependent inhibition on spontaneous activity of the myometrial strips.

The original trace obtained at a dose of 300 µM in the isolated organ bath is shown in Figure 2.

Discussion

In this study, the effect of mefenamic acid on in vitro rat spontaneous uterine smooth muscle contractility was investigated. The research revealed that mefenamic acid significantly reduced the frequency and amplitude of spontaneous uterine contractions. Mefenamic acid has an inhibitory effect on uterine contractions.

Mefenamic acid is used for many problems in gynecological practice and no significant side effects have been reported due to its use. Prostaglandins are well-known for their role in uterine contractions. Both prostaglandins of the prostaglandin F and prostaglandin E series (regardless of whether they are natural or artificial) are known to stimulate pregnant uterine contraction. Current evidence suggests that altered prostaglandin production may be responsible for preterm birth. Prostaglandin synthetase inhibitors such as mefenamic acid are known to affect normal

uterine activity, but the exact mechanisms are not clear [12]. Mefenamic acid has been demonstrated to be effective in preventing preterm labor, lowering the incidence from 40% to 15% in the control and experimental groups, respectively [12]. Arachidonic acid and prostaglandins released from the endometrium in the postpartum period are the main causes of postpartum pain. Therefore, the aim of the programmed treatments in this period is to reduce the prostaglandin level [13]. One of the current methods for pain management is the use of NSAIDs or opioids, which have relatively significant side effects. However, because of their negative effects, NSAI medicines should be used with caution [14]. However, the beneficial effect of mefenamic acid has also been demonstrated in terms of pain relief after hysteroscopy [15]. This conclusion is consistent not just with Siddle et al.'s observations, but also with the large number of articles documenting the postoperative analgesic benefits of prostaglandin synthesis inhibitors after surgery under general anesthesia [15]. A double-blind randomized study found that NSAIDs significantly decreased postoperative pain and analgesic consumption, and reduced hospital stay and abdominal discomfort after discharge [15].

Micromolar concentrations of mefenamic acid and its derivatives in the intercellular spaces of smooth muscle cells in the small intestine could have a significant impact on cellular excitability and motility. Studies show that this group of drugs causes hyperpolarization by activating potassium current, which is the main regulator of resting membrane potential. Thus, by activating this current, they strongly increase cellular excitability [16]. Fenamates have a variety of impacts on membrane transport proteins. When administered to the cytosolic side of non-selective cation channels in rat pancreatic exocrine cells, it has been demonstrated to block them [17]. Flufenamic acid prevents Ca^{2+}-activated Cl^- channels in Xenopus oocytes [16]. In addition, a large conductivity K^+ channel in the rabbit corneal epithelium was found to be potent activators [18]. Some NSAIDs analyzed induce smooth muscle relaxation by mechanisms independent of inhibition of prostaglandin synthesis, however are associated with inhibition of extracellular calcium influx through mechanisms related or unrelated to pertussis toxin-sensitive G proteins [19].

This drug, which is widely used in the clinic for menstrual pain caused by abnormal uterine contractions, especially in young women, may show its analgesic and effects on other problems such as preterm labor by inhibiting uterine contractions. It can be thought that different cellular pathway activations of mefenamic acid at the molecular level may also be responsible for this effect.

Ethical approval

Experimental research was allowed with the decision numbered 04-01 dated 08.03.2023 of Fırat University Experimental Animals Local Ethics Committee.

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