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Research Article

Relaxing Effects of Paracetamol on Uterine Spontaneous Contraction in Rats in Vitro

Parasetamolün Sıçanlarda In Vitro Uterus Spontan Kasılmaları Üzerindeki Gevşetici Etkileri

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Abstract

Aim: Paracetamol is a commonly used drug in acute and chronic pain. It is known that paracetamol, which is a pain reliever and antipyretic drug, is safe to use during pregnancy. The aim of this study was to investigate the effects of paracetamol on the uterine smooth muscle contraction- relaxation mechanism in female rats in diestrus.

Material and Methods: Wistar-albino intact female rats were used in the study. Longitudinal strips of myometrium obtained from animals at the diestroeus stage. Stripes were suspended in an isolated organ bath containing crebs solution under 1 g passive tension. After the regulation period, paracetamol were added non-cumulatively at 1000μM and 2000μM concentrations. Before and after the application, the area under the curve (AUC) and peak to peak (p-p) values were normalized as % change.

Results: Paracetamol caused a statistically significant decrease in p-p and area under the curve parameters of spontaneous uterine contractions at 1000 and 2000 μM doses ($p < 0.001$).

Conclusion: Paracetamol causes uterine relaxation by inhibiting uterine contraction. This effect should be taken into account in clinical use.

Keywords: Paracetamol, uterus, isometric contraction, rat

Öz

Amaç: Parasetamol akut ve kronik ağrıda yaygın olarak kullanılan bir ilaçtır. Ağrı kesici ve ateş düşürücü bir ilaç olan parasetamolün gebelikte kullanımının güvenli olduğu bilinmektedir. Bu çalışmanın amacı, diöstrustaki dişi sıçanlarda parasetamolün uterus düz kas kasılma gevşeme mekanizması üzerindeki etkilerini araştırmaktır.

Materyal ve Metot: Çalışmada Wistar-albino intak dişi sıçanlar kullanıldı. Longitudinal miyometriyum şeritleri diöstrustaki hayvanlardan elde edildi. Şeritler, 1 g pasif gerilim altında krebs solüsyonu içeren izole organ banyosuna asıldı. Regülasyon periyodundan sonra, parasetamol 1000μM ve 2000μM konsantrasyonlarda kümülatif olmayan şekilde eklendi. Uygulama öncesi ve sonrası eğri altında kalan alan (AUC) ve zirveden zirveye (p-p) değerleri % değişim olarak normalize edilmiştir.

Bulgular: Parasetamol spontan uterus kontraksiyonlarının p-p ve eğri altında kalan alan parametrelerinde 1000 ve 2000 μM dozlarda istatistiksel olarak anlamlı azalmaya neden oldu ($p < 0.001$).

Sonuçlar: Parasetamol uterus kasılmasını inhibe ederek uterus gevşemesine neden olur. Klinik kullanımında bu etki göz önünde bulundurulmalıdır.

Anahtar Kelimeler: Parasetamol, uterus, izometrik kontraksiyon, sıçan

INTRODUCTION

Smooth muscles line the walls of hollow organs, including vessels, gastrointestinal tract, uterus, and bladder, and respond to signals and maintain the homeostasis (1). Smooth muscle contraction is initiated by the cross-bridge action of myosin II-based thick and F-actin-based thin filaments. Myosin activity is significantly stimulated by regulatory light chain (RLC) phosphorylation in contact with actin (2). Therefore, smooth muscle RLC phosphorylation by calcium/calmodulin-dependent myosin light chain kinase is required mainly for smooth

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muscle contraction and physiological activities of organs lined with smooth muscle (3,4). It is known that the uterus, which is a smooth muscle, shows spontaneous contraction and many hormones and drugs affects their activity.

Paracetamol (acetaminophen) is a commonly used drug in acute and chronic pain in the world (5). The mechanism of action of paracetamol is related to the inhibition of cyclooxygenases and the endocannabinoid system and serotonergic pathways (6). Paracetamol is widely used for the relief of a number of acute pain conditions such as dysmenorrhea, muscle and joint pain, headache and toothache. Unlike non-steroidal anti-inflammatory drugs, paracetamol has no anti-inflammatory activity and is thought to exert antipyretic and analgesic effects through various mechanisms, including activation of selective and variable inhibition of prostaglandin synthesis and serotonergic pathways and cannabinoid receptors (7-9). Due to World of Health Organization's inclusion of this drug in the analgesic drug step (10) and also due to its decades of clinical experience, it is frequently prescribed in chronic pain conditions such as low back pain and osteoarthritis. Recently, meta-analyzes of randomized controlled trials covering these conditions have shown that effect sizes are moderate but still statistically significant compared to placebo (11-14).

Paracetamol is preferred over other analgesics, such as non steroid anti inflamatory drugs, which have a less favorable risk profile and aspirin, concerning about its effect on the fetus with limited use in pregnant women (15,16). Paracetamol is considered to be safe to use at all stages of pregnancy, making it the first-choice pain reliever and antipyretic drug for pregnant women (17). In addition, the United States Food and Drug Administration has classified paracetamol as Pregnancy Category B, and supports that these drugs can be used without maternal health and fetal development concerns (18). Paracetamol crosses the placenta freely and creates a direct effect on the fetus (19). It decreases the production of prostacyclin both in the culture of endothelial cells isolated from umbilical vessels and in the third trimester of pregnancy (20). It has been used by pregnant women for years without any obvious harmful effects on the developing baby. Therefore, paracetamol is generally recommended as the first choice among pain relievers and antipyretic drugs for pregnant women (21).

In recent years, there has been an increasing review regarding the safety of paracetamol in pregnancy. Various embryo-fetal and neonatal effects of paracetamol have been demonstrated depending on the duration of treatment, dose, and trimester of exposure. Large cohort studies have not found a relationship between maternal paracetamol use in the first trimester and congenital malformations or adverse pregnancy outcomes (22,23). A recent study has shown that when paracetamol was administered to a pregnant rat at doses in the clinical range used in patients, approximately 40% of the drug levels in the maternal circulation reached the fetus by crossing the placenta (24). Thus, the placenta provides of protection for the developing fetus, but the mechanisms involved or the effects of paracetamol on placental functions are not yet understood (24). Besides these known effects of paracetamol, its effects on the uterine smooth muscle contraction-relaxation mechanism are unknown. For this purpose, this study aimed to investigate the effects of paracetamol on the contraction-relaxation activity of rat uterine strips in diestrus.

MATERIAL AND METHOD

Animals and Tissue Preparation

In the study, intact female wistar rats (200-220 g) in diestrus were used. All procedures were approved by the Local Ethics Committee of Firat University Experimental Animals (14.04.2021, Issue:07). Rats were housed in the Firat University Experimental Animal Unit at 12: 12-h light-dark cycle, in rooms that were regularly ventilated at 21^{0}C room temperature. Animals were given free access to standard rat feed and tap water. All experiments on rats were carried out during the diestrus phase to control the reproductive cycle and endocrine hormones. Oestrus cycle was determined by taking a vaginal smear with a pasteur pipette in the every morning. On the day of the experiment, rats were euthanized without anesthesia. The abdominal cavities of the animals were quickly opened and their uterus rapidly excised. The excised uterine tissues were taken into Krebs solution and the connective tissue was carefully cut and cleaned. The uterine tissues were removed and divided into strips of 1.2x2x1 mm along its longitudinal axis. Cumulative data from 8 strips were collected.

Isometric Contraction

The myometrial sections were immediately suspended from each end in the isolated organ bath using surgical thread. Each strip of myometrium was suspended in a double wall tissue bath (MAY IOBS 99) filled with Krebs solution. System was continuously ventilated with a mixture of 95% O_2 and 5% CO_2 at 37°C. The upper side of the uterine strip was connected to an isometric power transducer and the other end was attached to a fixed hook under the tissue bath. To establish the basal tension-contraction relationship, the uterine strips were suspended under 1 g tension. The isometric power transducer senses the physical forces caused by isometric contractions in the smooth muscle strands in the chambers and converts them into electrical signals. These signals are delivered to the amplifier simultaneously. Amplified electrical signals are transmitted to the recording unit as frequency and amplitude parameters compatible with those in the original trace. Then the data were analyzed on the computer.

The myometrium strips exhibited spontaneous contractile activity of uneven frequency and intensity while washing every 10 minutes until equilibrated in crebs solution for 90 minutes. After the regulation period, paracetamol was

applied non-cumulatively in two separate doses of 1000μM and 2000μM. The effects of paracetamol on spontaneous uterine contractions were measured by mean peak to peak changes (p-p) and area under the contraction curve (AUC). During the control period, contraction activity (mean p-p and AUC) was taken as 100%. Before and after the application, the AUC and p-p values were normalized as% change.

Statistical Analysis

Statistical analysis of the data was evaluated using the paired sample T test in the SPSS 22.0 program. All values were determined as mean ± standard deviation (mean ± SD). For all analysis, p <0.05 was considered statistically significant.

RESULTS

Paracetamol exerted a relaxing effect on spontaneous contraction of uterine smooth muscle at 1000 μM and 2000 μM doses. Relaxing effects were statistically significant at 1000 μM and 2000 μM doses (p<0.001, Figure 1 and Figure 2). AUC and p-p values of spontaneous myometrial contractions were measured as 100±0. The mean values of p-p and AUC were 40.2±22.5 and 23.2±19.5, respectively, after the application of 1000 μM dose in uterine contraction. The mean values of p-p and AUC were 12.6±23.8 and 8.9±16.5, respectively, after uterine contraction at a dose of 2000 μM. Measurements were given as percent inhibition of the control period. The original traces obtained in the isolated organ bath at 1000 μM and 2000 μM doses were shown in Figure 3 and Figure 4, respectively.

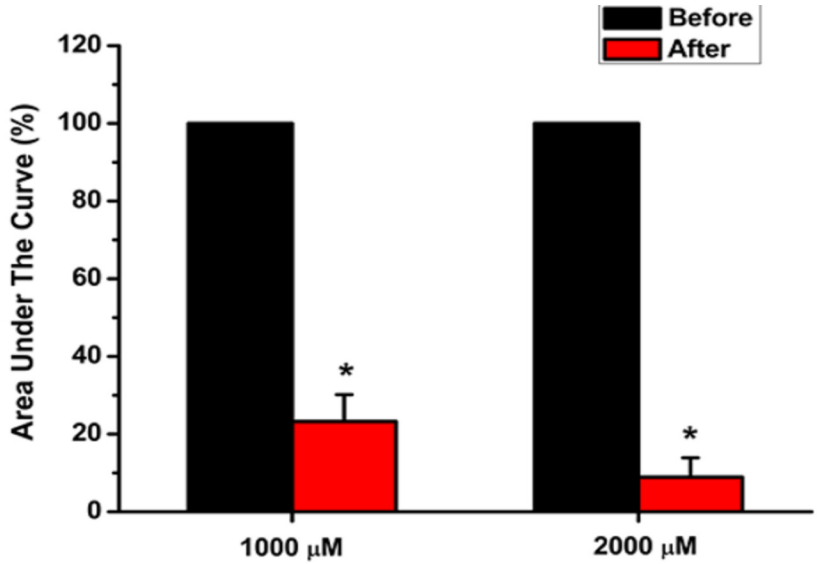

Figure 1. Effects of paracetamol on the area under the contraction curve (AUC) measurements in myometrial contractions. *p <0.001

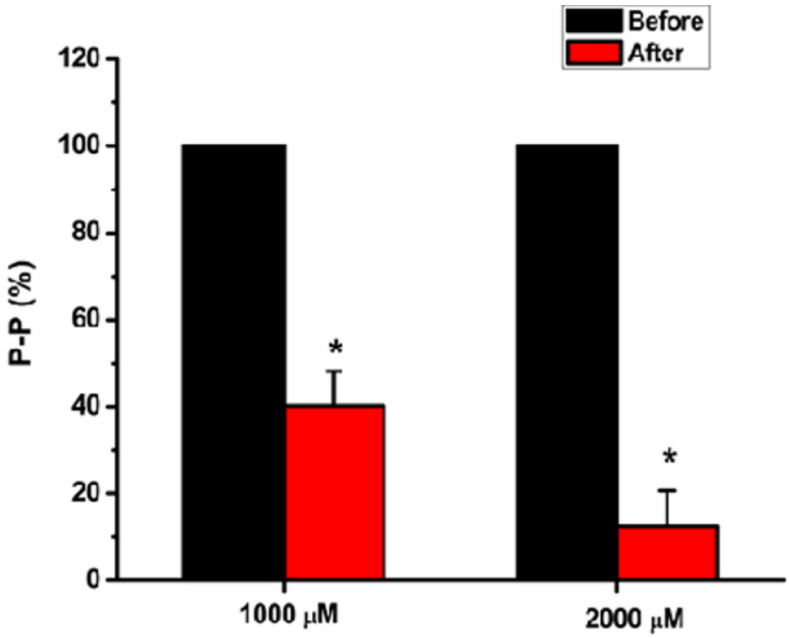

Figure 2. Effects of paracetamol on the peak to peak (p-p) measurements in myometrial contractions. *p<0.001

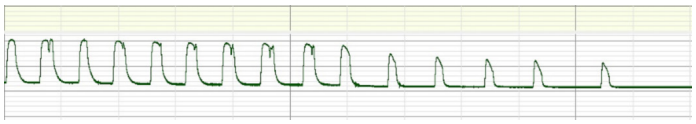

Figure 3. Original trace obtained when a 1000 μM dose of paracetamol was administered in an isolated organ bath

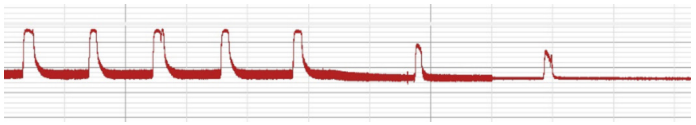

Figure 4. Original trace obtained when a 2000 μM dose of paracetamol was administered in an isolated organ bath

DISCUSSION

The study showed that paracetamol has an inhibitory effect on uterine spontaneous contractions. It was observed that the inhibitory effect was greater as the dose increased. In a study, it was shown that paracetamol exposure in female rats was associated with a significantly longer gestational period (mean 2.8 days) and a trend towards a lower incidence of preterm labor (4.7% versus 0.7%) (25). Notably, in a retrospective study, a similar increase in mean gestational age and a significant decrease in preterm births were reported in babies exposed to paracetamol during pregnancy, but no difference was observed between female and male babies (26). The authors predicted that paracetamol could prolong pregnancy through a decrease in prostacyclin synthesis, consistent with the findings of a randomized controlled trial that showed that low-dose aspirin (another cyclooxygenase inhibitor) also reduced preterm labor (27). However, another observational study did not find a significant association between paracetamol and preterm labor (28). One large prospective study reported that women taking paracetamol in their third trimester had a significantly increased risk of preterm labor, and analysis showed that this only applies to mothers with preeclampsia who took paracetamol for hypertension-related headache (22). Today, preterm birth is an important problem in obstetrics and accounts for 70% of perinatal deaths and almost half of long-term neurological morbidity (29,30). Therefore, prevention of spontaneous onset of preterm labor or rupture of membranes (31) will be of great public health importance. In the literature, it has been reported that the risk of preterm birth (26) and stillbirth (32) decreases after the use of paracetamol, and no association with miscarriage has been reported (33).The imbalance between Prostaglandin I2 (PGI2), or prostacyclin and Thromboxane A2 (TXA2, a vasoconstrictor) and inhibition of PGI2 synthesis has been proposed as an explanation for this situation (26,33).

It has been shown that paracetamol can be used after gynecological surgical procedures (34). Preoperative or intraoperative intravenous paracetamol was administered to hysterectomy patients and postoperative analgesic effects were evaluated. Paracetamol has been shown to provide significant postoperative analgesia with a reduction in morphine consumption and minimal side effects (35). Similarly, the pain score was significantly decreased by using paracetamol in the postoperative period of suction curettage (36). In our study, the inhibition of rat uterine contractions with paracetamol may shed light on the molecular causes underlying the results obtained in these studies. It can be said that paracetamol, which can also be used during pregnancy, can be reliable in pregnancies with clinical miscarriage risk and preterm birth risk.

CONCLUSION

In conclusion, it can be said that the use of paracetamol may have a potential preventive effect on preterm labor and prevent the risk of miscarriage. This effect may be associated with a decrease in the production of paracetamol-induced prostacyclin in women during pregnancy. However, the clinical effects of paracetamol using can be supported by studies using larger animal numbers of our findings. Although the findings of this study are based solely on experiments conducted in the rat uterus, must be careful when advising pregnant patients on the use of paracetamol in pregnancy.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *All procedures were approved by the Local Ethics Committee of Firat University Experimental Animals (14.04.2021, Issue:07).*

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