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Original Article

Clopidogrel inhibits acetylcholine-induced contractions of urinary bladder in rat

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Abstract

Aim: Clopidogrel as thrombocyte Adenosine Diphosphate (ADP) receptor antagonist is used especially in peripheric artery diseases by lengthening hemorrhage time, disrupting thrombocyte aggregation and decreasing blood viscosity. It shows its antagonist effects through glycoprotein (Gp) IIb/IIIa complex ADP by preventing its activation. Although the usage area and activity is in the vascular system, there are no adequate studies showing the activity of Clopidogrel on smooth muscle contraction-relaxation mechanism. This study was conducted to investigate the effects of Clopidogrel on bladder contraction-relaxation mechanism.

Material and Methods: In the present study, the bladder tissues taken from Wistar-Albino (n=7) intact female rats were used. After the decapitation, the longitudinal bladder tissues that were received 1-mm-thick, 8-mm length, and 2-mm-width, were hung in the 5-ml isolated organ bath that had Krebs-Ringer bicarbonate solution by applying 1.5 gr strain. After the bladder contractions were induced with 10 µM dose Acetylcholine (Ach), Clopidogrel was applied as two doses 0.1µM and 10µM in a noncumulative manner. The peak-to-peak (p-p) values and the values below the curve before and after the Clopidogrel application in the contraction induced with Ach were normalized as % change. The statistical analyses of the data were made in the SPSS 22.0 program by applying Paired T-Test. The $p<0.05$ value was accepted to be statistically significant.

Results: When the bladder contractions induced with Ach and the values after Clopidogrel applications were compared, it was determined that there was 79% inhibition in the area values with Clopidogrel at 0.1µM dose; 87% at 10µM dose. In the p-p values, there was inhibition at 0.1µM dose with 63%, at 10µM dose with 64%, each of the two doses, the p-p and area values were found to be statically significant ($P<0.001$).

Conclusions: Clopidogrel, which is used as an anti-aggregate especially in cardio-vascular diseases in clinical practice, has an inhibitory effect on bladder contraction (p-p and area), and the strongest inhibitor effect was observed at 10µM dose.

Keywords: Clopidogrel; Isolated Organ Bath; Contraction; Bladder, Rat.

INTRODUCTION

Excessively active bladder, urinary incontinence, urinary urgency and frequency are urinary system pathologies that affect millions of people and disrupt the life quality (1). The contractile activity of the smooth muscles of the bladder is of vital importance in the filling of the bladder in urination physiology. The coordinated and gradual contraction of the smooth muscle of the bladder is extremely important for the filling of the bladder and urination. In case this coordination is disrupted, it is probable that the above-mentioned and similar urinary pathologies might occur (2, 3). For this reason, it is extremely important that the contractile activity of the smooth muscle of the bladder is realized in physiological limits. 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 (4). 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 (5-7).

Clopidogrel is a Thrombocyte Adenosine Diphosphate (ADP) receptor antagonist and is a thienopyridine

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derivative, which is employed in peripheral arterial diseases to prolong bleeding times, disrupting platelet aggregation and reducing blood viscosity. It shows its antagonist effects by avoiding the activation of the glycoprotein (Gp) IIb/IIIa complex through ADP (8). The clopidogrel, which is inactive, is activated with hepatic and/or intestinal cytochrome P450 - CYP3A4 isozyme. It shows its anti-thrombocyte effect by inhibiting the P2Y12, which is the subtype of the thrombocyte ADP receptor (9). The clopidogrel inhibition of the adenosine diphosphatinin causes that there appears a significant reduction in the thrombocyte activation (10). Clopidogrel and its metabolite bind to the recycling plasma proteins, and are converted into carboxylic acid derivatives by passing through a fast hydrolysis process (11). Clopidogrel is a medicine whose effectiveness has been proven like other platelet inhibitors in reducing the diseases or deaths that are caused by acute coronary syndrome (12-14).

Clopidogrel is an important antiaggregant medicine that was approved by the FDA for treating unstable angina, non-ST Myocardial Infarction (MI), MI disease with ST elevation as a secondary protective agent for MI, store and peripheral artery disease (15). Studies conducted so far on clopidogrel have dealt with the efficacy of the cardiovascular system in general (16). There are not adequate studies that examine the efficiency of clopidogrel on smooth muscle contraction pattern. In a study conducted by Guglielmina et al., it was shown that clopidogrel had an endothelium-independent vasodilator effect on caudal artery (17).

In the above-given data, it was mentioned that P2Y receptor family might be influential on the contractile activity of the bladder, and the importance of determining which receptor subtype of this family might be influential. In the light of all these data, in the present study of ours, we aimed to examine the efficiency of clopidogrel on the contractile activity of the bladder of rats, and to show the efficacy of P2Y12 receptor on the contractile activity of the bladder. Is it possible that the results obtained in the present study enlighten an important dark point in the physiology of the bladder contractile activity, and be a treatment option in the bladder contractile activity disorders by revealing the effectiveness of clopidogrel on the smooth muscle of the bladder contractile activity as well as its antiaggregant activity?

MATERIAL and METHODS

Animals

Seven adult (3-5 months old) female Wistar-albino rats, weighing 250-300 grams were obtained from the University of Firat Experimental Research Unit (Elazig, Turkey). Female rats used in the experiments were involved in the diöstrus cycle. cycles were determined by vaginal smear. All the experiments were approved by Firat University, Ethical Committee, and the rats were used in compliance with the guidelines for the ethical use of laboratory animals. The female rats used in the study had vaginal smear before the experiment and female rats in the diostrus period were used in the experiments.

Drugs

The drugs used were acetylcholine chloride (Sigma-Aldrich, USA) and Clopidogrel (Sanofı Pharmaceuticals, Turkey). Clopidogrel tablets, containing 75 mg clopidogrel hydrogen sulfate were used to imitate human usage and supplied from a local pharmacy. clopidogrel hydrogen sulfate was crushed in a mortar and solved in Krebs- Henseleit solution and prepared just before starting the experiment.

Preparations

For examination of the functional role of clopidogrel in the bladder, firstly The female rats used in the study had vaginal smear before the experiment and female rats in the diostrus period were used in the experiments. rats were decapitated one by one by guillotine and then the abdominal wall was cut through midline and opened. Then whole urinary bladder was quickly removed, cleaned from fat and other tissues, and placed in Krebs-Henseleit solution (composition in mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.25, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, EDTA 0.03) and it was formed into a flat sheet. Afterward, longitudinal strips were formed from sheets, measuring 2–4 × 6–12 mm. Every strip was fixed by a needle to a petri dish, containing solid paraffin. Then they were tied by silk suture and suspended in isolated tissue organ baths (5, 10 and 20 mL) containing Krebs- Henseleit solution and bubbled with 95% O2/ 5 % CO2 at 37°C. The tension formed by the tissues was measured using transducers MP150 instrument (Biopac Systems, Inc., U.S.A.).

Protocol

All tissues were left to equilibrate for 60 minutes with rinsing every 30 minutes under a resting tension of 1.5 g before starting the experiment. After equilibration, various concentrations of acetylcholine 10 µM was added to all baths. After one hour of rinsing, 0.1µM, clopidogrel was given and incubated 30 minutes. After 30 minutes, again acetylcholine was added the same dose without rinsing and then this protocol was repeated with 10µM clopidogrel doses.

Statistical Analysis

All values were expressed as mean ± standard deviation. The conformity with the normal distribution was examined through Shapiro Wilk test. Statistical evaluation was performed using the Paired T-Test. For all analyses, $P < 0.05$ was considered to be statistically significant. The SPSS statistical program, version 22.0 for Windows (licensed by Firat University, Elazig, Turkey) was used for data analysis.

RESULTS

First Findings (0.1µM Clopidogrel)

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 0.1μM clopidogrel was administered (Figure 1, 2). This dose was about 1/100 of the dose administered per kilogram in human. The mean area values before and after administration of 0.1μM clopidogrel were 100±0.0 and 21±11, respectively (Figure 1). The mean peak-to-peak values before and after administration of 0.1μM clopidogrel were 100±0.0 and 37±2, respectively (Figure 2). According to these results, it was observed that 0.1μM Clopidogrel led to a statistically significant reduction in the area (Figure 1), peak-to-peak (Figure 2) values of bladder contractions when compared with pre-administration (p<0.001).

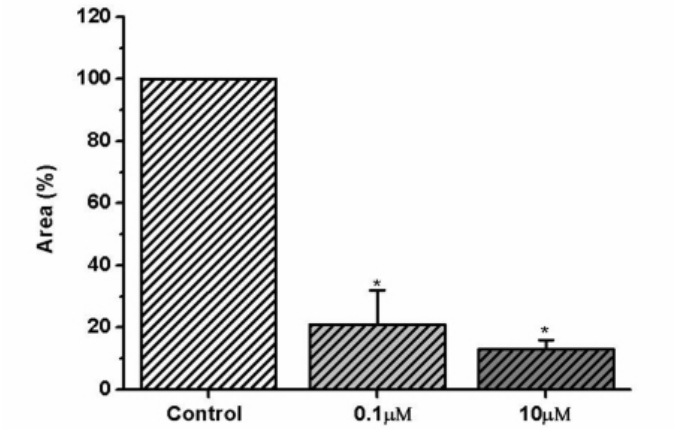

Figure 1. Effects of clopidogrel on contractions of the urinary bladder strips. The area of strips was decreased by clopidogrel at both 0.1μM and 10μM dose (*p < 0.001; n = 7)

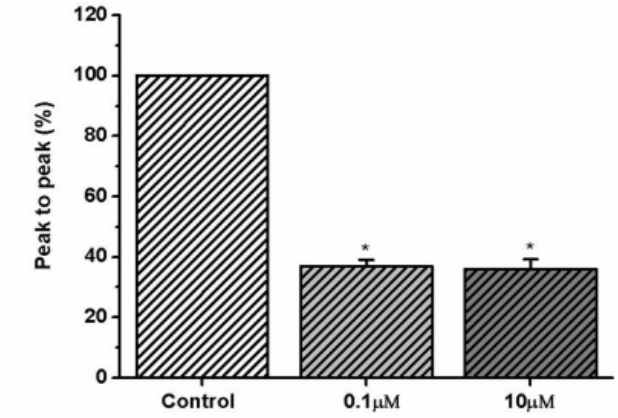

Figure 2. Effects of clopidogrel on contractions of the urinary bladder strips. The peak to peak amplitude of strips was decreased by clopidogrel at both 0.1μM and 10μM dose (*p < 0.001; n = 7)

Second Findings (10μM Clopidogrel)

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 10μM Clopidogrel was administered (Figure 1, 2) This dose was about the dose administered per kilogram in human. The mean area values before and after administration of 10μM Clopidogrel were 100±0.0 and 13±3, respectively (Figure 1). The mean peak-to-peak values before and after administration of 10μM Clopidogrel were 100±0.0 and 36±3.2, respectively (Figure 2). According to these results, 10μM Clopidogrel led to a statistically significant reduction in the area (Figure 1), peak-to-peak (Figure 2) values of bladder contractions when compared with pre-administration (p<0.05).

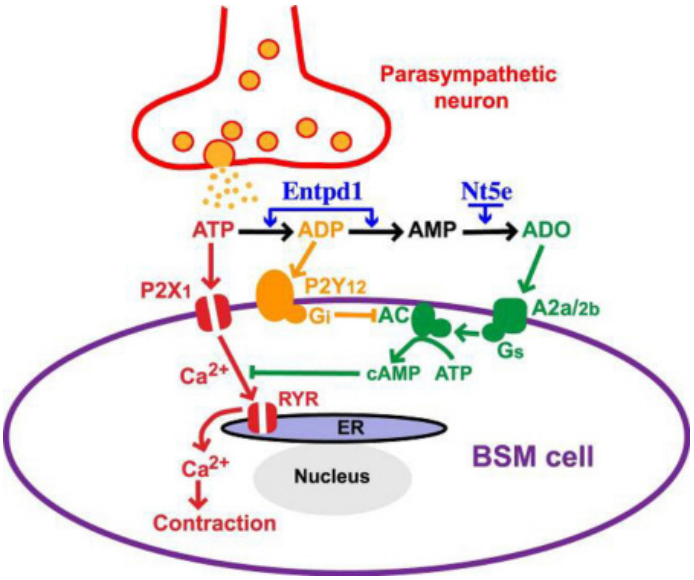

Figure 3. Proposed working model. In addition to activating P2X1 receptor on BSM, parasympathetically released ATP during micturition will also be converted to ADP quickly, which further activates P2Y12R, and inhibits adenylyl cyclase activity to decrease intracellular cAMP level. This will cause BSM contraction by an unknown mechanism and may also potentiate P2X-mediated BSM contraction force, possibly by inhibiting adenosine-mediated relaxation. ADP will be further converted by Entpd1 and Nt5e on BSM to adenosine, which binds to adenosine receptors and activates adenylyl cyclase to increase intracellular cAMP level, and eventually relaxes BSM after urinary voiding. Thus, adenylyl cyclase may function as a key protein and common pathway for the crosstalk between P2Y12R and adenosine receptors, and Entpd1 and Nt5e may serve as the temporal regulators for the crosstalk between P2Y12R and adenosine receptors. The dynamic interplay of these positive and negative signals may play a crucial role in modulating BSM purinergic contractility and could result in disordered bladder contractility when disrupted (18).

DISCUSSION

According to the results we obtained from thisstudy that were conducted in in vitro fashion, clopidogrel has an inhibitory effect on the contractions induced by ach in the smooth muscle of the bladder. In the light of these findings, clopidogrel might be used in urinary pathologies like excessively active bladder since it inhibits the contractions of the bladder and has an antiaggregant activity in clinical use. In addition to these, since clopidogrel is a P2Y12 receptor antagonist, we believe that P2Y12 receptor pathway might be influential in physiological mechanisms of the contractile activity of the bladder.

Coordinated and proper contraction of the smooth muscle

of the bladder is extremely important in the filling of the bladder and in the physiology of urination (3). According to the results obtained so far in previous studies, the contraction of the smooth muscle of the bladder is regulated partly by the purinergic signal (4,5,14). The extracellular purines like ATP and UTP might be excreted in neuromuscular junctions as neurotransmitters or from somatic cells as a response to the environmental stress; and might also regulate the bladder contractile activity after it binds to P2X (1, en7) and P2Y (1, 2, 4, 6, 11, - 14). The P2Y12 receptor serves especially in the physiological pathway, which ensures that the bladder relaxation occurs (Figure 3). In the present study, we planned the fashion of the study based on this information; and investigated the role of clopidogrel, which is a P2Y12 receptor antagonist, in the contractile activity of the smooth muscle of the bladder. The findings we obtained in our study show that -in line with this physiological mechanism- clopidogrel inhibits the contractions of the bladder in a dose-dependent manner. Based on these data, we believe that clopidogrel is a pharmacological agent, which might be employed in conditions like excessively active bladder with its antiaggregant effect in clinical use. In a previous study that was conducted with Entpdl and Nt5, which serve on the P2Y12 receptor pathway, in knock-out rats, showed that the inactivation of this pathway led to the emergence of constant bladder contractions (Figure 3) (18).

The present data suggest the possibility of employing the inhibition of P2Y12 receptor pathway in a new treatment option in pathologies that appear in advanced age due to impaired bladder function like excessively active bladder, urinary incontinence, urinary urgency and frequency. Excessively active bladder is a urinary system disorder, which is characterized by urgent urinary incontinence, urgency, frequency and nocturia especially in further ages (19). Symptoms might be very disturbing and affect the life quality of patients negatively (20, 21). Although its etiology is not known well, the prevalence of it is higher in patients who have advanced diabetes mellitus and conjunctival heart failure (22,23). One of the pharmacological treatment options employed most commonly is antimuscarinic drug treatment (24). It is extremely important to develop treatment approaches for the treatment of this disease, which influences the life quality of people at such a great extent. In the light of the results obtained in the present study, the question has come to the agenda "Is it possible that clopidogrel might be a new pharmacological agent, which may be employed in the treatment of excessively active bladder?". The fact that the dose of clopidogrel, which we used in the present study, was approximately 1% of the antiaggregant dose used in humans made us consider that it might be quite favorable for using in excessively active bladder in terms of dose safety as well.

CONCLUSION

Consequently, the findings of the in vitro study of ours showed that clopidogrel, which is a P2Y12 receptor antagonist, has inhibitory effects on the contractions of the bladder induced by Ach. In the light of these data, we believe that clopidogrel, which is used as an antiaggregant agent, might be a pharmacological agent employed in the treatment of excessively active bladder..

Competing interests: The authors declare that they have no competing interest.
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Ethical approval: All the experiments were approved by Firat University, Ethical Committee, and the rats were used in compliance with the guidelines for the ethical use of laboratory animals.

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