

Original article

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Effectiveness of platelet aggregation with different cyclooxygenase sensitivity to non-steroidal anti-inflammatory drugs in patients with nephrolithiasis

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Abstract:

Objective: to reveal the dependence of the efficiency of platelet (PL) aggregation on crosstalk between signaling pathways associated with stimulation of the thromboxane receptor (TxR) and purine P2 receptors at different activity of cyclooxygenase (COX) during the administration of non-steroidal anti-inflammatory drugs (NSAIDs) in patients with nephrolithiasis (NLT). **Materials and Methods.** Our study was prospective in its nature. It included 60 patients with NLT who were prescribed non-selective NSAIDs as part of medical expulsive therapy (MET) for analgesia. The cohort of patients was distributed among two groups: with effective (Group 1, n=30) and ineffective (Group 2, n=30) COX inhibition. The activity of the TxR and purine P2 receptors (P2X1 and P2Y receptors) of PL was assessed on a Chrono-Log Hematology Analyzer (USA). Agonists (adenosine triphosphate, adenosine diphosphate and arachidonic acid) were used at EC50 and EC10 concentrations. **Results.** In Group 1, after 72 hours of MET, the activity of the TxR and P2 receptors of PL decreased to the level of hyporeactivity. Regulation of the compensatory response of PL to hematuria was ensured through the synergism of P2X1 and P2Y receptors, TxR, and P2X1 receptors. In Group 2, after 72 hours of MET, the hyperreactivity of the TxR and P2 receptors persisted. The maximum increase in PL aggregation was achieved with synergism between the TxR and P2Y receptors. **Conclusion.** The effectiveness of PL aggregation in hematuria of patients with NLT during the administration of NSAIDs is determined by intracellular signaling associated with the TxR and P2 receptors.

Keywords: nephrolithiasis, hematuria, non-steroidal anti-inflammatory drugs, platelet receptors

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Introduction

Until now, there is no understanding of the reasons for the different functional activity of platelets (PL) during long-term administration of non-steroidal anti-inflammatory drugs (NSAIDs). Moreover, the lack of knowledge regarding the mechanisms of regulation of the PL component of hemostasis during cyclooxygenase (COX) blockade encodes a high risk of hemorrhage in patients with cardiovascular diseases. The severity of hematuria in patients with nephrolithiasis (NLT) is likely due to the varying sensitivity of the COX enzyme to NSAIDs, which are prescribed for analgesia in patients with NLT. With long-term prescription of high doses of non-selective NSAIDs to patients to relieve renal colic, heterogeneity in the activity of PL receptors is possible due to both the individual reactivity (resistance) of the body and the avoidance of the inhibitory effects of pharmaceuticals by COX [1]. Under physiological conditions, activation of COX-1 and the synthesis of thromboxane A2 (TxA2) ensure aggregation of PL, while the secretion of adenosine diphosphate (ADP) from α-granules enhances the inducing signal of the thromboxane receptor (TxR) [2]. This

phenomenon is caused by the coupling of the TxR and purine P2Y receptors with the G-protein of the Gq signaling pathway, which ensures an increase in the intracellular content of Ca²⁺. If hemostasis is impaired due to the administration of non-selective NSAIDs, it is possible to limit hemorrhage by turning on compensatory mechanisms of PL. However, it is not clear whether the efficacy of PL aggregation is related to the optimization of intracellular signaling during COX inhibition. In the context of deciphering the mechanisms for optimizing signaling pathways during a compensatory response, the synergism of PL receptors attracts attention. Of particular interest is the interaction of the TxR and purine P2 receptors, since the crosstalk between signaling pathways associated with stimulation of the TxR (via Gq and Gq12/13 proteins), P2Y receptors (through Gi and Gq proteins), and P2X1 receptor (ion channel dependent on adenosine triphosphate, ATP) is possible.

Objective – to establish the dependence of the efficiency of PL aggregation on crosstalk between signaling pathways associated with stimulation of the TR receptor and purine P2

receptors at different activity of COX against the background of the prescription of non-selective NSAIDs in patients with NLT.

Materials and Methods

The study included 60 patients with evidence of calculi in the urinary tract provided by the imaging technique. During hospital admission, all patients underwent a comprehensive clinical examination according to the traditional scheme adopted for diagnosing urolithiasis: patient complaints, history taking, physical examination, clinical and instrumental studies, ultrasound imaging and computed tomography of the kidneys, microbiological urine culture, and laboratory tests of blood and urine. Inclusion criteria: men and women ≥ 18 years of age with a calculus or calculi in their urinary tract. Exclusion criteria: use of antiplatelet agents and statins within the last 6 months; coagulopathy, thrombocytopenia, menstruation; acute vascular complications within 6 months of the hospitalization date; the presence of an artificial pacemaker or heart valve, coronary stents; severe concomitant pathology.

The mean calculus size was 13.4 ± 1.1 mm (min–max: 8.0–30.0 mm). Standard medical expulsive therapy (MET) included a non-selective NSAID (diclofenac sodium, 100–150 mg/day), an $\alpha 1A$ blocker (tamsulosin, 0.4 mg/day) and antibiotics. The cohort of patients was distributed among two groups depending on the level of residual activity of COX achieved after 72 hours of MET. In Group 1 ($n=30$) with effective inhibition of COX, hyporeactivity of the TxR was reproduced (reduced PL aggregation $<45\%$ caused by arachidonic acid, AA); while in Group 2 ($n=30$) with ineffective inhibition, we recorded the hyperreactivity of TxR (increased PL aggregation exceeding 55.0%).

The study was approved by the Ethics Committee of Donetsk State Medical University of the Russian Ministry of Healthcare. The protocol for studying the PL aggregation complied with the European recommendations for the standardization of aggregometry [3]. Analysis of the functional activity of receptors was carried out in vitro on a PL suspension for 5 days of MET, for which PL-enriched plasma was isolated from peripheral blood by centrifugation; the PL content in $1 \mu\text{l}$ was $200,000 \pm 20,000$. To determine the ability of NSAIDs to inhibit COX, PL was preincubated with acetylsalicylic acid, after which AA was added. In this test, the criterion for residual activity of COX is the sensitivity of the receptor to Tx_{A2}, the synthesis of which during the metabolism of AA depends on the inhibitory effect of acetylsalicylic acid.

The activity of purine P_{2Y} receptors (P_{2Y1} and P_{2Y12}), the sensitivity of which reflects the effectiveness of the autocrine response of PL, was studied. PL aggregation was assessed using the turbidimetric method on a Chrono-Log Hematology Analyzer (USA). ADP, ATP and AA were used as inducers. The study used threshold and subthreshold concentrations of agonists, which in the control group ($n=5$ healthy donors) caused PL aggregation at the level of 50.0% (EC₅₀) and 10.0% (EC₁₀), respectively. The use of an agonist at a concentration of EC₅₀ is substantiated by the necessity to assess the sensitivity of the PL receptor (normo-, hypo- and hyperreactivity), while EC₁₀ allows analyzing the interaction of several receptors (synergism), which is

manifested by potentiation of PL aggregation values upon stimulation of each receptor.

The characteristics of aggregograms were assessed by the amplitude of platelet aggregation (APL, %), the maximum slope of the curve (Slope, % per min) and the area under the curve (AUC, U). Microhematuria was established by the presence of three or more red blood cells (rbc) in the field of view (FOV) by microscopy of urine sediment and classified as mild (3–10 rbc/FOV), moderate (11–49 rbc/FOV) or severe (50–100 rbc/FOV).

The nature of distribution of individual values for each characteristic in the samples was assessed using the Shapiro–Wilk test. Depending on the distribution of characteristics, the Student's t-test, the Mann–Whitney U test, or the Wilcoxon W test were employed. In all cases, differences were considered statistically significant at $p < 0.05$. Statistical analyses were performed using the MedCalc Version 20.019 software. Data are presented as the mean () and standard deviation ($\pm$ SD) or the minimum and maximum values of the indicator (min–max) in the case of a normal distribution law, as well as the median (Me) and interquartile range (Q₁–Q₃) in the case of a distribution other than normal.

Results

After 72 hours of MET, the severity of hematuria in Group 1 was threefold of its severity in Group 2, 54.4 ± 1.3 rbc/FOV and 18.2 ± 1.2 rbc/FOV, respectively ($p < 0.001$). The activity of the TxR decreased by 33.8% ($p < 0.001$) vs. the initial observation period; as a result, receptor hyporeactivity was observed ($42. \pm 1.8\%$). The activity of the P_{2X1} receptor and P_{2Y} receptors decreased as well, by 44.4% and 31.5% ($p < 0.001$), respectively, compared with its initial level, thereby reaching the level characteristic of hyporeactivity ($30.2 \pm 1.0\%$ and $37.6 \pm 1.5\%$, correspondingly). In Group 2, the activity of TxR decreased by 13.4% ($p = 0.008$), while the hyperreactivity of the receptor remained ($59.7 \pm 0.5\%$), which could be due to the resistance of COX to the action of NSAIDs. The activity of the P_{2X1} receptor and P_{2Y} receptors decreased by 13.4% and 10.6% , respectively ($p < 0.001$) by, but remained in the range of hyperreactivity ($60.8 \pm 0.4\%$ and $61.7 \pm 0.7\%$, correspondingly).

After 5 days, the severity of hematuria in Group 1 was twice higher than in Group 2 ($p < 0.001$), 45.6 ± 1.9 rbc/FOV (min–max: 20.0–64.0) and 22.3 ± 1.2 rbc/FOV (min–max: 12.0–32.0), respectively, which can be explained by the different effectiveness of PL aggregation in patients when NSAIDs are prescribed.

In Group 1, there was an increase in TxR activity (by 18.3%) vs. an observation period of 72 hours ($p < 0.001$), i.e., to the level of normoreactivity, which can be understood as a compensatory response of PL aimed at limiting hematuria; the values of the latter decreased by 16.2% ($p = 0.044$) compared with the previous observation period. The activity of P_{2X1} and P_{2Y} receptors increased by 37.1% ($p = 0.028$) and 10.9% ($p = 0.037$), respectively, remaining in the hyporesponsive range of values.

In Group 2, after 5 days, the sensitivity of the TxR was maintained at the level of hyperreactivity ($58.2 \pm 0.7\%$). The activity of P₂ receptors decreased by 8.0% ($p < 0.001$), compared with the observation period of 72 hours, and

remained in the range of hyperreactivity (P2X1 receptors: $55.9 \pm 0.6\%$; P2Y receptors: $56.8 \pm 0.5\%$).

We intended to answer the following research question, "Does the potentiation effect of purine P2 receptors and TxR differ with different sensitivity of COX-1 to NSAIDs?"

In Group 1, after 5 days of MET, it was established that selective stimulation of the P2X1 receptor, P2Y receptors and TxR (agonists of ATP, ADP and AA) yielded similar ($p > 0.05$) values of the APL, Slope and AUC (Table). With simultaneous stimulation of several receptors, the maximum effect of aggregation was generated by the interaction of P2X1 and P2Y receptors. E.g., the values of APL, Slope and AUC exceeded those with isolated stimulation of P2Y receptors by 40.0, 54.5 and 54.0%, respectively ($p < 0.001$), and with isolated stimulation of the P2X1 receptor, by 51.0; 54.5 and 66.2% ($p < 0.001$).

Table. Platelet aggregation parameters with different cyclooxygenase activity in patients with nephrolithiasis upon administration of non-selective non-steroidal anti-inflammatory drugs

Agonists (EC ₁₀)	Indicators of aggregation		
	Aggregation amplitude, %	Slope, % per min	AUC (U)
Group 1			
ADP	11.0 ± 0.5 (9.9–12.1)	13.2 ± 0.8 (11.4–15.0)	17.6 ± 1.1 (15.2–20.0)
AA	10.4 ± 0.4 (9.4–11.4)	13.5 ± 0.8 (11.7–15.3)	17.1 ± 1.1 (14.7–19.5)
ATP	10.2 ± 0.4 (9.3–11.1)	13.2 ± 0.7 (11.7–14.7)	16.3 ± 0.8 (14.5–18.1)
ADP + ATP	15.4 ± 0.6 (14.0–16.7)	20.4 ± 0.8 (18.6–22.1)	27.1 ± 1.2 (24.5–29.6)
ATP + AA	$12.8 \pm 0.6^*$ (11.3–14.3) $P=0.011$	$17.5 \pm 0.7^*$ (15.1–18.9) $P=0.011$	$21.9 \pm 0.8^{**}$ (20.2–23.5) $P=0.001$
ADP + AA	$10.9 \pm 0.4^*$ (9.9–11.8)	$15.2 \pm 0.5^{**}$ (14.1–16.3)	$18.6 \pm 0.8^{**}$ (16.9–20.2)
Group 2			
ADP	11.9 ± 0.5 (10.7–13.1)	16.1 ± 0.9 (14.3–18.1) $P=0.023$	24.2 ± 0.9 (22.1–26.3) $P=0.011$
AA	10.8 ± 0.4 (9.8–11.7)	15.8 ± 1.0 (13.6–18.0) $P=0.005$	20.8 ± 1.0 (18.4–23.1) $P=0.023$
ATP	10.1 ± 0.4 (9.2–11.0)	$12.7 \pm 0.9^*$ (10.7–14.7)	$13.6 \pm 0.6^{***}$ (12.2–15.0) $P=0.016$
ADP + AA	17.5 ± 0.5 (16.5–18.5) $P=0.001$	22.4 ± 0.6 (21.2–23.6) $P=0.001$	34.6 ± 0.7 (33.0–36.1) $P=0.001$
ATP + AA	$14.3 \pm 0.3^{***}$ (13.9–15.1)	$17.9 \pm 0.5^*$ (16.8–19.1)	$25.2 \pm 0.7^{***}$ (23.8–26.7) $P=0.003$
ADP + ATP	14.4 ± 0.5 (13.4–15.3)	16.5 ± 0.5 (15.5–17.5) $P=0.001$	21.3 ± 0.9 (19.5–23.1) $P=0.001$

Data are presented as mean ($\bar{X}$) and standard deviation ($\pm SD$), as well as interquartile range (Q_1 – Q_3). Asterisks indicate the difference in the values of an aggregogram parameter with an indicated combination of agonists at the level of $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) vs. the values for the previous combination of agonists. P, statistically significant differences between the values of the parameter in Group 2 vs. Group 1.

With simultaneous stimulation of the P2X1 receptor and TxR, the values of APL, Slope and AUC exceeded those with isolated stimulation of P2X1 receptors by 25.5% ($p=0.006$), 32.6% ($p=0.002$) and 34.3% ($p<0.004$), respectively, while with isolated stimulation of the TxR, by 23.1% ($p=0.007$), 29.6% ($p=0.005$) and 28.1% ($p=0.003$). It should be noted that the values of APL, Slope and AUC with simultaneous stimulation of the P2X1 receptor and TxR were smaller than those with stimulation of the P2Y receptors and P2X1 receptor by 16.9% ($p=0.040$), 14.2% ($p=0.043$) and 19.2% ($p=0.035$), respectively. Values of PL aggregation with simultaneous stimulation of the TxR and P2Y receptors were comparable to the effect of isolated stimulation of the TxR and P2Y receptors ($p > 0.05$). The values of APL, Slope and AUC with simultaneous stimulation of the TxR and P2Y receptors were smaller than those with simultaneous stimulation of P2Y receptors and P2X1 receptor by 29.2, 25.5 and 31.4% ($p<0.001$), respectively, and also smaller by 14.9% ($p=0.041$), 13.4% ($p=0.039$) and 15.1% ($p=0.020$) than with simultaneous stimulation of the P2X1 receptor and the TxR.

In Group 2, after 5 days of MET with isolated stimulation of PL, judging by the number of PL involved in aggregation (AUC values of aggregograms), the ranking of receptor activity can be presented as follows: P2Y receptors > TxR > P2X1 receptor. When stimulating P2Y receptors, the AUC values were 16.3% higher ($p=0.029$) than when stimulating the TxR, while the values of APL and Slope were the same. The minimal effect of aggregation was yielded upon stimulation of the P2X1 receptor: the Slope and AUC values were smaller than those with isolated stimulation of P2Y receptors by 21.1% and 43.8%, respectively ($p=0.033$ and $p=0.010$), and of the TxR, by 19.6% ($p=0.038$) and 34.6% ($p=0.017$). Compared with Group 1, the values of Slope and AUC were higher: when stimulating P2Y receptors, by 22.0% ($p=0.025$) and 37.5% ($p=0.016$), respectively, while during stimulation of TxR, by 17.0% ($p=0.024$) and 21.6% ($p=0.039$); with stimulation of the P2X1 receptor, AUC values were 16.6% lower ($p=0.043$).

With simultaneous stimulation of P2Y receptors and the TxR, the values of APL, Slope and AUC exceeded those with isolated stimulation of P2Y receptors by 47.1, 39.1 and 43.0% ($p<0.001$), respectively; and with isolated stimulation of the TxR, by 62.0, 41.8 and 66.3% ($p<0.001$). The values of APL, Slope and AUC with simultaneous stimulation of the P2X1 receptor and TxR were smaller than those with stimulation of P2Y receptors and TxR by 18.3% ($p=0.041$), 20.0% ($p=0.013$) and 27.2% ($p=0.029$), respectively. A minimal effect of aggregation was observed during the interaction of P2Y receptors with P2X1 receptor. Values of aggregation with simultaneous stimulation of these receptors were similar to the effect of isolated stimulation of P2Y receptors ($p > 0.05$), i.e., signaling via Gi and Gq proteins was not enhanced.

Relative to the parameters of isolated stimulation of the P2X1 receptor, the values of APL, Slope and AUC were higher by 42.6, 29.9 and 56.6% ($p<0.001$), respectively, which implied an increase in the aggregation effect associated with the opening of the Ca²⁺ channel in the case of simultaneous activation of the P2X1 receptor and P2Y receptors. When stimulating the TxR and P2Y receptors, the values of APL, Slope and AUC exceeded those in Group 1 by 60.5% ($p=0.010$), 47.4% ($p=0.020$) and 86.0% ($p<0.001$), respectively; when stimulating the P2X1 receptor and TxR, the AUC values were 15.1% higher than in Group 1 ($p=0.030$); upon stimulation of P2Y receptors and P2X1 receptor, the values of Slope and AUC were lower by 19.1% and 21.4%

($p < 0.001$), respectively, than in Group 1. The differences in potentiation of the considered combinations of receptors were due to the fact that in Group 2, while maintaining COX-dependent signaling pathway: (a) the synergism of the TxR with P2Y receptors and P2X1 receptor was manifested, due to which the most significant values of aggregation were achieved; (b) the interaction of the signaling pathways of purine P2 receptors was not detected, probably, due to multidirectional changes in activity (the sensitivity of the P2Y receptors increased, whereas the sensitivity of the P2X1 receptor decreased).

Discussion

The sensitivity of patients to non-selective NSAIDs is a matter of debate [4]. Despite the proven risk of hemorrhage in the case of COX-1 inhibition, the causes of different severity of hematuria in patients with NLT remain unexamined. The lack of interest on the part of urologists in the regulating the functional activity of PL is explained by the spontaneous cessation of hematuria within a few days after discontinuation of NSAIDs. Our study made it possible to verify the severity of hematuria, and also established the fact of COX activity restoration upon administration of high doses of non-selective NSAIDs. Previously, a pronounced decrease in COX-1 activity 10 hours after administration of low doses of aspirin and an increase after 24 hours was established [5], but the practical significance of this finding remained unclear.

It is logical to assume that an increase in the activity of the COX enzyme with long-term administration of NSAIDs is accompanied by increased aggregation and limited hematuria. As for patients with hematuria and simultaneous excessive reactivity of the TxR in patients with resistance to NSAIDs, the effect of COX inhibition on the effectiveness of the functioning of signaling pathways in the PL should be recognized. The latter is determined by the synergism of receptors that ensure an increase in the level of intracellular Ca^{2+} in PL. In this regard, it is of interest to study the crosstalk between signaling pathways of the TxR associated with Gq and Gq12/13 proteins, the P2X1 receptor (which is an ionotropic channel), and P2Y receptors associated with the Gi-Gq protein.

In Group 1, when COX activity was restored due to the administration of NSAIDs, compensatory responses of PL with the participation of the TxR and purine P2 receptors were produced, which were manifested by an increase in the rate of intracellular signaling (Slope) and the number of PL involved in aggregation (AUC). Synergisms between the TxR and the P2X1 receptor, and between the purine P2X1 and P2Y receptors were revealed. Since the synergistic effect was evident with simultaneous stimulation of the TxR and P2X1 receptor, while it was absent with simultaneous stimulation of the TxR and P2Y receptors, it is logical to assume the key role of the P2X1 receptor (ATP-dependent ion channel) in increasing Ca^{2+} levels.

The interaction of purine P2X1 and P2Y receptors was manifested by the most pronounced effect of enhanced aggregation; therefore, optimal signaling in the PL was achieved with the simultaneous functioning of the ATP-dependent channel and P2Y1–P2Y12 receptors. It has been proven that the P2X1 receptor enhances the increase in the level of intracellular Ca^{2+} in the PL caused by the P2Y1

receptor [6]. It is likely that P2X1-dependent amplification of signaling associated with the Gq protein occurs as a result of potentiation of inositol-1,4,5-triphosphate receptors and/or phospholipase C. The increased effect of extracellular ATP on PL aggregation upon administration of NSAIDs may reflect the plasticity of purinergic signaling ensuring optimization of signaling pathways depending on the residual COX activity. The persistence of hematuria despite the achieved optimization of signaling pathways indicates the following: (a) changes in TxA2 synthesis may distort the response to other agonists; (b) signaling associated with the functioning of the TxR, P2X1 receptor, and P2Y1 and P2Y12 receptors does not ensure restoration of the proaggregant status of PL. Studying the synergism of other PL receptors involved in aggregation (in particular, the GPVI receptor for collagen) will allow developing new ways to maintain hemostasis in patients with COX inhibition.

In Group 2, regulation of PL reactivity with COX resistance to NSAIDs (preservation of TxR normoreactivity) is characterized, compared with Group 1, by higher levels of PL aggregation caused due to stimulation of P2Y receptors and TxR, and by lower values upon stimulation of P2X1 receptor, when COX inhibition was generated (TxR hyporeactivity was achieved).

Synergism of the TxR with purine P2Y receptors yielded increased PL aggregation, while simultaneous stimulation of the TxR and P2X1 receptor did not significantly change the aggregation parameters. Consequently, the absence of a significant effect with simultaneous stimulation of P2X1 and P2Y receptors allows concluding that the opening of the ATP-dependent ion channel and signaling associated with Gi and Gq proteins does not provide a critical increase in the level of intracellular Ca^{2+} in the PL for increasing the PL aggregation.

Probably, with excessive stimulation of receptors (receptor hyperreactivity), the achieved level of signaling does not undergo subsequent changes. Increased aggregation is achieved via signaling associated with Gq12/13 protein, as well as Gi and Gq proteins. Hence, the interaction of signaling pathways associated with the Gi protein (P2Y receptor) and Gq12/13 protein (TxR) plays a key role in increasing PL aggregation during COX resistance to non-selective NSAIDs. Previously, it was established that the TxA2 analog (U46619) causes tyrosine phosphorylation of various proteins, including FAK (focal adhesion kinase), Src and Syk kinases, regardless of signals emitted by $\alpha I\text{Ib}\beta 3$ integrin or P2Y receptors [7].

Synergism between the TxR and trimeric G protein-coupled receptors is implemented through activation of ERK2 (extracellular signal-regulated kinase) [8], as well as Rho kinase, independently of p38 MAP kinase (p38 mitogen-activated protein kinase) [9]. It has been proven that signaling through the Gq12/13 protein is necessary for the activation of the ERK2 enzyme, which modulates increased PL aggregation [10].

Conclusion

Against the background of the administration of NSAIDs, PL aggregation can be ensured simultaneously by several signaling pathways associated with the opening of the ATP-dependent Ca^{2+} channel (P2X1 receptor), Gi and Gq proteins (P2Y receptors), and Gq and Gq12/13 proteins (TxR for TxA2). In Group 1 (effective COX inhibition), after 72 hours

of MET, the activity of TxR, purine P2X1 receptor and P2Y PL receptors decreased and reached the level of hyporeactivity. Against the background of NSAID administration, restoration of TxR activity to the level of normoreactivity was observed after 5 days; the activity of P2X1 and P2Y receptors remained in the range of hyporeactivity. The key role in enhancing PL aggregation was played by the P2X1 receptor. The stimulation of the latter increased the efficiency of the signaling pathways associated with both the P2Y receptors and the TxR. The interaction of purine P2X1 and P2Y receptors had a more pronounced inducing effect on aggregation parameters than simultaneous stimulation of the TxR and P2X1 receptor.

In Group 2, with COX resistance, hyperreactivity of the TxR and P2 receptors persisted during the administration of NSAIDs. Regulation of PL aggregation on day 5 with COX resistance to NSAIDs (preservation of TxR hyperreactivity) was characterized by higher parameter values of the aggregogram when stimulating P2Y receptors and TxR, or by lower values when stimulating the P2X1 receptor, vs. those in Group 1 given that COX inhibition was reproduced (TxR hyporeactivity). The increase in the rate of intracellular signaling (Slope) and the amount of PL involved in aggregation (AUC) was reproduced when the TxR synergized with the P2Y receptors and the P2X1 receptor. Optimal modulation of the compensatory response of PL to hematuria was ensured via the synergism of the TxR and P2Y receptors. These results confirmed the possibility of enhancing PL aggregation through stereotypical signaling mechanisms associated with stimulation of P2Y receptors (via Gi and Gq proteins) and TxR (via Gq and Gq12/13 proteins) in the case of intact synthesis of ThA2 in PL. The complexity of interactions between the signaling pathways of the TxR and purine P2 receptors, which determines the aggregation activity of PL under conditions of blockade of COX-1 and COX-2, makes it possible to establish promising directions for pharmacological correction aimed at preventing hematuria in NLT.

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Conflict of interest: none declared by the authors.

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