Quinolinic acid (QA) is synthesized from L-tryptophan via the kynurenine pathway throughout the sequential conversion to 2-amino-3-carboxymuconate-semialdehyde (ACMS). Further conversion of QA to the essential cofactor NAD+ is catalyzed by quinolinate phosphoribosyltransferase (QPRT) (1). Under inflammatory conditions, QA accumulates within the central nervous system following immune activation and inflammation (2, 3) where mediates N-methyl-D-aspartate neuronal excitotoxicity leading to neural cell damage and dysfunction (4). QA may also enhance the release of synapticosomal glutamate as a consequence of the inhibition of glutamate uptake into the astrocytes, can decrease the activity of antioxidant enzymes promoting ROS production and generating lipid peroxidation, inhibits the activity of mitochondrial complexes leading to energetic dysfunction, activating caspasas and releasing cytochrome c (5, 6) and thereby play a crucial role in induction, development and progression of many neurodegenerative diseases (7).

Increased concentration of QA was also observed in mononuclear cells in Alzheimer’s patients (8) and serum of patients with liver cirrhosis as an indicator of hepatic dysfunction (9). Recent papers reported that QA, as tryptophan-derived uremic toxin, could be considered as a factor linking chronic kidney disease in CKD patients with disturbances in hemostatic profile (10-12). Studies in CKD patients pointed to the a contribution of QA to carotid atherosclerosis (13), endothelial dysfunction (14), hypercoagulability (15) or hyperfibrinolysis (16) and revealed QUINOLINIC ACID DOES NOT INFLUENCE COAGULATION PROFILE, NOR FIBRINOLYTIC ACTIVITY, UNDER PHYSIOLOGICAL CONDITIONS IN RATS

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Abstract: Disturbances of the hemostatic system are commonly observed in chronic kidney disease (CKD) patients. Along with CKD progression, the levels of tryptophan-derived uremic toxins increase, including quinolinic acid (QA). The objective of the study was to evaluate the effect of QA, a representative of tryptophan metabolites, on coagulation and fibrinolytic activity in male Wistar rats in vivo and platelet activity in vitro. Rats received QA dissolved in drinking water in doses of 3, 10, and 30 mg/kg (or water, VEH) for 14 days. Next, the following parameters were measured in the rat’s whole blood or plasma ex vivo: thromboelastometric (ROTEM) parameters, standard coagulation parameters and fibrinolytic parameters. To evaluate the direct effect of QA on coagulation and platelet activity, blood from control rats was drawn and analyzed in vitro in the following scheme: samples of whole blood were incubated with QA (100 µM) before thromboelastometric (ROTEM) analysis and collagen-induced platelet aggregation, or samples of platelet-rich plasma (PRP) were incubated with QA (100 µM, 1 and 2 mM) 10 min before collagen (1 µg/mL) or ADP (10 µM)-induced platelet aggregation. QA administrated for 14 days in drinking water had no effect per se on activation of coagulation and fibrinolytic parameters in rats ex vivo. Similarly, no changes were observed in whole blood incubated directly with QA regarding coagulation parameters or collagen-induced platelet aggregation. QA inhibited ADP-induced platelets aggregation in PRP only at higher concentrations of 1 and 2 mM and when aggregation was initiated by the addition of 10 µM ADP in vitro.

Keywords: quinolinic acid (QA), uremic toxin, rats, hemostasis, platelets, coagulation, fibrinolysis

Quinolinic acid (QA) is synthesized from L-tryptophan via the kynurenine pathway throughout the sequential conversion to 2-amino-3-carboxymuconate-semialdehyde (ACMS). Further conversion of QA to the essential cofactor NAD+ is catalyzed by quinolinate phosphoribosyltransferase (QPRT) (1). Under inflammatory conditions, QA accumulates within the central nervous system following immune activation and inflammation (2, 3) where mediates N-methyl-D-aspartate neuronal excitotoxicity leading to neural cell damage and dysfunction (4). QA may also enhance the release of synapticosomal glutamate as a consequence of the inhibition of glutamate uptake into the astrocytes, can decrease the activity of antioxidant enzymes promoting ROS production and generating lipid peroxidation, inhibits the activity of mitochondrial complexes leading to energetic dysfunction, activating caspasas and releasing cytochrome c (5, 6) and thereby play a crucial role in induction, development and progression of many neurodegenerative diseases (7). Increased concentration of QA was also observed in mononuclear cells in Alzheimer’s patients (8) and serum of patients with liver cirrhosis as an indicator of hepatic dysfunction (9). Recent papers reported that QA, as tryptophan-derived uremic toxin, could be considered as a factor linking chronic kidney disease in CKD patients with disturbances in hemostatic profile (10-12). Studies in CKD patients pointed to the a contribution of QA to carotid atherosclerosis (13), endothelial dysfunction (14), hypercoagulability (15) or hyperfibrinolysis (16) and revealed

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that QA, among other uremic toxins (17, 18) may participate in the dysregulation of hemostasis and thereby progression of cardiovascular disease (CVD) (19). Investigations regarding associations between uremic toxins and hemostasis revealed that tryptophan metabolites exerted distinct effects on hemostasis, for example, elevated concentrations of anthranilic acid (AA) were associated with elevated parameters of fibrinolysis in CKD patients (20).

Hemostasis is a complex physiological process that maintains normal blood flow in the circulation (21) it also reacts to a vessel injury by activating platelets and coagulation factors to produce platelet thrombus stabilized by insoluble fibrin (22). Hemostasis is also controlled by endothelial cells which under physiological conditions are required to prevent thrombus formation by exerting anti-coagulant properties and counteracting platelet activation and aggregation (23). During fibrinolysis, the accumulation of fibrin is counteracted by activation of tissue plasminogen activator (t-PA) and balanced by the presence of fibrinolysis inhibitors among which plasminogen activator inhibitor (PAI-1) plays the particularly important role (24). A variety of thrombogenic agents, including uremic toxins, can impair these balanced antithrombotic properties and can contribute to thrombotic complications (25, 26).

Taking the above-mentioned studies into consideration, we undertook these studies to investigate the influence of QA on hemostasis using two experimental approaches. The first approach in the study was aimed to investigate the influence of QA per se, administrated for 14 days in normal rats, on coagulation and fibrinolytic parameters ex vivo. The second approach was aimed to evaluate the direct influence of QA on coagulation parameters and platelet aggregation in blood samples from normal rats in vitro. The results were presented and discussed in this paper.

**EXPERIMENTAL**

**Animals**

Adult male Wistar rats (Wistar Cmdb: Wi) six weeks old, weighting 190 – 200 g were purchased from the Centre of Experimental Medicine (CEM), Medical University of Bialystok (UMB), Poland. Rats were grouped in individually ventilated cages and maintained in an environment-controlled room (12 h light/12 h dark cycle, 21 ± 2°C, 55% relative humidity, 15 air changes/h) and had access to sterilized tap water and standard rat or mice chow ad libitum. All experimental procedures involving animals were approved by the Local Bioethics Committee on Animal Testing UMB (Permit Number: 125/2015) and were conducted in accordance with the institutional guidelines, which are in compliance with national and international laws, including EU
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Directive 2010/63/EU for animal experiments and the Guidelines for the Care and the Use of Animals in Biomedical Research (Giles 1987). The 3R rule ("Replacement, Reduction and Refinement") was respected in the study.

Chemicals
Quinolinic acid (QA) (Sigma-Aldrich); pentobarbital (Biowet, Poland); calcium chloride (CaCl₂), sodium chloride (NaCl) (Sigma-Aldrich, Germany); reagents for prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT) and fibrinogen (Fg) levels (HemosIL Instrumentation Laboratory, USA); ready-to-use ELISA kits for rat plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (t-PA) (Innovative Research, USA); collagen (Chrono-Log Corp., USA); ADP (Sigma Chemical Co, St. Louis, MO, U.S.A).

Experimental design for in vivo experiment
Wistar rats were randomly assigned into the control group (VEH, n = 16) and the QA group (n = 16 rats/group). The 14-day supplementation timing and QA dosage in rats were chosen on the basis of our previous experiments in rats with induced carotid arterial injury thrombosis model. The results showed that QA affected hemostatic parameters in rats receiving QA for 14 days. Therefore, we repeated the same supplementation timing and QA dosage in the presented study. The experimental procedures were illustrated in Figure 1.

Selection of QA dose for in vivo experiment
Doses in the 14-day experiment were selected in logarithmic dose sequences of 3, 10, and 30 mg/kg. Accordingly to our preliminary experiments with rats, we determined that the average daily consumption of water, with or without QA, did not exceed 30 mL/day/rat weighting 190-200 g. The average daily consumption of water was measured under the same experimental conditions for 2 rats housed in one cage. According to these calculations, each dose of QA in drinking water was prepared ex tempore each day of the 14 days of the experiment and were adjusted to the rats’ average daily water consumption and body weight. The rats were observed daily for survival, behavioral and clinical signs of toxicity.

Blood collection and samples preparation for ex vivo analysis
In the 14th day of the experiment, the rats were anesthetized with pentobarbital (50 mg/kg). The volume of blood needed for ex vivo analysis, including thromboelastometric (ROTEM) parameters was taken from controls and QA treated groups. Blood samples were collected into anticoagulant 3.13% trisodium citrate from right ventricle. Plasma for ex vivo analysis of coagulation parameters and fibrinolysis parameters was prepared by centrifuging the blood at 3500 × g for 20 min at room temperature.

Thromboelastometry (ROTEM) parameters (ex vivo analysis)
Thromboelastometry analysis was performed using the ROTEM system (Tem International GmbH, Manheim, Germany) according to the method described here (27, 28) in a whole blood by measuring: clotting time (CT), clot formation time (CFT), alpha angle (α), and maximum clot firmness (MCF). Coagulation was initiated with calcium chloride (12 mM final concentration in samples) and examined with the NATEM test.

Coagulation parameters (ex vivo analysis)
Prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), and fibrinogen (Fg) levels (Clauss method) were determined in rat plasma according to the manufacturer’s instructions using the Coag-Chrom 3003 Coagulometer and standard reagents (Bio-ksel, Poland).

Fibrinolytic parameters (ex vivo analysis)
Total concentration and concentration of the active form of tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) were determined by ELISA techniques, at 25°C according to the manufacturer’s instructions using a microplate reader (ELx808, BioTek Instruments, Inc., United States).

Selection of QA concentration for in vitro analysis
The amount of QA was selected empirically to achieve final concentration in the volume of the sample in vitro (100 µM) and to be relevant to the highest dose used in vivo (30 mg/kg). In the analysis of aggregation in PRP the amount of QA was increased to a higher final concentration of 1 mM and 2 mM to determine the action threshold of QA under in vitro conditions.

Blood collection and samples preparation for in vitro analysis
The volume of blood needed for in vitro analysis was taken from the control group into anticoagulant 3.13% trisodium citrate from the right ventricle.
Next, blood specimens were divided into three cuvettes to prepare samples for thromboelastometry analysis, platelet aggregation in a whole blood and PRP analysis. Samples were mixed with QA or VEH and were incubated for 10 minutes and vigorously stirred in cuvettes at 37°C at 1000 rpm before in vitro analysis.

**In vitro analysis of thromboelastometric (ROTEM) parameters and platelet aggregation**

Thromboelastometry analysis was performed using the ROTEM system (Tem International GmbH, Manheim, Germany) according to the method described in (27, 28). Samples were mixed with QA (100 µM) or VEH (0.9% NaCl), and incubated for 10 minutes and vigorously stirred in cuvettes at 37°C at 1000 rpm before analysis. Coagulation was initiated with calcium chloride (12 mM final concentration in samples) and examined with the NATEM test. Measured parameters included: CT, CFT, α angle, and MCF.

Collagen-induced platelet aggregation in whole blood was analyzed using the impedance method in a Whole Blood Lumi-Aggregometer (Chrono-log Corp., USA) according to manufacturer’s protocol. Samples were mixed with QA (100 µM) or VEH (0.9% NaCl), and incubated for 10 minutes and vigorously stirred in cuvettes at 37°C at 1000 rpm before analysis. Aggregation was initiated by the addition of collagen (2 µg/mL). Changes in electric resistance (impedance) were registered during 6 min and were expressed as the maximal extent of aggregation (%; or maximal amplitude), slope of the curve, the latency time (lag phase) and area under the curve (AUC).

Collagen-stimulated and ADP-stimulated platelet aggregation in PRP was analyzed with the optical method in a Lumi-Aggregometer (Chrono-log Corp., USA) according to manufacturer’s protocol. Samples were mixed with QA (100 µM, 1 mM or 2 mM) or VEH (0.9% NaCl), and incubated for 10 minutes and vigorously stirred in cuvettes at 37°C at 1000 rpm before analysis. Aggregation was initiated by the addition of collagen (1 µg/mL) or ADP (10 µM). Changes in light transmission during the platelet aggregation were registered during 6 min. The maximal extension of the aggregation curve at the 6th min was expressed as maximal amplitude (%).

**Statistical analysis of results in rats experiment**

The data were analyzed using GraphPad Prism 6 (La Jolla, CA, USA). Graphic design presentation of the results was performed using GraphPad Prism 6. Normally distributed data were presented as mean ± SD, while the non-Gaussian data as median (full-

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![Figure 2](image.png)

**Figure 2.** Effect of 14 days of QA administration in drinking water on thromboelastometric parameters. CT – clotting time; CFT – clot formation time; α – alpha angle, MCF – maximum clot firmness. VEH – control group; QA3, QA10, QA30 – doses of quinolinic acid (mg/kg/24 h)
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The normality of distribution was tested using the Shapiro-Wilk test. The Student t-test or nonparametric Mann-Whitney test were used to compare differences between experimental groups and control group. A two-tailed p < 0.05 was considered to indicate significance.

RESULTS

**Thromboelastometric (ROTEM) parameters (ex vivo analysis)**

Thromboelastometry parameters including: CT (clotting time to the beginning of the fibrin polymerization); CFT (clot formation time displaying kinetics of clot formation); α (alpha angle displaying the dynamics of clot formation); MCF (maximum clot firmness displaying the strength of clot stabilization) were not significantly affected in blood of VEH rats versus QA 3, 10 and 30 mg/kg administrated rats regarding the following ROTEM parameters: CT (220.1 ± 9.49 vs. 225.9 ± 16.27; 243.8 ± 13.78 and 218.1 ± 11.02, respectively); CFT (46.5 ± 2.82 vs. 50.96 ± 4.80; 51.3 ± 3.10 and 46.96 ± 2.08, respectively); α (alpha angle (80.88 ± 0.73 vs. 80.85 ± 0.73; 80.43 ± 0.53 and 81.11 ± 0.44) as well as MCF (76.19 ± 0.48 vs. 77.42 ± 0.73; 76.16 ± 0.49 and 76.07 ± 0.58, respectively). Data were presented in Figure 2.

**Coagulation parameters (ex vivo analysis)**

Standard clotting assay of coagulation did not detect significant differences in starting times of clotting in plasma as follows: PT values were comparable in VEH rats (9.45 ± 0.11) and QA (3; 10; 30 mg/kg) administrated rats (9.62 ± 0.08; 9.28 ± 0.08; and 9.47 ± 0.16, respectively). Similarly, aPTT in VEH rats (23.26 ± 1.23) and QA (3; 10; 30 mg/kg) administrated rats (23.95 ± 0.84; 26.66 ± 1.74 and 28.22 ± 1.70, respectively). TT in VEH rats (32.33 ± 0.90) and QA (3; 10; 30 mg/kg) administrated rats (32.02 ± 1.50; 32.83 ± 0.84; and 30.44 ± 1.08, respectively) did not show statistical significance. Also, fibrinogen concentration had comparable values in VEH rats (1.17 ± 0.02) and all the other groups of rats administrated with QA 3 mg/kg (1.23 ± 0.04), QA 10 mg/kg (1.18 ± 0.03) and QA 30 mg/kg (1.14 ± 0.04). Data were presented in Figure 3.

**Fibrinolytic parameters (ex vivo analysis)**

Fibrinolytic activity of plasma is modulated by its activator (tPA) and inhibitor (PAI-1). No significant differences were found between VEH rats versus QA 3, 10 and 30 mg/kg administrated rats in total concentration of tPA (0.72 ± 0.04 vs. 0.72 ± 0.07 and 0.73 ± 0.06; 0.73 ± 0.06, respectively) and concentration of active form of tPA (0.43 ± 0.02 vs.
0.45 ± 0.02; 0.47 ± 0.03 and 0.48 ± 0.03, respectively). Similarly, there were no significant differences between total concentration of PAI-1 (1.50 ± 0.12 vs. 1.63 ± 0.17; 1.67 ± 0.11; 1.55 ± 0.10, respectively) and concentration of active form of PAI-1 (0.45 ± 0.02 vs. 0.42 ± 0.04; 0.46 ± 0.02 and 0.41 ± 0.03, respectively) in rats plasma. Data were presented in Figure 4.

**In vitro analysis of thromboelastometric (ROTEM) parameters and platelet aggregation**

None of the thromboelastometric parameters assessing clot formation in whole blood from initial stages to stabilized clot formation were changed following 10 min of incubation with 100 µM QA (Table 1). Collagen-induced platelet aggregation in whole blood after incubation with 100 µM QA did not significantly differ from the parameters of control samples (Table 2). Similarly, collagen-induced platelet aggregation parameters in PRP did not differ from the parameters of control samples when were incubated 10 min with 100 µM QA (Table 3). In turn, ADP-induced aggregation was significantly inhibited in samples of PRP incubated 10 min with 1mM QA and 2 mM QA versus controls (39.94 ± 9.02; 29.82 ± 11.51 vs. 51.91 ± 9.268, respectively) and remained without significant changes in PRP samples incubated with 100 µM QA (Table 3).

**DISCUSSION AND CONCLUSION**

Tryptophan-derived uremic toxins accumulate and exert abnormal effects on various systems, including hemostasis in CKD patients (29, 10-12, 15, 16). Taking into consideration reported data and the results of our previous work we decided to examine the effect of tryptophan metabolite – QA – on coagulation, fibrinolysis parameters and platelet function in rats. The present study represents the continuation of the previous experiments regarding investigations of QA on hemostasis in experimental models of thrombosis in rodents with activated coagulation and platelets. The results showed that QA modulates hemostatic parameters in rodents with activated thrombosis. There is a wide spectrum of evidence that the experimental model of thrombosis by induction of arterial injury using electric current can lead to the formation of platelet plug and activation of coagulation to form fibrin (30, 31). Uremic toxins also may represent a variety of thrombogenic agents and can cause thrombotic complications (25, 26). Therefore, the present study was undertaken to evaluate the influence of QA on coagulation, fibrinolysis parameters and platelet function ex vivo and in vitro in normal rats without experimentally induced thrombosis. Accordingly to the previous study, the first approach to determine if QA...
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per se could modulate any of the investigated parameters was to administrate QA in the same, as previously used, doses of 3, 10 and 30 mg/kg/24 h for 14 days and analyze blood samples using thromboelastometry and standard coagulation and fibrinolytic tests. Presented results showed that none of thromboelastometric parameters, assessing clot formation in a whole blood from initial stages (CT, CFT, alpha angle) to stabilized clot formation (MCF), classical blood coagulation parameters indicating the times to plasma clotting in different pathways of coagulation (PT, PTT, TT, and Fg) or fibrinolytic parameters which are mainly dependent on concentration and activity of tPA and PAI-1 were changed after 14-day of QA administration in normal rats.

The results presented in the study may indicate that QA exerts its toxic effect on a cardiovascular system only in the presence of injured endothelium. This assumption is consistent with the results from our previous experiment in rats with induced carotid arterial injury thrombosis model. In the model, QA affected hemostatic parameters in rats receiving QA for 14 days with injured endothelium, whereas in rats receiving only water hemostatic parameters were unchanged despite of injured endothelial cells. Probably, QA toxicity in CKD patients may also be

### Table 1. Effect of QA on whole blood thromboelastometric (ROTEM) parameters after 10 min of incubation in vitro.

<table>
<thead>
<tr>
<th>NATEM parameters</th>
<th>VEH</th>
<th>QA100 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT (s)</td>
<td>108.5 (60-135)</td>
<td>109.5 (67-130)</td>
</tr>
<tr>
<td>CFT (s)</td>
<td>28 (20-33)</td>
<td>26.5 (21-30)</td>
</tr>
<tr>
<td>Alfa (angle)</td>
<td>84 (83-86)</td>
<td>85 (84-86)</td>
</tr>
<tr>
<td>MCF (mm)</td>
<td>76 (74-78)</td>
<td>76 (75-77)</td>
</tr>
</tbody>
</table>

VEH - control group; QA - quinolinic acid; CT - clotting time; CFT - clot formation time; α - alpha angle; MCF - maximum clot firmness; There were no statistically significance changes between VEH and QA100 groups.

### Table 2. Effect of QA on collagen-induced (2µg/ml) platelet aggregation in whole blood after 10 min of incubation in vitro.

<table>
<thead>
<tr>
<th>Platelet aggregation (whole blood)</th>
<th>VEH</th>
<th>QA100 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude</td>
<td>9.25 (7.5-14)</td>
<td>10.5 (9-11.5)</td>
</tr>
<tr>
<td>Slope</td>
<td>4 (3-6)</td>
<td>4 (4-5)</td>
</tr>
<tr>
<td>Lag Time</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>AUC</td>
<td>23.2 (16.2-40.35)</td>
<td>26.8 (19.35-31.35)</td>
</tr>
</tbody>
</table>

VEH - control group; QA - quinolinic acid; amplitude - maximal extent of aggregation (%); slope of the curve; lag time - the latency time; AUC - area under the curve; There were no statistically significant changes between VEH and QA100 groups.

### Table 3. Effect of QA on collagen-induced (1µg/ml) and ADP-induced (10µM) platelet aggregation in PRP after 10 min of incubation in vitro.

<table>
<thead>
<tr>
<th>PRP aggregation</th>
<th>VEH</th>
<th>QA 100 µM</th>
<th>QA 1 mM</th>
<th>QA 2 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude (%) of collagen-induced aggregation</td>
<td>69.5 (52-91)</td>
<td>76 (73-80)</td>
<td>68 (53-79)</td>
<td>73 (50-79)</td>
</tr>
<tr>
<td>Amplitude (%) of ADP-induced aggregation</td>
<td>51.91 ± 9.27</td>
<td>53.5 ± 5.54</td>
<td>39.94 ± 9.02**</td>
<td>29.82 ± 11.51**</td>
</tr>
</tbody>
</table>

VEH - control group; QA - quinolinic acid; ADP - adenosine diphosphate; PRP - platelet rich plasma; amplitude - maximal extent of aggregation (%); ** - p value < 0.001 compared with VEH
related to endothelial dysfunction, which is a well-known instigator of cardiovascular morbidity and it develops in CKD with a remarkable frequency (32). Moreover, QA was also linked with many other pathological states related to endothelial dysfunction, including oxidative stress, inflammation or atherosclerosis (5, 13, 14). In the present study, the endothelium seemed to be intact by QA, as none of the measured parameters of coagulation and fibrinolysis remained unchanged.

In light of the results presented above the next approach in the study was to investigate the influence of QA on coagulation parameters and platelet function in vitro. The whole blood and PRP samples were incubated with 100 µM QA and no significant differences in either thromboelastometric parameters or collagen-induced platelet aggregation were observed. Further, we used 1 mM and 2 mM concentrations of QA in PRP samples to investigate if QA is able to exert any modulatory action on platelets suspended in plasma and to determine the QA action threshold in such conditions in vitro. Additionally, using different agonists, such as collagen and ADP, which triggers different platelet activation pathways (33), would give the information about platelet response to the particular agonist. The results showed that QA even at higher concentrations failed to affect platelet function in the collagen-induced aggregation but exerted an inhibitory effect on ADP-induced aggregation measured in PRP. Collagen and ADP trigger different signaling pathways leading to platelet aggregation (34). Following GP VI interactions with collagen, platelets undergo strong activation and release the content of α- and dense granules (35), activating several different adhesive receptors, such as α<sub>IIb</sub>β<sub>3</sub> and α<sub>2</sub>β<sub>1v</sub> (36). GP VI acts also via the non-receptor tyrosine kinase pathways activating gamma isoform of phospholipase C (PLC<sub>γ</sub>) (37). In turn, for normal-ADP induced platelet aggregation a co-activation of P2Y<sub>12</sub> and P2Y<sub>1</sub> receptors is necessary (38), while the inhibition of P2Y<sub>1</sub> by antagonist inhibits ADP-induced platelet aggregation (39). P2Y<sub>12</sub> is coupled with Gi protein which inactivates adenylate cyclase while P2Y<sub>1</sub> activates PLC<sub>γ</sub> isoform via Gq/11 protein (40). P2Y<sub>1</sub> signaling is responsible for initial platelet activation, shape change, and transient platelet aggregation (41), whereas P2Y<sub>12</sub> signaling more potently amplifies platelet aggregation induced by ADP and other agonists (42, 43). In the present study, ADP-induced platelet aggregation in PRP was selectively inhibited by QA (1 and 2 mM) in vitro. Nevertheless, it still remains to be investigated whether QA acts as a potential inhibitor of ADP-dependent platelet aggregation and displays normal inhibitory action or if the action of QA at higher concentrations is shifted into toxicity.

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Conflict of interest

The authors declare no conflicts of interest.

REFERENCES


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