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Increase in antiplatelet effects of prostaglandins by α-tocopherol and quercetin

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Abstract. Prostacyclin (PGI_2) and prostaglandin E_1 (PGE_1) are effective inhibitors of platelet aggregation. Therefore, they are attractive compounds for the treatment of cardiovascular diseases. However, their use is restricted due to unwanted side effects. α-Tocopherol and quercetin are weak antiplatelet agents, which have no side effects. The aim of this work was to study possible potentiation of antiplatelet effects of PGs with α-tocopherol or quercetin. In this work, platelet-rich plasma was prepared from human blood and the inhibition of adenosine diphosphate-induced platelet aggregation by PGs in the presence and absence of α-tocopherol or quercetin was studied. At a subthreshold concentration 200 μM and 2 μM α-tocopherol and quercetin, respectively, increased the antiplatelet effects of PGI_2, PGE_1, and a synthetic analogue of PGI_2, iloprost. Thus, combination of PGs with α-tocopherol or quercetin allows using prostaglandins at lower concentrations to inhibit platelet aggregation.

Key words: platelet aggregation, α-tocopherol, quercetin, prostacyclin, prostaglandin E_1, iloprost.

INTRODUCTION

Prostacyclin (PGI_2) and prostaglandin E_1 (PGE_1) are formed from arachidonic acid by cyclooxygenase and corresponding synthases (Funk, 2001). They participate in various functions and exert their effects through specific membrane receptors (Kobayashi & Narumiya, 2002). PGI_2, PGE_2, and their analogues are potent inhibitors of platelet aggregation (Whittle et al., 1978), which is responsible for thrombosis and cardiovascular diseases. PGs are attractive substances for the treatment of these diseases. However, the clinical use of PGI_2 and its pharmacological analogues is restricted due to their adverse side effects.

α-Tocopherol (Steiner & Anastasi, 1976; Freedman et al., 1996) and quercetin (Pace-Asciak et al., 1995; Pignatelli et al., 2000) were also found to inhibit platelet

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aggregation and platelet dependent thrombosis (Mruk et al., 2000). α-Tocopherol and quercetin are essentially weaker inhibitors of platelet aggregation than PGs. At the same time, the combination of PGs with these compounds might increase their antiplatelet effect and decrease the therapeutic concentrations of PGs and their side effects. To test this possibility, we studied the combined effect of PGI_2, PGE_1, and iloprost with α-tocopherol or quercetin and found that the two last compounds essentially potentiate the effect of the PGs in vitro.

MATERIALS AND METHODS

Adenosine diphosphate (ADP), α-tocopherol, and quercetin were obtained from Sigma Chemical Co. (St. Louis, MO, USA), Na salt of PGI_2 from Kemasol Ltd. (Tallinn, Estonia). (E)-(3aS, 4R, 5R, 6aS)-hexahydro-5-hydroxy-4-[(E)-(3S, 4RS)-2-hydroxy-4-methyl-1-octan-6-ynyl]-$\Delta^{2(1H),\delta}$-pentalenevaleric acid (iloprost) was a generous gift of Schering AG (Berlin, Germany). ADP was dissolved in saline, α-tocopherol in ethanol, and quercetin in 1 M NaOH solution. The Na salt of PGI_2 was dissolved in glycine buffer (pH 10.4) before each experiment and kept on ice. All the compounds were diluted in saline.

Blood of healthy volunteers of both gender aged from 19 to 53 years was used in this work. Blood was anticoagulated with one-tenth volume of 3.8% trisodium citrate solution. Platelet-rich plasma (PRP) was obtained after the centrifugation of the citrated blood at $160 \times g$ for 10 min at room temperature.

Platelet aggregation was measured photometrically in an aggregometer (Chrono-Log Corporation, USA). An aggregometer cuvette containing 0.5 mL of an aliquot of PRP was maintained at 37 °C and stirred with a Teflon-coated bar. Aggregation was induced by ADP (Born, 1962), which was added to platelets at a concentration just sufficient to induce submaximum aggregation (95–85% of maximum). This concentration varied from 2 to 10 μM in different samples of PRP. The inhibition of platelet aggregation was determined by the incubation of quercetin, PGI_2, PGE_1, and iloprost for 1 min prior to the addition of ADP into a cuvette with PRP. α-Tocopherol was incubated for 5 min because it acts inside the platelets and it takes more time for inhibition of platelet aggregation. A longer incubation time for each of the compounds did not give a higher effect. The aggregation studies were concluded within 3 h after blood collection. The sensitivity of platelets did not change during this period.

The values of IC_{50} concentration necessary to reduce the ADP-induced aggregation by 50% were obtained from concentration–effect curves. Data were expressed as mean ± standard error of the mean (SEM). The effects of PG in the presence and absence of α-tocopherol or quercetin were measured in the same sample of PRP. Each point was obtained as a mean of measurements at least in 4 samples. Student's *t*-test for paired data was used to determine statistical differences between the points for the same concentration of PG in the absence or presence of α-tocopherol or quercetin. Results were considered to be statistically significant at a probability level of $p < 0.05$.

RESULTS

α-Tocopherol inhibited platelet aggregation at concentrations of 1 mM and higher. Added to PRP alone at a concentration of 200 μM it did not inhibit ADP-induced platelet aggregation but significantly increased the effect of PGs. The values of IC_{50} for PGE_1, PGI_2, and iloprost in the absence and in the presence of α-tocopherol are presented in Table 1 with α-tocopherol potentiated effects of PGE_1, PGI_2, and iloprost shown.

Quercetin alone inhibited platelet aggregation at concentrations of 20 μM and higher. Subthreshold concentration (2 μM) of quercetin potentiated effects of PGs decreasing the values of IC_{50} for PGE_1, PGI_2, and iloprost (Table 2).

Table 1. Potentiation of the antiaggregatory effects of PGs by α-tocopherol

PG	IC_{50} (nM) ± SEM		
	In the absence of α-tocopherol (I)	In the presence of α-tocopherol 0.2 mM (II)	(I)/(II)
PGE_1	50 ± 8.1 (7)	29 ± 5.4** (7)	1.7
PGI_2	1.4 ± 0.2 (5)	0.58 ± 0.07** (5)	2.4
Iloprost	1.3 ± 0.1 (12)	0.30 ± 0.07*** (12)	4.3

The data are presented as means ± standard error of the mean (SEM) of the concentration of prostaglandin (PG) that causes inhibition of platelet aggregation by 50% (IC_{50}). Platelet aggregation was induced by adenosine diphosphate. The number of experiments is shown in parentheses.
** – $p < 0.01$.
*** – $p < 0.001$.

Table 2. Potentiation of the antiaggregatory effects of PGs by quercetin

PG	IC_{50} (nM) ± SEM		
	In the absence of quercetin (I)	In the presence of quercetin 2 μM (II)	(I)/(II)
PGE_1	32 ± 0.9 (4)	19 ± 1.3*** (4)	1.7
PGI_2	1.4 ± 0.2 (7)	0.8 ± 0.2* (7)	1.8
Iloprost	1.7 ± 0.2 (12)	0.68 ± 0.09*** (12)	2.1

The data are presented as means ± standard error of the mean (SEM) of the concentration of prostaglandin (PG) that causes inhibition of platelet aggregation by 50% (IC_{50}). Platelet aggregation was induced by adenosine diphosphate. The number of experiments is shown in parentheses.
* – $p < 0.05$.
*** – $p < 0.001$.

DISCUSSION

In several works, α-tocopherol and quercetin were found to be able to inhibit platelet aggregation in vitro. The effective concentrations were found to vary from 50 μM (Pignatelli et al., 1999) to 1 mM (Steiner & Anastasi, 1976) for α-tocopherol and from 10 μM (Pignatelli et al., 2000) to 2500 μM (Janssen et al., 1998) for quercetin. The difference in concentrations depends on the compound used to induce aggregation, the incubation time of α-tocopherol or quercetin, and the method of platelet preparation. In our experimental conditions, α-tocopherol or quercetin, when used alone, inhibited platelet aggregation at concentrations 1 mM and 20 μM, respectively (not shown). In combination with PGs, we used 5 and 10 times lower concentrations of α-tocopherol and quercetin, respectively. At these concentrations, neither of the compounds inhibited platelet aggregation when used alone, but essentially potentiated the inhibition induced by each of the three PGs.

The characteristic actions of PGs studied are mediated by specific cell-surface receptors, the activation of which leads to an increase of the synthesis of intracellular cyclic adenosine monophosphate (cAMP) through Gs protein coupling to adenylate cyclase (Wise & Jones, 1996). cAMP, in turn, activates cAMP-dependent protein kinase and this causes the inhibition of platelet aggregation (Schwarz et al., 2001). α-Tocopherol is found to act inside the platelets through the inhibition of protein kinase C (PKC) (Freedman et al., 1996). The latter is a key enzyme in signal transduction and its activation leads to platelet aggregation. In our experiments, the simultaneous synthesis of cAMP and the inhibition of PKC probably increased the sensitivity to PGs in the presence of α-tocopherol.

Quercetin interacts with many cell enzymes (Middleton et al., 2000) and the mechanism of its inhibitory effect on platelet aggregation may be more complicated. Tzeng and co-workers (Tzeng et al., 1991) concluded from the result of their work that the major antiplatelet effect of flavonoids might be the inhibition of thromboxane A_2 (TxA_2) receptors together with the inhibition of TxA_2 formation in platelets. This may be caused by the inhibition of cyclooxygenase (Chi et al., 2001). It was also suggested that the antiplatelet effect of quercetin might be caused by the inhibition of nucleotide phosphodiesterase leading to an increase of cAMP (Lanza et al., 1987). In addition, quercetin inhibits the activity of PKC and phosphatidylinositol 3-kinase (Agullo et al., 1997; Gamet-Payrastre et al., 1999). This is important for the control of the reversible aggregation of platelets induced by a low concentration of an agonist (Lauener et al., 1999).

This study confirms and extends the previous evidence on the antiplatelet effect of α-tocopherol and quercetin and demonstrates that the subthreshold concentrations of α-tocopherol and quercetin potentiate the antiplatelet effects of PGI_2, PGE_1, and iloprost. Especially effective is the combination of α-tocopherol with the lower concentrations of iloprost. The combination of α-tocopherol or quercetin allows using PGs at lower concentrations to inhibit platelet aggregation and might decrease adverse side effects of the latter. Ex vivo experiments are necessary to conclude on practical applications of the data obtained in this work.

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Prostaglandiinide antiagregatiivse efekti suurendamine α-tokoferooli ja kvertsetiiniga

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Prostatsükliin (PGI_2) ja prostaglandiin E_1 (PGE_1) on efektiivsed trombotsüütide agregatsiooni inhibiitorid, mistõttu on need olulised ühendid südame-veresoonkonnahaiguste ravis. Nende kasutamine on aga piiratud ebasoodsate kõrvaltoimete tõttu. α-tokoferool ja kvertsetiin on nõrgad antiagregandid. Samal ajal puuduvad neil ebasoodsad kõrvaltoimed. Töö eesmärgiks on uurida võimalikke PG-de potentseeritavaid koostoimeid α-tokoferooli või kvertsetiiniga vereliistakute agregatsiooni inhibitsioonis. Inimverest valmistati vereliistakuterikas vereplasma (PRP) ja uuriti adenosiindifosfaadi poolt indutseeritud trombotsüütide agregatsiooni inhibitsiooni prostaglandiinidega α-tokoferooli ja kvertsetiini juuresolekul või puudumisel. α-tokoferool ja kvertsetiin vastavates lävikontsentratsioonides 200 μM ja 2 μM suurendasid tunduvalt PGI_2, PGE_1 ja PGI_2 sünteetilise analoogi iloprosti trombotsüütide agregatsiooni pidurdavat efekti. Seega võimaldab PG-de kombineerimine α-tokoferooli või kvertsetiiniga kasutada prostaglandiine madalamates kontsentratsioonides trombotsüütide agregatsiooni pidurdamiseks.