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Inhibition of Human Platelet Aggregation by Amides and Ester of Salicylic Acid with Platelet-Activating Factor Analogs

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Abstract—The influence of acetyl salicylic acid (ASA) derivatives with platelet-activating factor (PAF) lipid analogs on PAF-induced human platelet aggregation has been studied. It was found that the ASA amide with an ethanolamine plasmalogen PAF analog (1-0-alk-1′-enyl-2-acetyl-sn-glycero-3-phospho-(N-2′-acetoxybenzoyl)ethanolamine) and the ASA ester with a choline plasmalogen PAF analog (1-0-alk-1′-enyl-2-(2′-acetoxybenzoyl)-sn-glycero-3-phosphocholine) at concentrations of 10^{-7}-10^{-6} M effectively inhibit PAF-induced aggregation of human platelets. In contrast to these compounds, the ASA amide with an alkyl PAF analog (1-0-alkyl-2-acetyl-sn-glycero-3-phospho-(N-2′-acetoxybenzoyl)ethanolamine) did not inhibit PAF-induced platelet aggregation. As possible mechanisms of action of the studied compounds, the blockade of PAF-receptor and cyclooxygenase inhibition are proposed.

Key words: platelet-activating factor analogs, acetyl salicylic acid, platelets

Cyclooxygenase is a key enzyme of thromboxane and prostaglandin synthesis and catalyzes the conversion of arachidonic acid into prostaglandin endoperoxides (PGG_2, PGH_2) [1]. Recently, a considerable number of studies are devoted to the inducible form of cyclooxygenase, cyclooxygenase-2, which is expressed in a number of cells (human lung fibroblasts, endometrial stromal cells, human amniotic cells, mouse macrophages, human neutrophils) by such factors as inflammatory agonists [2], γ-interferon [3], interleukin-1β [4], transforming growth factor of keratinocytes [3], and prostaglandin E_2 [5]. Some recent studies have considered the mechanism of action of cyclooxygenase-2 and searched for new specific inhibitors of this enzyme [1, 6]. New inhibitors of cyclooxygenase-2 were found among the metabolites of nonsteroidal antiinflammatory drugs [7], triterprenoids [8] and synthetic compounds (celecoxib) [9].

Previously, we found that ASA ester with 1-0-acyl-lyso-sn-glycero-3-phosphocholine inhibits superoxide production by leukocytes [10] and aggregation of platelets obtained from blood of hypercholesterolemia patients [11]. Considering these results [10, 11] and data of studies of lipid PAF antagonists [12], it can be supposed that the molecular design of such compounds may be useful in the search for inhibitors of PAF and cyclooxygenase. It is known that the initial stage of PAF-induced platelet activation including PAF binding with receptors, phosphoinositide cycle activation, second messenger formation, and protein kinase C activation are usually accomplished without cyclooxygenase metabolites, and ASA as such did not inhibit these processes [12]. However, the subsequent steps of PAF-induced platelet activation, such as the release reactions of ADP and serotonin, depend on the formation of cyclooxygenase metabolites and are inhibited by ASA [13]. It is know that lipid antagonists of PAF-receptors are effective PAF inhibitors [12], whereas ASA is a concurrent inhibitor of cyclooxygenase and covalently modifies the enzyme by acetylation of the Ser530 residue [14]. Such modification results in the blockade of arachidonic acid binding with the substrate-binding site of cyclooxygenase [14]. Since ASA analogs having a substituent on the carboxyl group are able to acetylate the cyclooxygenase [14], it is reasonable to suppose that derivatives of ASA and structural PAF analogs might exert a simultaneous action on the platelet PAF receptors and cyclooxygenase.

In this work, we studied the influence of ASA derivatives with PAF analogs on PAF-induced aggregation of human platelets.

Abbreviations: PAF) platelet-activating factor (1-0-alkyl-2-acetyl-sn-glycero-3-phosphocholine); ASA) acetyl salicylic acid (2-acetoxybenzoic acid).

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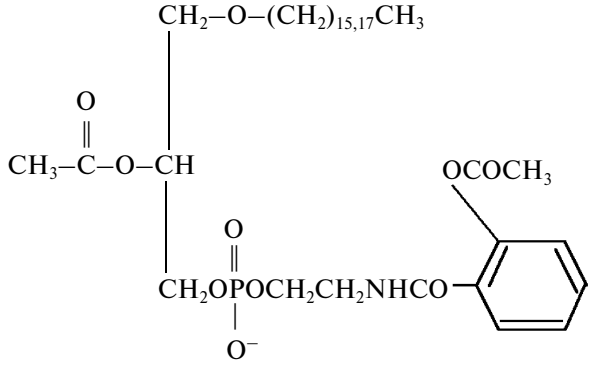
MATERIALS AND METHODS

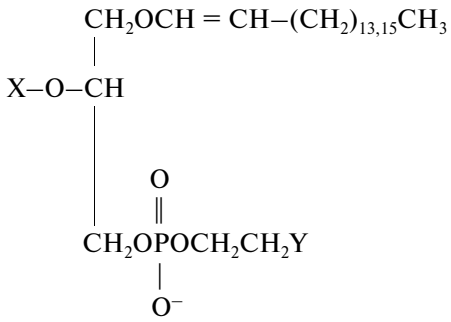
PAF was obtained from beef heart choline plasmalogen by a standard method [15].

1-0-alk-1′-enyl-2-acetyl-sn-glycero-3-phosphoethanolamine was obtained by phospholipase D-catalyzed trans-esterification of 1-0-alk-1′-enyl-2-acetyl-sn-glycero-3-phosphocholine in the presence of ethanolamine as described previously [16].

In this study the alkyl PAF analog 1-0-alkyl-2-acetyl-sn-glycero-3-phospho(N-2′-acetoxybenzoyl) ethanolamine (compound 1) of chemical structure:

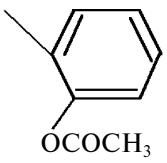


as well as the plasmalogenic PAF analogs of the chemical structure:

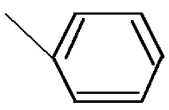


were obtained.

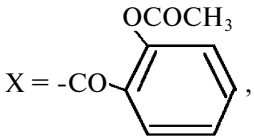
Compound 2: 1-0-alk-1′-enyl-2-acetyl-sn-glycero-3-phospho(N-2′-acetoxybenzoyl)ethanolamine: X = $-COCH_3$; Y = -NHCO



Compound 3: 1-0-alk-1′-enyl-2-acetyl-sn-glycero-3-phospho(N-2′-benzoyl)ethanolamine: X = $-COCH_3$; Y = -NHCO



Compound 4: 1-0-alk-1′-enyl-2-(2′-acetoxybenzoyl)-sn-glycero-3-phosphocholine:



1-0-alk-1′-enyl-2-acetyl-sn-glycero-3-phospho(N-2′-acetoxybenzoyl)ethanolamine (compound 2) was obtained by treatment of 1-0-alk-1′-enyl-2-acetyl-sn-glycero-3-phosphoethanolamine (25 mg, 0.056 mmol) with the chloroanhydride of 2-acetoxybenzoic acid (50 mg, 0.25 mmol) in the presence of chloroform and triethylamine (0.2 ml) and purified by column chromatography on silica gel 100/160 μm.

The following physicochemical characteristics were found for compound 2: R_f = 0.42 in $CHCl_3$–CH_3OH–H_2O (66 : 15 : 2 v/v); IR spectrum (ν_{max}, cm^{-1}): 2930, 2850, 1740, 1660, 1620, 1540, 1470, 1375, 1240, 1070, 960, 775; UV spectrum (λ_{max}, nm (ε) in C_2H_5OH): 207 ($3\cdot10^4$), 262 ($3.6\cdot10^4$).

1-0-alkyl-2-acetyl-sn-glycero-3-phospho(N-2′-acetoxybenzoyl)ethanolamine (compound 1) was obtained by acylation of 1-0-alkyl-2-acetyl-sn-glycero-3-phosphoethanolamine with the chloroanhydride of 2-acetoxybenzoic acid as described for synthesis of compound 2.

The following physicochemical characteristics were found for compound 1: R_f = 0.40 in $CHCl_3$–CH_3OH–H_2O (66 : 15 : 2 v/v); IR spectrum (ν_{max}, cm^{-1}): 2940, 2880, 1755, 1670, 1620, 1580, 1470, 1260, 1070, 955, 760; UV spectrum (λ_{max}, nm (ε) in C_2H_5OH): 209 ($3.14\cdot10^4$), 259 ($3.7\cdot10^4$).

1-0-alk-1′-enyl-2-acetyl-sn-3-phospho-(N-benzoyl)ethanolamine (compound 3) was obtained by treatment of 1-0-alk-1′-enyl-2-acetyl-sn-glycero-3-phosphoethanolamine (30 mg, 0.067 mmol) with the chloroanhydride of benzoic acid (40 mg, 0.27 mmol) in the presence of triethylamine (0.2 ml) and purified by column chromatography on silica gel 100/160 μm.

The following physicochemical characteristics were found for compound 3: R_f = 0.38 in $CHCl_3$–CH_3OH–H_2O (66 : 15 : 2 v/v); IR spectrum (ν_{max}, cm^{-1}): 2930, 2860, 1730, 1640, 1540, 1460, 1220, 1070, 760; UV spectrum (λ_{max}, nm (ε) in C_2H_5OH): 200 ($3.16\cdot10^4$), 225 ($3.7\cdot10^4$).

1-0-alk-1′-enyl-2-(2′-acetoxybenzoyl)-sn-glycero-3-phosphocholine (compound 4) was obtained by treatment of 1-0-alk-1′-enyllyso-sn-glycero-3-phosphocholine with chloroanhydride of 2-acetoxybenzoic acid as described previously [11].

The following physicochemical characteristics were found for compound 3: R_f = 0.12 in $CHCl_3$–CH_3OH–H_2O (66 : 25 : 2 v/v); IR spectrum (ν_{max}, cm^{-1}): 3400, 2920, 2860, 1745, 1670, 1470, 1230, 1060, 980, 760; UV spec-

trum (λ_{max}, nm (ε) in C_2H_5OH): 229 ($1.95 \cdot 10^4$), 280 ($0.61 \cdot 10^4$).

Platelets were isolated from blood of healthy donors with 3.8% trisodium citrate solution as anticoagulant (blood/anticoagulant ratio 9 : 1). Platelet-rich plasma was obtained by centrifugation of blood samples at 200*g* for 15 min. PAF-induced platelet aggregation was measured turbidimetrically in platelet-rich plasma at 520 nm as described previously [17]. Results given in the table are the means of three parallel measurements ± the standard error of the means. Similar results were obtained in 4-5 independent experiments.

RESULTS AND DISCUSSION

There can be considerable variations in the construction of derivatives of ASA with PAF analogs due to the existence of three types of phosphoglycerides (alkyl-acyl-, diacyl- and plasmalogenic phosphoglycerides), the presence of a free amine group in phosphatidylethanolamine and phosphatidylserine, as well as due to the sn-2 and sn-1 positions of phosphoglycerides. We obtained two types of ASA derivatives with PAF analogs: the ASA amides with ethanolamine plasmalogen and ethanolamine alkyl PAF analogs (compounds 2 and 1, respectively) and the ester of ASA with choline plasmalogen PAF analog (compound 4). The choice of ester 4 is due to presence in sn-2 position the acetyl group bound with the salicylic acid residue that to some extent may imitate the acetyl residue of PAF. At the same time, ASA amides with PAF analogs (compounds 1 and 4) contain the acetyl residue in sn-2 position as in PAF and the ASA residue is localized in the polar part of the molecule.

Influence of ASA derivatives with structural PAF analogs on the PAF-induced aggregation of human platelets in the platelet-rich plasma

Tested compound	Concentration of compounds, M		
	10^{-8}	10^{-7}	10^{-6}
	Extent of aggregation inhibition, %		
1-0-alkyl-2-acetyl-sn-glycero-3-phospho(N-2′-acetoxybenzoyl)ethanolamine (1)	0	0	0 ± 5
1-0-alk-1′-enyl-2-acetyl-sn-glycero-3-phospho(N-2′-acetoxybenzoyl)ethanolamine (2)	0 ± 10	90 ± 5	100 ± 4
1-0-alk-1′-enyl-2-acetyl-sn-3-phospho-(N-benzoyl)ethanolamine (3)	0	0 ± 5	80 ± 3
1-0-alk-1′-enyl-2-(2′-acetoxybenzoyl)-sn-glycero-3-phosphocholine (4)	0 ± 5	70 ± 5	100 ± 5

Note: As a control for inhibition of PAF-induced platelet aggregation, the choline plasmalogen PAF analog (1-0-alk-1′-enyl-2-acetyl-sn-glycero-3-phosphocholine) was used. At concentration 10^{-6} M, it induces complete (95-100%) inhibition of PAF-induced platelet aggregation [19].

The results of the study of anti-aggregatory activity of these compounds are presented in the table. The data in the table show the differences in the anti-aggregatory activity of two ASA amides—compounds 1 and 2. Compound 1 does not reveal any anti-aggregatory activity and has a small proaggregatory activity (data not shown), whereas compound 2 at concentration 10^{-7}-10^{-6} M significantly decrease the PAF-induced aggregation in platelet-rich plasma. The observed difference in activities of two compounds having very similar chemical structures appears to be due to the identity of the residues in sn-1 and sn-2 positions in compound 1 with that of PAF. At the same time, compound 2 resembles plasmalogen PAF analogs that, as described by us previously, usually have anti-aggregatory activity toward human platelets [17]. The inhibition of platelet aggregation by compound 2 may be due to the phospholipid part of the molecule as well as to the presence of the ASA residue. To reveal the influence of the ASA residue, we synthesized model compound 3 (1-0-alk-1′-enyl-2-acetyl-sn-3-phospho-(N-benzoyl)ethanolamine) having the benzoic acid residue instead of the ASA residue in the polar part of the molecule and which is not an inhibitor of cyclooxygenase [14]. The data show that compound 3 inhibits the PAF-induced aggregation only at concentration of 10^{-6} M, whereas compound 2 caused 90%-inhibition already at 10^{-7} M. Since the ethanolamine plasmalogen PAF analog (1-0-alk-1′-enyl-2-acetyl-sn-glycero-3-phosphoethanolamine) having a free amino group caused 50%-inhibition of aggregation [17], it is obvious that the ASA residue of compound 2 has a definite role in the process of inhibition of platelet aggregation. It will be noted that under our conditions ASA itself at concentrations 10^{-7}-10^{-6} M had no influence on platelet aggregation, confirming published data [13, 18].

Compound 4 is an ester of ASA and 1-0-alk-1′-enyllyso-sn-glycero-3-phosphocholine and has high anti-aggregatory activity. The chemical structure of this compound closely corresponds to the structure of choline plasmalogen PAF analog (1-0-alk-1′-enyl-2-acetyl-sn-glycero-3-phosphocholine) that induces the desensitization of PAF-receptors to PAF and inhibits PAF-induced platelet aggregation [19]. It was shown previously that the benzoyl analog of PAF (1-0-alkyl-2-

benzoyl-sn-glycero-3-phosphocholine) has an extremely low proaggregatory activity with respect to platelets [20]. Our data show that the presence of the 2-acetoxybenzoyl residue in sn-2 position instead of the benzoyl residue results in the appearance the properties of inhibitor of PAF-induced platelet aggregation in compound 4.

It is known that many lipid compounds are agonists as well as antagonists of PAF receptors [12]. It has been shown recently that the amide of prostaglandin E_1 and ethanolamine plasmalogen PAF analog (1-0-alk-1′-enyl-2-acetyl-sn-glycero-3-phospho-(N-11α, 15α-dioxy-9-keto-13-prostenoyl)ethanolamine) has high biological activity and inhibits several pathways of human platelet aggregation [21]. The results presented above show that the lipid PAF analogs that have an ASA residue attached in a particular position reveal properties of inhibitors of PAF-induced platelet aggregation. Since a possible mechanism of action of these compounds may include the blockade of PAF receptors as well as the inhibition of platelet cyclooxygenase, these compounds can be considered as promising in principle in the search for new inhibitors of PAF and cyclooxygenase-2.

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