PLATELETS AND VASCULAR OCCLUSION

A role for PAF-acether (platelet-activating factor) in platelet-dependent vascular diseases?

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ABSTRACT Platelets — isolated or in conjunction with leukocytes — interact with vessel walls in many experimental and human diseases. Several mediators are held responsible for platelet activation and interaction with leukocytes, among which PAF-acether (platelet-activating factor) is a prime candidate. This phospholipid mediator is released by most inflammatory cells, including neutrophils, by isolated organs such as kidney and heart, is a potent platelet and neutrophil agonist, and exerts major vasoactive properties. Its biosynthesis involves a two-step enzymatic process yielding the active molecule from the membrane alkyl-ether choline-containing phospholipids. The first step imparts a phospholipase A2 that hydrolyzes a long-chain fatty acid (which can be arachidonic acid) from membrane phospholipids, leaving the intermediate compound lysO-P Af-acether, a PAF-acether precursor that is acetylated by an acetyltransferase in a second step. It can also result from deacetylation of PAF-acether by an acetylhydrolase. PAF-acether release might explain the intervention of platelets in diseases such as glomerulonephritis and allergic vasculitis, in which the involvement of neutrophils and platelets is frequently noted. The end result of these complex sets of cell-to-cell interactions is the release of most known inflammatory mediators, influencing vascular permeability, cell infiltration, and smooth muscle contraction. Nevertheless, direct evidence for the implication of these rather well-defined cellular and molecular interactions in human pathologic states remains to be obtained.


THE INVOLVEMENT OF PLATELETS is a common feature in various human or experimental vascular diseases. Indeed, activated platelets release coagulation and growth factors, enzymes, vasoactive amines, lipid mediators, and basic proteins, all endowed with properties that can result in vessel wall injury. In thrombi, neutrophil-platelet interaction is often seen, for which several mediators are held responsible because they are released from neutrophils and are potent inducers of platelet aggregation and release. One of them, PAF-acether (platelet-activating factor), is a phospholipid that is synthesized by various cell types and organs. Therefore, platelets can be instrumental in physiologic and pathologic phenomena, not only via the known pathways of hemostasis and coagulation but also as a result of their interaction with, for example, stimulated leukocytes.

A phospholipid mediator of platelet activation. In 1971, it was recognized that antigen-induced degranulation of immunoglobulin E (IgE)-sensitized basophils provoked the release in the extracellular medium of a soluble mediator, which was named platelet-activating factor (PAF).1 We subsequently demonstrated its presence in human leukocytes and its ability to activate human platelets. Later, its physicochemical characteristics, including its phospholipid nature and the backbone of its molecular structure, were discovered, and we thereby proposed the recognition of a new class of phospholipid mediator.2 Finally, its structure was elucidated and its full synthesis achieved.3-5 PAF (1-O-alkyl-2-O-acetyl-sn-glyceryl-3-phosphorylcholine) is now termed "PAF-acether" to emphasize the structural features necessary for biological activity (figure 1).

Synthetic PAF-acether with C16 or C18 chains at position 1 have an equipotent activity in inducing platelet aggregation and desensitization or granule enzyme release from neutrophils.6 By contrast, some structural features are mandatory for PAF-acether activity, such as an ether-linkage at position 1, the presence and the steric position of the short 2-acyl chain, and the presence of the choline head group.4, 7, 6-11 These data are in favor of a specific platelet receptor for PAF-acether. Indeed, recent studies show PAF-acether-specific platelet binding sites.

PAF-acether aggregates rabbit and human platelets at a concentration of 1 x 10^{-9}M to 10^{-11}M. Circulating basophils are a source for PAF-acether in the rabbit1 but probably not in man.12, 13 Release of PAF-acether in the presence of the ionophore A 23187 or phagocytotic particles was observed from murine mac-
rophages but not from mastocytes. PAF-acether is also released by the platelets themselves, therefore providing a molecular basis for the suggested third way of platelet aggregation. PAF-acether has been recovered from rat, rabbit, and human alveolar macrophages, human blood monocytes, and neutrophils, whereas 100% pure lymphocyte preparations failed to release the mediator. Therefore, PAF-acether, once described as a mediator of immediate hypersensitivity, is in fact formed by several cells, mostly proinflammatory ones, under various immune and nonimmune stimuli. Release in vivo in the vasculature of PAF-acether from IgE-sensitized basophils has been documented, and platelets returning to the circulation, after PAF-acether-induced thrombocytopenia, are specifically desensitized to PAF-acether. First reports on the presence of PAF-acether in human blood in allergic and renal diseases are appearing. These results indicate that leukocyte-platelet interaction via the release of PAF-acether might take place in vivo.

**Assay and characterization of PAF-acether.** PAF-acether is quantified by aggregation of (or radiolabeled serotonin release from) washed rabbit platelets. Platelets are made refractory to adenosine-5'-diphosphate (ADP) and to arachidonic acid (AA). Using this very sensitive platelet bioassay (aggregation), one can detect as low as 5 pg of PAF-acether. The concentrations of PAF-acether are calculated over a standard curve established from aggregations of the rabbit washed platelets triggered by increasing concentrations of synthetic PAF-acether.

Besides aggregation of AA- and ADP-insensitive platelets, criteria for PAF-acether identification are (1) elution pattern identical to that of synthetic PAF-acether on silicic acid thin-layer or high-pressure liquid chromatography, (2) inactivation by phospholipases A₂, C, and D and insensitivity to lipase from Rhizopus arrhizus, and (3) absence of aggregation of PAF-acether–desensitized platelets.

**PAF-acether formation in platelets.** It has been known since 1977 that when the two pathways of platelet aggregation (AA- and ADP-dependent) are inoperative, aggregations can still be launched by the calcium ionophore or thrombin, thus calling for the existence of more than two pathways. We indeed observed PAF-acether formation by rabbit and human washed platelets challenged by ionophore A 23187. It was shown that this formation occurred even in the presence of inhibitors of the ADP and AA pathways. Later on we demonstrated that this synthesis was also triggered by thrombin and collagen but not by ADP, AA, or PAF-acether itself. Other authors have reported on the formation of PAF-acether by calcium ionophore-stimulated rabbit or human platelets.

**PAF-acether biosynthesis.** Involvement of a phospholipase A₁ (PLA₁) in PAF-acether formation was quickly suspected, mainly because all platelet agonists that trigger PAF-acether formation are potent PLA₁ activators. Accordingly, all tested PLA₁ inhibitors (EDTA, EGTA, bromophenacyl bromide, dibutyl cyclic AMP, and compound 874CB) totally suppressed PAF-acether formation from washed rabbit platelets challenged either by the calcium ionophore A 23187 or by thrombin. Although these different inhibitors are not specific for PLA₁, the fact that they are all active in spite of their different chemical structures allows one to conclude that PLA₁ is most probably involved in PAF-acether formation. The result of PLA₁ action on ether-linked choline-containing phospholipids, lyso PAF-acether, has no platelet aggregating properties, but after acetylation it is transformed into a compound with biological activity and chromatographic behavior indistinguishable from that of native PAF-acether. Lyso PAF-acether is formed upon calcium ionophore A 23187- or thrombin-induced rabbit platelet activation. Interestingly, the use of PLA₁ inhibitors mentioned above also led to the total suppression of lyso PAF-acether formation. Furthermore, an enzyme capable of transferring an acetyl group on position 2 of the lyso PAF-acether has been demonstrated in spleen, leukocytes, and murine macrophages. It is indeed present in rabbit and human platelets because incubation of platelet lysates with synthetic lyso PAF-acether and acetyl-coenzyme A led to the formation of PAF-acether. This acetyltransferase activity was increased when platelets were activated by thrombin before lysis, reached a maximum between 1 and 2 min after thrombin stimulation, and returned to near its basal level within 5 min. The peak of activity preceded the plateau of PAF-acether formation.

The simultaneous release of PAF-acether and arachidonate metabolite from platelets. The probable precursor for
lyso PAF-acether and PAF-acether synthesis is an ether-linked glycerylphosphorylcholine (GPC), i.e., a phosphatidylcholine with an ether bond in position 1 of the glycerol. When platelet phospholipid extracts were subjected to alkaline hydrolysis followed by chemical acetylation, the potential precursor was successively transformed into lyso PAF-acether and PAF-acether. Rabbit platelets (5 x 10⁹) contained 2 to 3 nmol (i.e., 8 to 10 nmol considering a yield of 25%) of ether-linked GPC. Upon thrombin stimulation, around 0.3 nmol lyso PAF-acether and 5 pmol PAF-acether were formed (10% and less than 0.2%, respectively, of the total ether-linked GPC). In platelets, the ether-linked GPC represented 11%, 18%, 30 and 17% of total GPC.

The reaction leading to lyso PAF-acether via PLA₂ also triggers the freeing of fatty acid. This fatty acid linked to the glycerol backbone in position 2 could be AA, the mediator of the second pathway of platelet activation. Indeed, in rabbit platelets, alkylacyl GPC contains AA in a higher amount than diacyl GPC. Comparable differences were noted with human platelets, 44% and 23% for alkylacyl and diacyl GPC, respectively. This AA can be mobilized upon platelet activation. Similar results have been obtained in macrophages and neutrophils.

These data indicate that from the very same molecule, two mediators of platelet activation can be formed: thromboxane A₂ and PAF-acether. Thus PLA₂ appears to be a key enzyme for platelet activation.

Mechanisms of leukocyte-platelet interactions. Several mechanisms implicating various cell types with proinflammatory functions may explain the platelet-dependent amplification of vascular permeability and inflammatory lesions. Degranulation of basophils sensitized by IgE antibodies triggers activation of platelets, which leads to immune complex deposition in acute serum sickness in rabbits, a disease that involves mainly renal and peripheral small arteries.

The well-known interactions of neutrophils with platelets in various experimental preparations and in human pathologic states (reviewed in ref. 32) was poorly understood until it was found that activated neutrophils release thromboxane A₂ and PAF-acether. Moreover, since 1981 many studies have emphasized the role of PAF-acether as a neutrophil-activating agonist. Since the initial descriptions, most of the neutrophil functions have been shown to be triggered by PAF-acether (e.g., aggregation, adherence, degranulation, chemotaxis and chemokinesis, release of superoxide anions and leukotriene B₄, and increase in C₃ receptor expression).

Thus practically all known mechanisms of neutrophil activation can in turn bring platelets into the inflammatory site.

Another example of leukocyte-platelet interaction can be found in the onset of bronchoconstriction in the guinea pig, baboon, and man. Alveolar macrophages release PAF-acether when stimulated by a variety of agents such as zymosan particles, bacteria, immune complexes, and, in hyperimmunized animals, the specific antigen. PAF-acether injected either intravenously or administered intratracheally is capable of inducing a potent bronchoconstriction that appears to be temporally related to a decrease in the number of blood platelets and neutrophils and to cell deposition in lung capillaries. These observations have been made in the guinea pig, the rabbit, the baboon, and man. Therefore mediators released from alveolar macrophages upon antigenic or nonantigenic (phagocytosis) stimulation induce platelet and neutrophil aggregation within the lung vessels. This process triggers bronchoconstriction via unknown pathways. Recently, ultrastructural evidence for the presence of aggregated platelets in the extravascular space, close to the lung smooth muscle, has been obtained after intravenous administration of PAF-acether to guinea pigs. This cascade of events, successively implicating lung macrophages, platelets, capillaries, and bronchi, offers an alternative explanation for unexplained bronchospasm in human pathologic states.

Direct vascular effects of PAF-acether. When PAF-acether was administered intradermally to the rat or to humans it induced edema and in the latter case hyperalgesia. This effect appears to be independent of platelets and neutrophil activation. Recently, a dual response — early and late — was reported in human skin.

Intravenous injection of PAF-acether in rabbits, guinea pigs, baboons, and rats triggered hypotension. The phospholipid seems to be the same molecule as the antihypertensive polar renomedullary lipid described 25 years ago. In dogs PAF-acether induces an acute circulatory collapse. In all species the hypotensive effect is independent of platelet aggregation, this being particularly clear in the rat.

The only vascular response in vitro to PAF-acether stimulation that has been observed is in the rat portal vein preparation. There is no effect on isolated vascular muscle preparations. However, vessels respond to PAF-acether when present in an intact organ such as the heart. Injection of PAF-acether in isolated guinea pig heart Langendorff preparations triggers dramatic impairment in the mechanical performance of the heart.
and decrease in flow rate. These data would suggest an effect on the coronary circulation in the perfused organ. However, we have been unable to show any direct effect in isolated canine coronary arteries (R. Santamaria, unpublished observations). Several authors have pointed out the platelet-independent effect of PAF-acether. Platelet depletion abolished the lung mechanical changes but not ventilatory and circulatory alterations provoked by intravenous injection of PAF-acether. Furthermore, the phospholipid raised airway and vascular pressure and induced edema in guinea pig lungs perfused with platelet-free solution. However, most of the effects in vitro and those obtained after platelet depletion must be examined in caution, since the experimental conditions are far from being physiologic. Moreover, high doses of PAF-acether are used in most of these experiments. Therefore, even if these experiments are useful to define the potential role of PAF-acether and to unveil some of the involved mechanisms, they do not necessarily reflect its mode of action in physiology and pathology in experimental animals and in man.

Conclusion. Activation of platelets and neutrophils in the vascular bed is a common feature in vascular diseases. Because PAF-acether is formed by and acts on platelets and neutrophils, it may represent one of the critical links in cell-to-cell interactions, thus providing the cellular and molecular basis for potent amplifying injurious mechanisms. It also appears to have direct effect on the vasculature. However, only the development of new drugs capable of interacting specifically with the biosynthetic pathways or sites of action of the various potential mediators so far described will bring about the elucidation of their precise role in various pathologic situations.

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