The Role of Platelet-Activating Factor in Sepsis: A Bench-to-Bedside Review

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Platelet-activating factor (PAF) is a potent phospholipid mediator with pleiotropic effects. It is produced by a wide variety of cells including platelets, neutrophils, monocytes/macrophages, and endothelial cells. Multiple substances stimulate its production, including lipopolysaccharide and tissue factors released after endothelial disruption. Serum levels of PAF are elevated in both animal models and in human sepsis. The administration of PAF to experimental animals produces physiological dysfunction similar to that seen in sepsis, and various PAF receptor antagonists attenuate these manifestations. Guided by these observations, several clinical trials of such agents have recently been conducted. Although most have been negative to date, a Phase III trial of a recombinant PAF acetylhydrolase is currently underway.

The pathogenesis of sepsis, defined as a systemic inflammation in response to infection, continues to be an area of active research. Advances in our understanding of molecular signaling and cellular interactions have focused many studies of sepsis on inflammatory and anti-inflammatory pathways. Cytokines and other mediators, in conjunction with the offending infectious agent(s), are suspected to play a prominent role in the adaptive and maladaptive responses associated with sepsis in both animal models and humans. A prime example of one such molecule is platelet-activating factor (PAF).

PAF is a phospholipid, which was initially described when platelet aggregation was observed after immunoglobulin E stimulation of basophils [1]. The substance, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphatidyl choline, is now known to be produced by a wide variety of cells including platelets, neutrophils, monocytes/macrophages, and endothelial cells. Multiple substances stimulate PAF production, including lipopolysaccharide (LPS), and tissue factors released after endothelial disruption [2,3]. When PAF is injected into animals, it produces physiological dysfunction similar to

that seen in sepsis (Fig. 1). These effects are mediated by various cytokines and other molecules thought to play a role in sepsis. In several experimental animal models, PAF receptor antagonists (PAFras) attenuate the manifestations of sepsis. Serum levels of PAF are elevated in both animal models and in human sepsis. Guided by these observations, several clinical trials of PAFras and one of a recombinant PAF-acetylhydrolase (rPAF-AH) have been conducted.

This paper provides a more detailed review of these aspects of PAF biology, its potential role as a key contributor to the pathophysiology of sepsis, and the outcomes of recent clinical trials with PAFras and a rPAF-AH in human sepsis.

Biology of PAF

PAF is synthesized via two pathways: a *de novo* pathway and a remodeling pathway (in which PAF can be reconstituted from its major metabolite, lyso-PAF). This latter pathway appears to be the primary pathway involved in systemic inflammation/sepsis [4–6]. Once generated, PAF is distributed in two phases. While most white blood cells secrete PAF into the local extracellular environment, endothelial cells present PAF bound to the cellular membrane. PAF binds with a transmembrane receptor (PAFr) linked to a guanine 5′ triphosphate-

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Figure 1. The pleiotropic effects of PAF on multiple organ systems mimic the effects of sepsis.

PAF: platelet-activating factor.
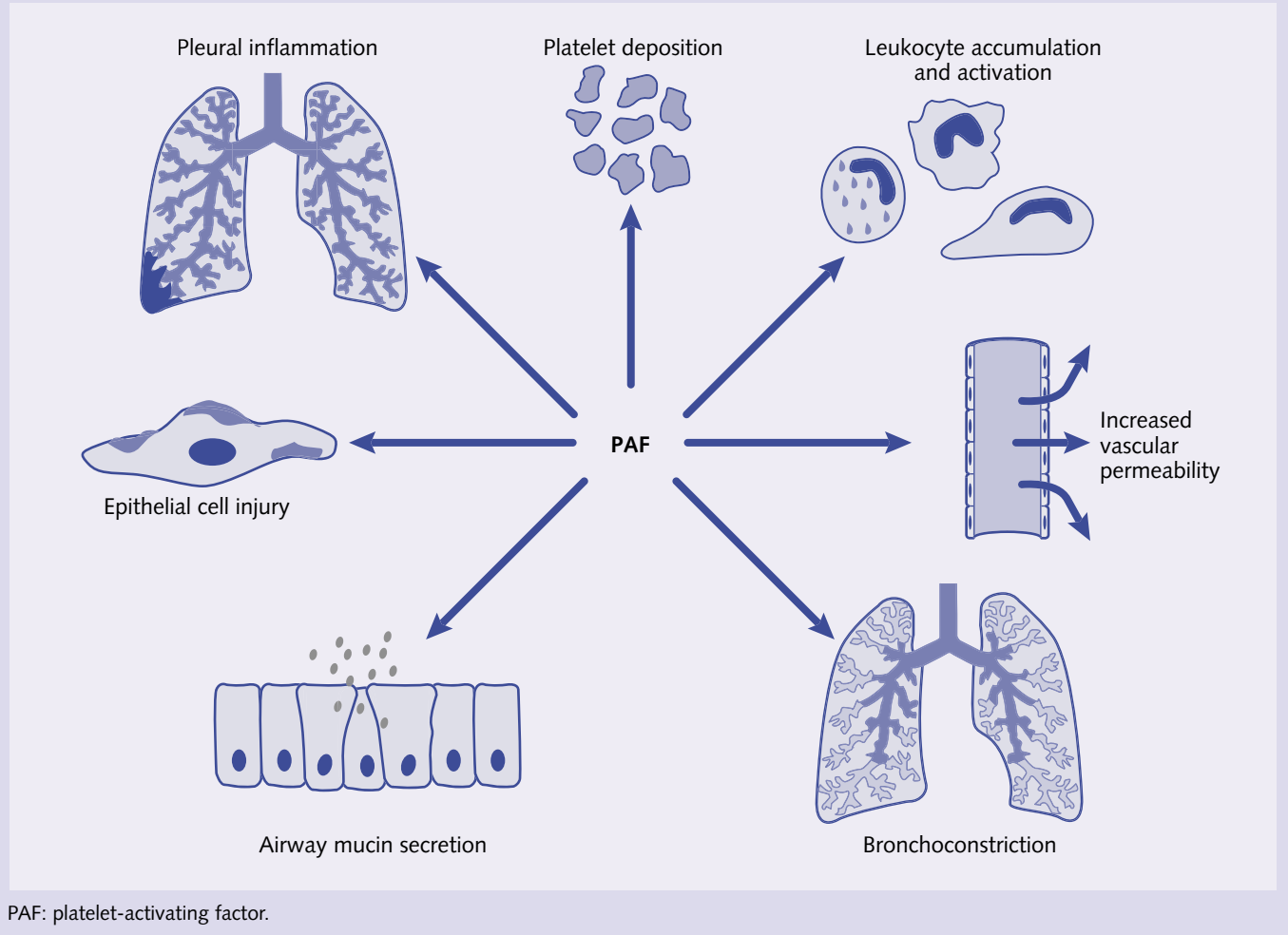
binding protein (G-protein). G-protein activation is followed by:

- intracellular calcium mobilization
- subsequent activation of protein kinases
- the generation of second messengers, such as diacylglycerol and inositol triphosphate [7–9].

PAF receptor activation is not limited to PAF stimulation alone. Oxidized phospholipids, similar in structure to PAF, are also able to bind and activate PAF receptors. These particles are generated by free-radical reactions rather than by regulated enzymatic pathways. Damaged endothelium with resultant oxidation can release these biologically active phospholipids.

Activation of PAF receptor is thought to initiate complex molecular signaling and cellular adhesion cascades. Priming mechanisms may be important for the induction of certain cellular responses; PAF alone may induce specific responses but may also modulate a cell's reactivity to secondary stimuli so that the eventual response is significantly enhanced. For example, in the presence of LPS, juxtacrine interactions may be required between PAF and other mediators to induce cytokine release (e.g. PAF and selectin induction of tumor necrosis factor-α release by monocytes) [10,11]. Paracrine signaling in the presence of PAF and LPS after interaction with specific cellular adhesion molecules (e.g. involving β_2-integrins and CD14 complexed with LPS-binding protein) may have a significant effect on downstream cellular signaling functions. Autocrine self-amplification from paracrine sources may also be important in regulating PAF concentrations. Numerous cytokines and mediators have been implicated in such PAF/LPS interactions, including interleukin-1 (IL-1), IL-2, IL-6, IL-8, interferon-γ, nitric oxide, complement, and granulocyte-colony stimulating factor [11].

Degradation of PAF is normally rapid (half-life of only minutes in plasma) and completed by PAF-AH [12]. This enzyme, produced largely by hepatocytes and

macrophages, circulates in human plasma and is bound to lipoproteins. PAF-AH is a member of the phospholipase family and hydrolyzes esterified acetates in a highly specific manner. This specificity for short and/or oxidized acyl groups at the *sn*-2 position of glycerol is believed to ensure the hydrolysis of PAF and products of lipid oxidation and fragmentation (oxidized phospholipids) rather than the phospholipid components of lipoproteins and cellular membranes. PAF-AH activity is calcium-independent, possibly enabling the enzyme to circulate systemically as a substrate scavenger [13].

PAF and PAF-AH levels in sepsis
Experimentally, LPS affects the expression of both PAF and its receptor. Serum PAF levels are elevated after systemic LPS administration in rat, pig, cat, and mouse models [14,15]. In mice, over-expression of the PAF receptor results in increased mortality from LPS administration [16]. In addition, LPS and other cytokines can inhibit PAF-AH secretion [17]. Local production of PAF has been identified in gastrointestinal and pulmonary tissue samples from septic animals [14,18].

In human studies, the results have been more variable. Statistically significant elevations in PAF levels have been reported in a few studies, while in others, elevated PAF levels in septic patients have not been detected [19–22]. The explanation for this variability remains unclear, but may relate to assays of PAF level concentrations and activity of differing accuracy and validity. Also, as previously noted, enhanced PAF receptor activation in sepsis could be the result of PAF expression, the generation of oxidized phospholipids, or both.

Differences in the efficacy of PAF degradation by PAF-AH in these experimental and clinical settings may also have contributed to these results. PAF-AH activity is decreased and its clearance increased in human sepsis [23]; these changes in patients compared with healthy subjects may be related, in part, to associated changes in lipoprotein concentrations during sepsis. Such changes in degradative activity could lead to differences in circulating PAF concentrations, and interestingly, the half-life of PAF has been reported to be significantly greater in those who died of sepsis as compared with controls and survivors [22].

Effects of PAF in animal sepsis models
Well-documented responses to PAF include [24]:

- enhanced platelet aggregation
- neutrophil chemotaxis with increased vascular adherence
- altered glucose metabolism

- impaired endothelial cell function (most notably increased microvascular permeability and thrombus formation).

Shock/circulatory system collapse and death are also among the effects observed when PAF is administered intravenously to experimental animals [25,26]. This PAF-induced septic shock is the result of diminished cardiac contractility, decreased preload, peripheral vasodilatation, pulmonary vasoconstriction, and bronchospasm.

Attenuation of PAF effects in septic animal models
PAFras have been used to attenuate the consequences of LPS-induced sepsis, providing evidence that PAF is an important mediator in experimental models. Multiple studies have shown that complete protection against LPS-induced sepsis can be achieved if the agent is administered prior to the onset of the experimental intervention causing sepsis (reviewed in [11]). When PAFras are given as a 'treatment' strategy (i.e. after administering the sepsis-causing intervention) the effects have been more variable; in some cases, a benefit has been noted, while in others, no apparent benefit was observed.

Studies demonstrating that rPAF-AH administration can attenuate PAF-induced manifestations of sepsis are limited. In one study, the local injection of PAF into rat paws reproducibly induced footpad edema, and the local or systemic administration of a rPAF-AH was able to block this PAF-induced edema [12]. Similarly, pretreatment with a rPAF-AH in a rat model, characterized by vascular leakage into the pleural space after PAF pleural injection, demonstrated a >80% reduction in vascular leakage compared with control animals [12]. Pretreatment also prevents the development of acute lung injury in experimental models of pancreatitis [27], and in models of PAF-induced acute lung injury (Fig. 2).

Human studies of PAF receptor antagonism in sepsis
To date, five studies have been published that report results of PAF receptor antagonism in human sepsis. No mortality benefit has yet been verified.

The first study was a multicenter, prospective, randomized, double-blind, placebo-controlled Phase III trial of a PAFra, named BN52021 [28]. All patients (n=262) were treated with standard supportive care and appropriate antimicrobial therapy. The primary outcome, 28-day all-cause mortality, was not significantly different between the two treatment arms. Mortality was 42% in the PAFra group and 51% in the placebo

Figure 2. Prevention of pulmonary edema and inflammation induced by PAF, after administration of rPAF-AH in guinea pigs.

| No drug | PAF | rPAF-AH + PAF |
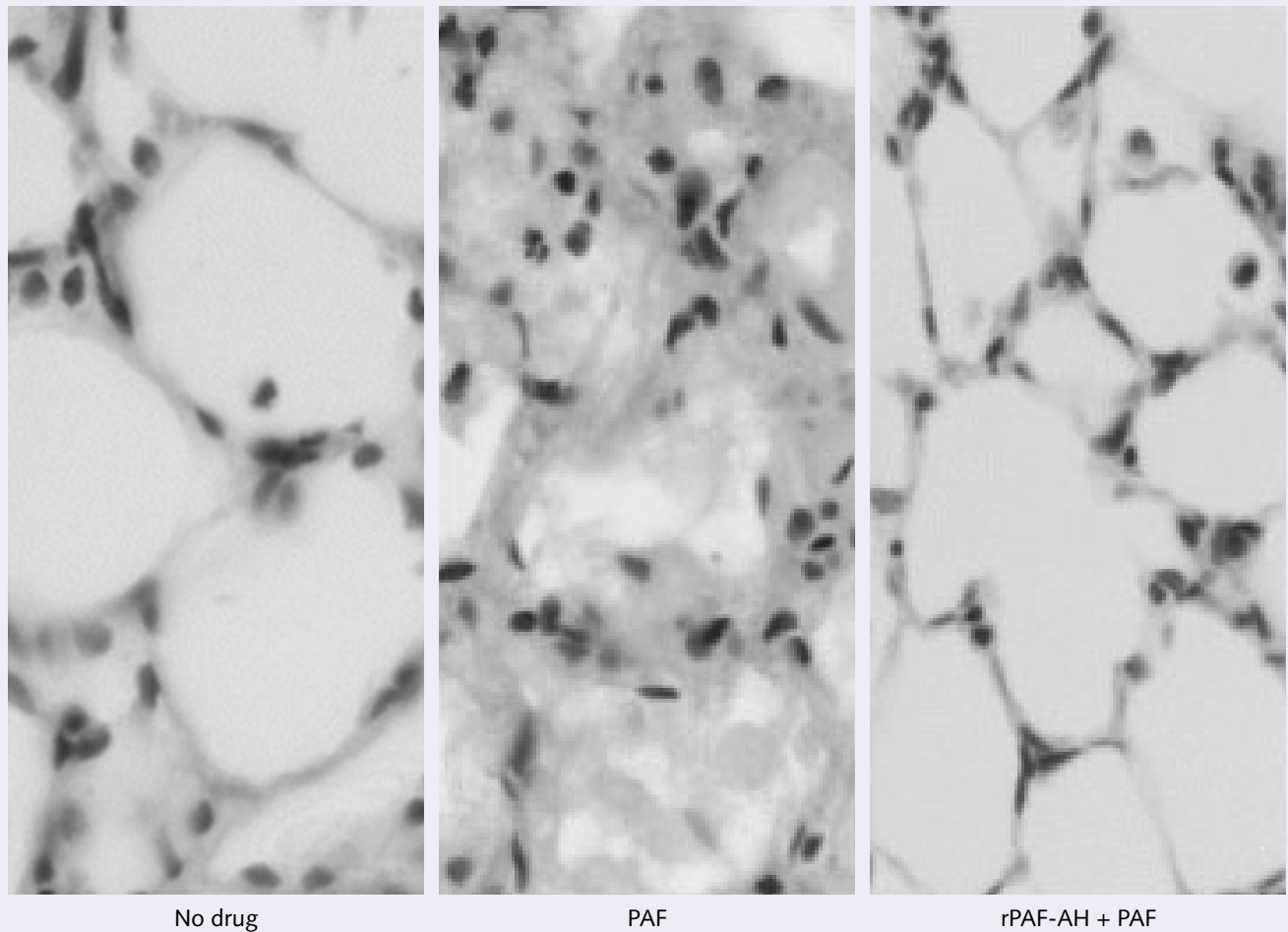
PAF: platelet-activating factor; rPAF-AH: recombinant PAF-acetylhydrolase.
Unpublished data provided by ICOS Corporation (Bothell, WA, USA).

group (p=0.17). Notably, a separate retrospective analysis, specifically of those patients with documented Gram-negative sepsis, was performed. The differences in 28-day mortality in this subgroup were statistically significant, with mortality rates of 33% in the PAFra group as compared with 57% in the placebo group (p=0.01).

Driven by the results of this retrospective subgroup analysis, a second multicenter, prospective, randomized, double-blind, placebo-controlled, Phase III study of the PAFra, BN52021, was conducted [29]. Patients with severe sepsis suspected to be Gram-negative in origin were enrolled (n=609) and treated with the best available supportive care and antimicrobial therapy. Once again, the primary outcome of 28-day all-cause mortality failed to demonstrate any mortality benefit (47% vs. 49% in the placebo group; p=0.50). An analysis of the target population (documented Gram-negative sepsis) yielded similar rates (44% vs. 50% in the placebo group; p=0.29).

Another PAFra, TCV-309, has also been tested in a small multicenter, prospective, randomized, double-blind, placebo-controlled, Phase II trial of 29 septic patients [30]. Mortality rates at 28 and 56 days were not statistically different (58.3% vs. 56.3% in placebo group for 56-day mortality).

A larger study (n=98) of TCV-309 in septic patients with systemic inflammatory response syndrome (SIRS), shock, and Acute Physiology and Chronic Health Evaluation (APACHE) II scores >15 has also been reported [31]. No mortality reduction was observed.

Finally, a third PAFra, BB-882, was investigated in a multicenter, prospective, randomized, double-blind, placebo-controlled, Phase II trial in 152 patients with clinical suspicion of infection and an APACHE II score of

15–35 [32]. Consistent with the previous studies, no statistically significant 28-day mortality difference was reported (53% vs. 45% in the placebo group; p=0.32).

The above studies were all designed as 'treatment' protocols for patients with established sepsis. However, as described above, most preclinical studies demonstrate greater effectiveness of the PAF antagonists when given in 'prevention' protocols. The prevention of acute lung injury in such studies has been particularly compelling. Thus, in contrast to the studies with PAFras, two Phase II trials with a rPAF-AH have been reported in preliminary form [23,33]. The first, a small trial, was not placebo controlled, and therefore no mortality data were provided. The more recent study was a multicenter, prospective, randomized, double-blind, placebo-controlled trial with 240 patients (127 with severe sepsis and 113 with major trauma). Patients received either the rPAF-AH or placebo. The study was designed with a primary endpoint of preventing the development of the acute respiratory distress syndrome (ARDS). Thus, patients were excluded if they met American–European Consensus Conference criteria for ARDS [34]. In addition, patients had to receive drug within 12 h onset of sepsis or trauma. Although no significant difference in the incidence of ARDS was detected (the primary endpoint), there was a statistically significant reduction in mortality associated with the use of the rPAF-AH (14.5% vs. 28.4% in the placebo group). Moreover, the reduction was especially dramatic in the sub-group of patients with severe sepsis (21.4% vs. 44.2% in the placebo group; p<0.05). Thus, in contrast to the results of studies that used PAFras, the effect on mortality in this study with a rPAF-AH may have been the result of one or more of the following:

- the difference in the drugs themselves
- the relatively early administration of the drug to severely septic patients
- excluding patients who already showed evidence of established ARDS (perhaps a 'marker' of early, and, therefore, still 'treatable' sepsis).

Validation of these results are now being tested in a large, Phase III, multicenter trial.

Conclusion

The potential role of PAF in sepsis has been well documented in both experimental models and patients. Despite compelling evidence that PAF is an important mediator in this disease, clinical trials with PAF antagonists have not shown that inhibiting the effects of PAF will improve outcome. These results are similar to many other trials of anti-inflammatory therapy in sepsis [35]. An exception appears to be a recent trial with a rPAF-AH, designed to treat septic patients at a very early stage in their illness. The results of the current validation Phase III trial will be of great interest to those concerned with finding new treatments for this continuing clinical problem.

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