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# PAF ANTAGONISTS:
## NEW DEVELOPMENTS FOR CLINICAL APPLICATION

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Joseph T. O'Flaherty Peter W. Ramwell

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**Joseph T. O'Flaherty** **Peter W. Ramwell**

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*On the Cover*

**Animal lung section showing an infiltration of eosinophils following antigen challenge (left), and the lung morphology observed in antigen-challenged animals after treatment with the PAF antagonist, BN52730 (right).**

*Color photographs*

**Photos provided by:** Dr. Jean Michel Mencia-Huerta of the Institut Henri Beaufour in Les Ulis, France.

# PLATELET-ACTIVATING FACTOR ANTAGONISTS:

## NEW DEVELOPMENTS FOR CLINICAL APPLICATION

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# Foreword

This Conference was convened by International Business Communications (IBC) to discuss the pathophysiology of the platelet-activating factor (PAF), and specifically to explore the clinical indications for PAF antagonists; consequently, most of the individuals attending the Conference were from the pharmaceutical industry.

It should be noted that PAF is a lipid mediator and this is important in many ways for it is very similar to thromboxane $A_2$ and the leukotrienes. They exhibit a wide range of biological activity as well as being ubiquitous in their distribution which always makes recognition of specific clinical indications difficult. The key to identifying their putative pathophysiologic roles of course has been the highly successful development of potent and specific receptor antagonists. The relationship between PAF and these two eicosanoids is a constant theme since many of PAF's effects can be blocked by thromboxane and leukotriene synthesis inhibitors and receptor antagonists. At this time, however, less progress has been made in developing inhibitors of PAF synthesis.

As with leukotriene antagonists it is not easy to identify convincing indications for PAF antagonists. Asthma is clearly the primary indication, but several other indications were considered at this Conference including septic shock, transplantation, CNS injury, some type of inflammation and cyclosporine-induced nephrotoxicity. As might be expected Phase II-III IND studies are being undertaken with respect to small airway disease.

A large number and variety of compounds have been successfully synthesized to obtain specific PAF antagonists which are orally active. However, some compounds in Phase I are not highly specific PAF antagonists since it is thought that a broader and less specific spectrum of antagonist activity, for example against histamine or leukotrienes as well as PAF, may be advantageous in diseases involving a variety of pathophysiological mediators.

Now, the physiological roles of PAF are arousing interest especially in nidation, lung maturation, and promotion of the immune response. However, the federal agencies and Industry need to encourage further exploratory research since much more information is still needed to provide a basis for more meaningful clinical studies.

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Platelet-activating factor (PAF) has far-reaching biological properties. Consider the following points. First, PAF activates thrombocytes, leukocytes, vascular endothelium, macrophages, smooth muscle, neurocytes, glandular cells, dermatocytes, and other tissues. Few, if any, organs are indifferent to the agonist's stimulating actions. Second, these same cell types, when stimulated with any one of various agents, produce PAF. The molecule may form at virtually any site of perturbation. Third, PAF stimulates target cells to release diverse bioactive principles including serotonin, histamine, tumor

necrosis factor, leukotrienes, prostaglandins, and, indeed, PAF itself. PAF thereby recruits many other mediators into tissue responses. Fourth, PAF is hydrophobic. It seems best designed to operate at or near to its sites of origin rather than remotely or systemically. Moreover, most body fluids and cell types convert PAF to its bioinactive *sn*-2 lyso derivative within minutes. This likely contributes to controlling spread of the product. Fifth, PAF is extremely toxic. When intravenously infused, it causes cardiovascular and pulmonary collapse, splanchnic edema, hypovolemia and hemoconcentration, and/or extensive intravascular thrombosis and shock. These anaphylactoid reactions can be rapidly lethal. Sixth, PAF is a simply structured 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine. Hence, the compound is readily generated from resident structural lipids and has a ubiquitous distribution not only among different cell types but also among different animal species.

PAF thus differs from conventional hormones, interleukins and growth factors. It lacks their narrow range of origins and target cell specificities; it acts upon many other mediator systems; it has catastrophic bioactions; it apparently operates preferentially near to its sites of origin but has the potential to influence remote tissues; and it functions in most animals. These considerations suggest that PAF evolved early in phylogeny as a universal signal to coordinate local responses. Its message depends upon its access to cells and the type of cells in its immediate environment. When PAF escapes regional confines, however, toxicity may result. Perhaps only such a view explains the status of current studies. This volume reports on findings implicating PAF in diverse allergic, immunologic, inflammatory, cardiovascular, and other pathological reactions. Presumably, in these instances PAF generation has become excessive. The compound disseminates and its actions are no longer expressed by a meaningful configuration of neighboring cells. Rather, PAF acts indiscriminately on multiple targets to produce chaotic effects. Agents that antagonize PAF, then, may prove therapeutic in an extraordinarily broad set of clinical conditions involving needless self-injury. The editors and authors hope that *PAF Antagonists: New Developments*

*for Clinical Application* will promote further studies that examine PAF in human disease.

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Two additional papers, not presented at this Conference, were submitted for this Volume by Dr. William L. Salzer of Wake Forest University and Dr. Giora Feuerstein and colleagues at the Department of Pharmacology, SmithKline Beecham Pharmaceuticals. We greatly appreciate their added contributions.

Portfolio Publishing Company wishes to express its gratitude to the staff of IBC for their continued support and cooperation in allowing these excellent compilations to be included in our Series on Advances in Applied Biotechnology. Papers delivered at other IBC Conferences were included in Volume 2, *Discoveries in Antisense Nucleic Acids*, of this Series and will be included in forthcoming volumes on Protein C and Related Anticoagulants and on Technologies and Strategies for Fat and Cholesterol Reduction in Food.

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# Quantitation of the Platelet-Activating Factor

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**Currently, quantitative analysis of 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (PAF) can be placed in one of approximately five categories: biological, enzymatic, mass spectrometric, chromatographic, and immunological assays. Bioassays utilizing platelet activation are sensitive but are subject to interference. Enzymatic assays measuring acetate incorporation are also sensitive but can only measure active biosynthesis via the deacylation-reacylation pathway. Radioimmunoassays are promising (picogram sensitivity) but show some cross-reactivity with common molecules and thus require considerable preparation. Chromatography with high sensitivity requires the conversion of PAF to a neutral chromophore. The mass spectrometric assays, the most fruitful assays, not only permit structural and quantitative information but also through molecular species analysis generate important information concerning biosynthetic specificity. Of the mass spectrometric methods, analysis of PAF as the pentafluorobenzoyl-diglyceride with chemical ionization in the negative ion mode provides the most sensitive and selective method of quantitating PAF molecular species as well as their precursors and metabolites. This methodology is expensive and restricted to low throughput. However, this degree of sensitivity is required to ascertain the role of PAF in health and disease.**

## Introduction

The platelet-activating factor (PAF) is a potent lipid autocoid first described nearly 30 years ago[1] that is involved in the etiology of a number of diseases as well as basic physiological responses. In fact, the role for PAF is immense, encompassing the entire life cycle. PAF is not only required for critical aspects of conception,[2] it also mediates a major cause of death, septic shock.[3] However, assigning a causative role to PAF has required quantitation, which has been problematic. Until recently, quantitation has relied on insensitive physicochemical or sensitive but nonspecific biological methods of PAF analysis. Current methodology will be divided into five areas and discussed with respect to their strengths and weaknesses.

## Bioassays and Enzymatic Assays of PAF

The original and most popular method of PAF analysis is platelet activation.[4] The relative sensitivity of this and the other assays discussed are shown in Figure 1. Typically, the release of serotonin or aggregation yield a log-linear response to PAF sensitive to the picogram level. Although the assay has been critical to the understanding of PAF, results have been confounded by a number of factors, including the presence of natural PAF antagonists, modulators of signal transduction, and lytic factors. Sphingomyelin[5] is a particularly menacing source of interference, because it is abundant and not easily separated by thin-layer chromatography or normal phase HPLC.

A useful complement to the bioassays for PAF is acetate incorporation,[6,7] which relies on the PAF biosynthetic apparatus to synthesize AcetylCoA and transfer (lysoPAF:acetylCoA transferase) the acetate to nascent lysoPAF. PAF is then isolated and assayed for tritium. Because acetate in high-specific activity is available, the synthesis of pg amounts of PAF can be detected. This assay is inexpensive and has been successfully employed by a number of