

The Therapeutic Potential of Phospholipase A₂ Inhibitors in Cardiovascular Disease

M.C. White and J. McHowat*

Saint Louis University School of Medicine, Department of Pathology, 1402 S. Grand Blvd., St. Louis, MO 63104, USA

Abstract: Leukocyte recruitment and the expression of pro-inflammatory cytokines are prevalent characteristics of early atherogenesis [1]. Recently, several inflammatory mediators have been linked to atheroma formation and inflammatory pathways have been shown to promote thrombosis [1]. The discovery of mast cells, activated T lymphocytes and macrophages in atherosclerotic lesions, the detection of human leukocyte antigen class II expression, and the finding of local secretion of several cytokines all suggest the involvement of immune and inflammatory mechanisms in the pathogenesis of atherosclerosis [2-5]. Recent research suggests activation of protease activated receptors (PAR) on the surface of endothelial cells may play a role in general mechanisms of inflammation. In previous studies, our laboratory has demonstrated that thrombin (which activates PAR-1) and trypsin (which activates PAR-2) stimulation of endothelial cells results in activation of calcium-independent phospholipase A₂ (iPLA₂) [6,7]. iPLA₂ plays a critical role in the synthesis of membrane phospholipid-derived inflammatory mediators such as arachidonic acid, platelet activating factor (PAF), and prostaglandins, all demonstrated to be central in both the initiation and propagation of the inflammatory response. Activation of iPLA₂ results in release of choline lysophospholipids from endothelial cells, these metabolites may contribute to the initiation of ventricular arrhythmias following myocardial ischemia as a direct result of incorporation into the myocyte sarcolemma. This biochemical event represents a direct link between occlusion of a coronary vessel and the nearly immediate initiation of arrhythmogenesis often seen in myocardial ischemia.

Key Words: Myocardial ischemia, arrhythmogenesis, thrombosis, endothelial cell, phospholipase A₂, inflammation, atherosclerosis.

PHOSPHOLIPASE A₂ IN THE CARDIOVASCULAR SYSTEM

Studies by numerous laboratories have identified phospholipase A₂ (PLA₂) as a critical enzyme in the progression of several cardiovascular diseases. PLA₂ are a large family of esterases responsible for the hydrolysis of *sn*-2 esterified fatty acids from membrane phospholipids resulting in the stoichiometric production of free fatty acid, most notably arachidonic acid, and lysophospholipid (Fig. 1). These metabolites are capable of exerting a direct effect on membrane properties and can serve as precursors for other biologically active metabolites. For example, subsequent removal of the polar headgroup of the lysophospholipid produces lysophosphatidic acid, leading to the activation of numerous signaling pathways [8,9]; platelet-activating factor (PAF), is formed following the acetylation of lyso PAF, a lysophospholipid formed following PLA₂-catalyzed hydrolysis of alkyl acyl glycerophospholipids [10-13]; and the formation of prostaglandins, leukotrienes and thromboxanes occurs when arachidonic acid is the fatty acid released by PLA₂.

Mammalian PLA₂s are classified into three main types: 1) secretory, Ca²⁺-dependent, 2) cytosolic, Ca²⁺-activated and 3) intracellular, Ca²⁺-independent [14-16]. While the three types of PLA₂s are distinct, they are known to coexist in mammalian cells and may interact with each other.

Secretory PLA₂ (sPLA₂) isoforms require the presence of millimolar concentrations of Ca²⁺ for hydrolysis of phospholipids and demonstrate no preference for the *sn*-2 fatty acid. Several roles are proposed for sPLA₂, including eicosanoid release in response to agonist stimulation in a variety of inflammatory conditions, including rheumatoid arthritis [17,18] and ulcerative colitis [19].

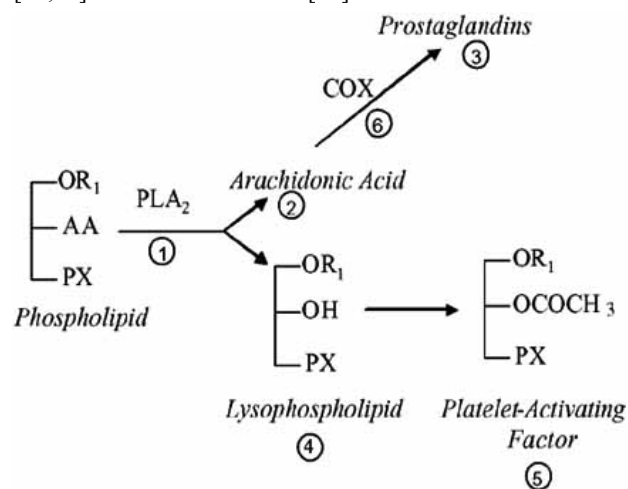


Fig. (1). Membrane phospholipids are hydrolyzed at the *sn*-2 position by iPLA₂ resulting in the stoichiometric production of a free fatty acid (in this case, arachidonic acid, AA) and a lysophospholipid. AA can then be oxidized by cyclooxygenase (COX) to form eicosanoids. Lysophospholipids can be acetylated to produce platelet activating factor (PAF).

*Address correspondence to this author at Department of Pathology, Saint Louis University School of Medicine, 1402 S. Grand Blvd., St. Louis, MO 63104, USA; Tel: 314-577-8302; Fax: 314-268-5649; E-mail: jane.mchowat@tenethealth.com

While Ca^{2+} is not required for the catalytic activity of cytosolic PLA_2 (c PLA_2), it is necessary for the binding of c PLA_2 to its phospholipid substrate. c PLA_2 is expressed constitutively in most cell types and has been demonstrated to play an important role in agonist-induced arachidonic acid release and subsequent eicosanoid production [20].

Calcium-independent PLA_2 (i PLA_2) activity has been observed in both the cytosol and membrane fractions of mammalian cells [21-25]. i PLA_2 enzymes do not require Ca^{2+} for substrate hydrolysis and demonstrate a preference for arachidonylated substrates. Research in our laboratory and others has shown that the majority of PLA_2 activity in both human myocardium [26-30] and coronary vasculature endothelial cells [6,25] occurs in the absence of Ca^{2+} , thus representing i PLA_2 , and is the focus of this review.

Arachidonic acid and platelet activating factor (PAF) are two of the important inflammatory metabolites produced following i PLA_2 -catalyzed hydrolysis of membrane phospholipids. Once liberated from phospholipids by i PLA_2 , arachidonic acid (② in Fig. 1) is converted to prostaglandins (③ in Fig. 1) by the action of cyclooxygenases (COX) (④ in Fig. 1). Liberated lysophospholipids (④ in Fig. 1) can be rapidly acetylated at the sn-2 position by lyso-PAF:acetyl CoA acetyltransferase to produce PAF (⑤ in Fig. 1).

PAF is a phospholipid with diverse and potent physiologic and pathophysiologic effects. It is produced by a variety of cells that participate in the development of inflammatory reactions, including endothelial cells, macrophages, polymorphonuclear neutrophils (PMN), eosinophils, basophils and platelets [31-34]. The primary inflammatory actions of PAF include stimulation of neutrophil adherence to the endothelium and increased vascular permeability [35]. In leukocytes, PAF promotes aggregation, chemotaxis, granule secretion and oxygen radical generation as well as adherence to the endothelium. PAF also increases the permeability of the endothelial cell monolayer and stimulates smooth muscle contraction [36].

During myocardial ischemia, microsomal i PLA_2 activity is increased ten-fold after 5 mins ischemia with no corresponding decrease in cytosolic PLA_2 activity until after more prolonged intervals, suggesting activation of a latent membrane-associated i PLA_2 rather than recruitment of PLA_2 from the cytosol to the microsomal fraction [37]. The increase in microsomal i PLA_2 activity is detected with plasmalogen substrates only; no increase in i PLA_2 activity is observed with phosphatidylcholine or phosphatidylethanolamine substrates [38]. In addition to activation of an integral membrane i PLA_2 , it is possible that longer periods of ischemia are associated with translocation of cytosolic i PLA_2 to the membrane as a result of ischemia-induced phosphofructokinase translocation [39]. More recently, it has been shown that cytosolic i PLA_2 is translocated to the nuclear membrane after 5 mins ischemia and remains detectable in the nucleus after 90 mins of reperfusion [40].

In isolated ventricular myocytes, short intervals of hypoxia also result in an increase in membrane-associated i PLA_2 activity that is selective for plasmalogen substrates with no detectable change in activity measured using phosphatidylcholine [41]. Although purified cytosolic i PLA_2

is activated and stabilized by ATP [42], both cytosolic and membrane-associated i PLA_2 in rabbit ventricular myocytes is inhibited by ATP [26,41] providing an interesting mechanism whereby i PLA_2 may be activated by decreased ATP concentrations in the ischemic myocardium.

Thrombotic occlusion of a major coronary artery at a site of preexisting atherosclerosis is the underlying cause of the vast majority of myocardial infarctions [43]. It has been demonstrated that the incidence of ventricular arrhythmias is greater when ischemia results from intracoronary thrombus formation as opposed to balloon occlusion [44], suggesting that products released from or associated with an intracoronary thrombus may directly or indirectly influence the electrophysiological properties of cardiac myocytes.

In response to thrombin stimulation, endothelial cells have been shown to release both lysophosphatidylcholine and lysoplasmenecholone [6]. The release of these metabolites from endothelial cells could result in incorporation into the cardiac myocyte sarcolemma and alterations in the electrophysiologic properties of cardiac myocytes in the ischemic area. In normoxic ventricular myocytes, lysoplasmenecholone produces action potential derangements, including early and delayed afterdepolarizations that may contribute to the initiation of arrhythmogenesis in the ischemic myocardium, demonstrating a direct relationship between activation of myocardial i PLA_2 and arrhythmogenesis in the ischemic heart [41]. The action potential derangements caused by lysoplasmenecholone occur as a result of the action of this amphiphilic compound on multiple membrane currents [45]. Lysophosphatidylcholine has been shown to induce increases in intracellular Ca^{2+} , stimulate protein kinase C and activate c PLA_2 in cardiac myoblastic H9c2 cells [8]. Also, in isolated cardiac myocytes, lysophosphatidylcholine increased intracellular Ca^{2+} , changed cell shape and resulted in creatine kinase release [9]. Accordingly, activation of i PLA_2 in endothelial cells by local thrombin generation at sites of vascular injury could indirectly contribute to cardiac myocyte dysfunction as a result of increased choline lysophospholipid generation [6].

ACTIVATION OF IPLA₂ IN CORONARY ARTERY ENDOTHELIAL CELLS BY SERINE PROTEASES

Myocardial infarction and the development of thrombotic coronary artery occlusion are associated with the presence of the serine proteases thrombin and tryptase. Thrombin generated at sites of vascular injury is the most potent activator of blood platelets [46,47] and its action on inflammatory cells has been well characterized, serving as a chemotactic agent for monocytes [48] and a mitogen for both lymphocytes [49] and vascular smooth muscle cells [50,51]. Acute thrombin activation occurring in response to vascular injury or wounding of the vascular endothelium is an important initial event in vascular repair. However, sustained thrombin activation has the potential to mediate a prolonged inflammatory response and proliferative cellular events in the blood vessel wall, such as those that occur in atherosclerosis and restenosis [52]. Similarly, increased numbers of degranulated mast cells were found in the adventitia of infarct-related coronary arteries [53] and the mediators released from these granules, including tryptase, are mitogens and co-mitogens for human

fibroblasts, stimulating collagen synthesis [54]. Though these studies demonstrate the presence of either thrombin or trypsin in several cardiac conditions, a defined role has yet to be established for these proteases.

Protease activated receptors represent a growing family of receptors that are activated by proteolytic cleavage of their N-terminus and are coupled to G proteins [see 55 for review]. Interaction of proteases with PAR may have far-reaching implications in diversified cellular responses, particularly in general mechanisms of inflammation and host defense [2,56]. These receptors couple to multiple intracellular signaling pathways including activation of phospholipases and MAP kinases [55]. We have determined that stimulation of human coronary artery endothelial cells (HCAEC) with either thrombin (which activates PAR-1) or trypsin (which activates PAR-2) increases HCAEC membrane-associated iPLA₂ activity [7,57]. Stimulation of EC with the peptide sequences representing the tethered ligands for PAR-1 and PAR-2 indicate that the thrombin- and trypsin-stimulated increases in iPLA₂ activity occur *via* proteolytic cleavage of PAR-1 and PAR-2, respectively [7,25]. Additionally, increases in arachidonic acid release and PAF production in HCAEC have been seen upon stimulation with both thrombin and trypsin [7,57].

The presence of P-selectin on an activated EC layer plays an essential role in the initiation of a tentative adhesive interaction between the circulating inflammatory cell and activated EC monolayer [56]. Subsequently, the enhanced expression of endothelial cell-associated PAF has been shown to cause transient adherence of neutrophils to the endothelial cells [58-60]. Several groups have highlighted the significance of the PAF/PAF-receptor interaction in cell adhesion to, and migration across, the endothelium. Prescott *et al.* [61] have correlated the adhesion of neutrophils to thrombin-activated endothelium with PAF synthesis and expression on the surface of endothelial cells. Additionally, Kuijpers *et al.* [62] were able to demonstrate the ability of PAF receptor antagonists to prevent neutrophil migration across cytokine pretreated endothelial cells by approximately 60 percent. The increase in PAF production and P-selectin expression in response to thrombin or trypsin stimulation suggest there would be an accompanying increase in neutrophil adherence to the stimulated HCAEC monolayer. Results from work in our laboratory has shown that trypsin stimulation of HCAEC monolayers increased neutrophil adherence more than 2-fold over levels of neutrophil adherence to unstimulated HCAEC [7]. Importantly, pretreatment of the HCAEC with bromoenol lactone (BEL) significantly inhibited trypsin induced increases in neutrophil adherence, demonstrating the role of iPLA₂ in the regulation of neutrophil adherence. Data from our laboratory demonstrates that activation of iPLA₂ *via* thrombin or trypsin stimulation is capable of inducing inflammatory changes, such as increases in cell surface P-selectin expression, arachidonic acid release and PAF production [7,57], in the endothelium suggesting a possible mechanism for the initiation of the inflammatory response present in coronary endothelium in cardiovascular diseases.

As the studies examining possible factors responsible for either the cause or progression of various cardiovascular diseases have intensified, an increasing amount of informa-

tion regarding the adverse effects of inflammation in the cardiovascular system has been identified. More recent studies have identified inflammation as a risk factor and potential propagative factor for a variety of cardiovascular diseases such as myocarditis, atherosclerosis, and myocardial ischemia. Clearly, the activation of iPLA₂ in HCAEC by mediators such as thrombin and trypsin in the progression cardiovascular disease represents an intriguing pathway that could be targeted for therapeutic intervention to alleviate several cardiovascular diseases.

PLA₂-CATALYZED PHOSPHOLIPID METABOLITE PRODUCTION IN THE CARDIOVASCULAR SYSTEM

Activation of PLA₂, to release arachidonic acid from membrane phospholipids, and cyclooxygenase (COX), to convert arachidonic acid to the prostaglandin precursor PGH₂, represent the two crucial rate limiting steps for the prostaglandin biosynthetic pathway. The presence of both immediate and delayed prostaglandin production suggests that different combinations of PLA₂/COX enzymes are involved in each type of response. It is proposed that immediate production of prostaglandins, occurring within minutes, is mediated by COX-1, the constitutive isoform, and more delayed prostaglandin production occurs *via* COX-2, the inducible isoform [63-67]. However, it remains controversial as to whether distinct PLA₂ isoforms are utilized specifically with each COX isoform.

Studies by Fujishima *et al.* [68] aimed at examining eicosanoid production in cPLA₂ α knockout mice have demonstrated the central role of this enzyme in immediate and delayed eicosanoid production. Although intracellular PLA₂ isoforms may be directly coupled to COX within the cell for eicosanoid production, several studies have suggested that immediate eicosanoid production involves both cPLA₂ and sPLA₂, with cPLA₂ being the activator of the response, but sPLA₂ providing the majority of arachidonic acid release [22, 69-72].

The synthesis of eicosanoids in the heart may have direct inotropic or chronotropic effects and their presence has been implicated in several pathological conditions in the heart [73]. Although little is known about the role of iPLA₂ in eicosanoid production in the heart, a possible role for iPLA₂ in immediate generation of eicosanoids has been described. Utilizing BEL, iPLA₂ antisense and Ca²⁺ depletion *via* BAPTA and EDTA, Tay and Melendez [74] have demonstrated that immunoglobulin G receptors (Fc γ R) are functionally coupled to iPLA₂ β for the release of arachidonic acid and the production of leukotriene B₄ and PGE₂. Murakami *et al.* [75] described a role for iPLA₂ β and COX-1 activity in ionophore-induced PGE₂ generation, however, no role for iPLA₂ was identified in the delayed response. In these experiments it was determined that iPLA₂ may release arachidonic acid in closer proximity to COX-1 than COX-2 or that iPLA₂-derived arachidonic acid was inaccessible to COX-2 for eicosanoid production.

iPLA₂ AND MEMBRANE PHOSPHOLIPID REMODELING

Thus far, this review has focused on the role of iPLA₂ in the production of inflammatory mediators. However, in addi-

tion to its signal transduction role, iPLA₂ has been demonstrated to be involved in membrane phospholipid remodeling and repair [22].

Plasmalogens protect cells from singlet oxygen and/or radical-initiated oxidation by functioning as free radical scavengers that can be subsequently catabolized to less toxic metabolites [76,77]. In the heart, where oxygen consumption is high, the cells are more prone to oxidative stress and have an increased requirement for protection against free radicals. Thus, plasmalogens would play a protective role by inhibiting peroxidation of polyunsaturated fatty acids as well as being substrates for free radicals. Nigam and Schewe [78] have indicated that sPLA₂ and cPLA₂ do not display any selectivity for oxidized phospholipid substrates, suggesting that the Ca²⁺-independent PAF acetylhydrolases or iPLA₂ may be responsible for membrane repair. Release of peroxidized fatty acids from the membrane by the action of PLA₂ was found to be an absolute requirement for glutathione peroxidase to reduce and detoxify fatty acid hydroperoxides in membranes, indicating that PLA₂ is essential in maintaining membrane integrity [78]. Thus, it follows that if hydrolysis of cytotoxic oxidized membrane phospholipids is catalyzed by iPLA₂, then iPLA₂ inhibition may augment cardiovascular oxidant injury. For example, we have demonstrated a marked inhibition of *in vivo* and *in vitro* myocardial membrane-associated iPLA₂ activity with clinical concentrations of doxorubicin, an anticancer drug associated with a high incidence of cardiotoxicity thought to be mediated by increased formation of oxygen free radicals and phospholipid peroxidation [30]. Doxorubicin alone caused little cell death, however inhibition of both cytosolic and membrane-associated iPLA₂ with BEL resulted in potentiation of doxorubicin-induced cell death. These results suggest that iPLA₂ inhibition may potentiate cell death in response to oxidative damage and may be potentially significant in reperfusion damage observed following myocardial ischemia.

THERAPEUTIC IMPLICATIONS OF PLA₂ INHIBITION IN CARDIOVASCULAR DISEASE

In conditions such as atherosclerosis and myocardial ischemia in which inhibition of cardiac inflammation would lead to improved cardiac function and prevention of further injury, the use of PLA₂ inhibitors has the potential to be beneficial. However, as new information about specific PLA₂ isoforms is discovered, many PLA₂ inhibitors originally designed to be selective for a specific PLA₂ isoform have subsequently been found to inhibit more than one isoform. For example, we have recently demonstrated that methyl arachidonyl fluorophosphonate (MAFP), a widely used inhibitor of cytosolic PLA₂ isoforms, is a potent inhibitor of PAF acetylhydrolase (PAF-AH) and potentiates PAF accumulation in thrombin and tryptase stimulated HCAEC [79]. Current research by our lab demonstrates MAFP augments thrombin and tryptase stimulated prostaglandin production in HCAEC as a direct consequence of increased arachidonic acid release (unpublished findings).

Due to the problems associated with the specificity of pharmacologic inhibitors, several molecular biology techniques have been used to support data obtained using pharmacologic inhibitors. While some of these techniques, such as overexpression of a specific PLA₂ isoform or the use of

knockout mice, are beneficial when studying the function of a specific isoform, their therapeutic potential is questionable. However, inhibition of specific PLA₂ isoform activity *via* antisense oligonucleotides [80-84], has a large therapeutic potential. Preliminary studies in human cell lines indicate that specific knock down of individual PLA₂ isoforms is possible. The utility of this approach for use with other isoforms is supported by the fact that the sequences encoding PLA₂ even within a single group can be vastly different ranging from only 8% protein sequence homology in human group IV to a maximum of 61% in group VIII [85]. This relative lack of sequence identity assures that for any given PLA₂ isoform hundreds of putative antisense sequences are available for comparison to minimize off-target effects and maximize on target reduction of gene expression. Furthermore, the investigation of antisense inhibition of PLA₂ could lay the groundwork for discovery of a nucleic acid-based anti-thrombotic drug.

CONCLUSION

In cardiovascular inflammation seen in thrombosis, ischemia, and atherosclerosis, either thrombin or tryptase are known to be elevated, demonstrating their potential to both initiate and propagate the inflammatory response. We have demonstrated that activation of iPLA₂ *via* either thrombin or tryptase stimulation leads to arachidonic acid release, PAF production, cell surface P-selectin expression and increased neutrophil adherence [7,57], all events contributing to inflammation following vascular injury. These findings demonstrate the significance of iPLA₂ in the initiation of the inflammatory response, emphasizing the possibility of preventing extensive cardiovascular damage by inhibiting iPLA₂ induced inflammation. The activation of iPLA₂ in HCAEC and the subsequent production of choline lysophospholipids may contribute directly to the initiation of ventricular arrhythmias due to their incorporation into the ischemic myocyte sarcolemma.

One of the major challenges of using PLA₂ inhibitors as potential therapeutic agents is to optimize maximal efficacy with minimal side effects. The emergence of a growing body of literature more elegantly describing and characterizing PLA₂-catalyzed membrane phospholipid hydrolysis under experimental conditions can only serve to further our understanding of this complicated process. In the future, the use of PLA₂ inhibitors may be governed more by the pharmaceutical aspect of therapy rather than the pharmacological one. For example, targeting the inhibitors to a specific cell type by introducing a homing tag, using localized application rather than systemic or targeting the enzyme to recognize a specific intracellular biochemical event (such as a decrease in pH as would be seen in myocardial ischemia) may broaden the potential for the use of PLA₂ inhibitors. Finally, the emerging role of PLA₂ enzymes in cell repair under certain disease conditions poses the intriguing possibility that we may eventually be more interested in preserving PLA₂ activity than inhibiting it.

REFERENCES

- [1] Libby, P. *Nature*, **2002**, *420*, 868.
- [2] Wick, J.; Schett, G.; Amberger, A.; Kleindienst, R.; Xu, Q. *Immunol. Today*, **1995**, *16*, 27.
- [3] Libby, P.; Hansson, G.K. *Lab. Invest.*, **1991**, *64*, 5.

- [4] Hansson, G.K.; Holm, J.; Jonasson, L. *Am. J. Pathol.*, **1989**, *135*, 169.
- [5] Jeziorska, M.; McCollum, C.; Woolley, D.E. *J. Pathol.*, **1997**, *182*, 115.
- [6] McHowat, J.; Kell, P.J.; O'Neill, H.B.; Creer, M.H. *Biochem.* **2001**, *40*, 14921.
- [7] Meyer, M.C.; Creer, M.H.; McHowat, J. *Am. J. Physiol.*, **2005**, *289*, C1485.
- [8] Golfman, L.S.; Haughey, N.J.; Wong, J.T.; Jiang, J.Y.; Geiger, J.D.; Choy, P.C. *J. Lipid Res.*, **1999**, *40*, 1818.
- [9] Chen, M.; Xiao, C.Y.; Hashizume, H.; Abiko, Y. *Am. J. Physiol.*, **1998**, *275*, H1782.
- [10] Snyder, F. *Am. J. Physiol.*, **1990**, *259*, C697.
- [11] Uemura, Y.; Lee, T.-C.; Snyder, F. *J. Biol. Chem.*, **1991**, *266*, 8268.
- [12] Lee, T.-C.; Uemura, Y.; Snyder, F. *J. Biol. Chem.*, **1992**, *267*, 19992.
- [13] Snyder, F. *Biochim. Biophys. Acta.*, **1995**, *1254*, 231.
- [14] Dennis, E.A. *Trends Biochem. Sci.* **1997**, *22*, 1.
- [15] Balsinde, J.; Balboa, M.A.; Insel, P.A.; Dennis, E.A. *Ann. Rev. Pharm. Tox.* **1999**, *39*, 175.
- [16] Dennis, E.A. *J. Biol. Chem.* **1994**, *269*, 13057.
- [17] Vadas, P.; Pruzanski, W. *Lab. Invest.*, **1986**, *55*, 391.
- [18] Pruzanski, W.; Vadas, P. *J. Rheumatol.*, **1988**, *15*, 1601.
- [19] Minami, T.; Tojo, H.; Shinomura, Y.; Matsuzama, Y.; Okamoto, M. *Gut*, **1994**, *35*, 1593.
- [20] McHowat, J. In *Recent Research Developments in Physiology*; Pandalai, Ed.; Research Signpost: India, **2003**, pp. 521-538.
- [21] Portilla, D.; Dai, G. *J. Biol. Chem.*, **1996**, *271*, 15451.
- [22] Balsinde, J.; Dennis, E.A. *J. Biol. Chem.*, **1996**, *271*, 6758.
- [23] Hazen, S.L.; Zupan, L.A.; Weiss, R.H.; Getman, D.P.; Gross, R.W. *J. Biol. Chem.*, **1991**, *266*, 7227.
- [24] Hazen, S.L.; Stuppy, R.J.; Gross, R.W. *J. Biol. Chem.*, **1990**, *265*, 10622.
- [25] Creer, M.H.; McHowat, J. *Am. J. Physiol.*, **1998**, *275*, C1498.
- [26] McHowat, J.; Creer, M.H. *Lipids*, **1998**, *33*, 1203.
- [27] McHowat, J.; Creer, M.H. *Am. J. Physiol.*, **1998**, *274*, C447.
- [28] McHowat, J.; Creer, M.H.; Hicks, K.K.; Jones, J.H.; McCrory, R.D.; Kennedy, R.H. *Am. J. Physiol.*, **2000**, *279*, E25.
- [29] McHowat, J.; Tappia, P.S.; Liu, S.-Y.; McCrory, R.D. Panagia, V. *Am. J. Physiol.* **2000**, *280*, C573.
- [30] McHowat, J.; Swift, L.M.; Arutunyan, A.; Sarvazyan, N. *Cancer Res.*, **2001**, *61*, 4024.
- [31] Bratton, D.; Henson, P.M.; In *Platelet-Activating Factor and Human Disease*; Barnes, Page, Henson, Eds.; Blackwell Scientific Publications: Oxford, **1989**, pp. 23-59.
- [32] Camussi, G.; Tetta, C.; Baglioni, C. *Clin. Immun. Immunopathol.*, **1990**, *57*, 331.
- [33] Lotner, G.Z.; Lynch, J.M.; Betz, S.J.; Henson, P.M. *J. Immunol.*, **1980**, *124*, 676.
- [34] Triggiani, M.; Schleimer, R.P.; Warner, J.A.; Chilton, F.H. *J. Immunol.*, **1991**, *146*, 660.
- [35] Bulger, E.M.; Maier, R.V. *Crit. Care Med.*, **2000**, *28*, N27.
- [36] Montrucchio, G.; Alloati, G.; Camussi, G. *Physiol. Rev.*, **2000**, *80*, 1669.
- [37] Ford, D.A.; Hazen, S.L.; Saffitz, J.E.; Gross, R.W. *J. Clin. Invest.*, **1991**, *88*, 331.
- [38] Hazen, S.L.; Ford, D.A.; Gross, R.W. *J. Biol. Chem.*, **1991**, *266*, 5629.
- [39] Hazen, S.L.; Wolf, M.J.; Ford, D.A.; Gross, R.W. *FEBS Lett.*, **1994**, *339*, 213.
- [40] Williams, S.D.; Hsu, F.-F.; Ford, D.A. *J. Lipid Res.*, **2000**, *41*, 1585.
- [41] McHowat, J.; Liu, S.; Creer, M.H. *Am. J. Physiol.*, **1998**, *274*, C1727.
- [42] Hazen, S.L.; Gross, R.W. *J. Biol. Chem.*, **1991**, *266*, 14526.
- [43] Davies, M.J.; Thomas, A. *N. Engl. J. Med.*, **1984**, *310*, 1137.
- [44] Goldstein, J.A.; Butterfield, M.C.; Onishi, Y.; Shelton, T.J.; Corr, P.B. *Circulation*, **1994**, *90*, 139.
- [45] Liu, S.J.; Creer, M.H.; Kennedy, R.H.; McHowat, J. *Am. J. Physiol.*, **2003**, *284*, C826.
- [46] Davey, M.G.; Luscher, E.F. *Nature*, **1967**, *216*, 857.
- [47] Berndt, M.C.; Phillips, D.R. In *Platelets in Biology and Pathology*; Gordon, Ed.; Elsevier/North Holland: Amsterdam, **1981**, pp. 43-74.
- [48] Bar-Shavit, R.; Kahn, A.; Wilner, G.D.; Fenton, J.W. *Science*, **1983**, *220*, 728.
- [49] Chen, L.B.; Teng, N.N.H.; Buchanan, J.M. *Exp. Cell Res.*, **1976**, *101*, 41.
- [50] Chen, L.B.; Buchanan, J.M. *Proc. Natl. Acad. Sci. USA*, **1975**, *72*, 131.
- [51] McNamara, C.A.; Sarembock, I.J.; Gimple, L.W.; Fenton II, J.W.; Coughlin, S.R.; Owens, G.K. *J. Clin. Invest.*, **1993**, *91*, 94.
- [52] Nelken, N.A.; Soifer, S.J.; O'Keefe, J.; Vu, T.-K.H.; Charo, I.F.; Coughlin, S.R. *J. Clin. Invest.*, **1992**, *90*, 1614.
- [53] Laine, P.; Kaartinen, M.; Penttillä, A.; Panula, P.; Paavonen, T.; Kovanen, P. *Circulation*, **1999**, *99*, 361.
- [54] Patella, V.; Marinò, I.; Arbustini, E.; Lamparter-Schummert, B.; Verga, L.; Monika, A.; Marone, G. *Circulation*, **1998**, *97*, 971.
- [55] Dery, O.; Corvera, C.U.; Steinhoff, M.; Bunnett, N.W. *Am. J. Physiol.*, **1998**, *274*, C1429.
- [56] Meyer, M.; McHowat, J. In *Recent Research Developments in Physiology*; Pandalai, Ed.; Research Signpost: India, **2004**, pp. 129-147.
- [57] Meyer, M.C.; Kell, P.J.; Creer, M.H.; McHowat, J. *Am. J. Physiol.*, **2005**, *288*, C475.
- [58] Gamble, J.R.; Skinner, M.P.; Berndt, M.C.; Vadas, M.A. *Science*, **1990**, *249*, 414.
- [59] Geng, J.G.; Bevilacqua, M.P.; Moore, K.L.; McIntyre, T.M.; Prescott, S.M.; Kim, J.M.; Biss, G.A.; Zimmerman, G.A.; McEver, R.P. *Nature*, **1990**, *343*, 757.
- [60] Lorant, D.E.; Patel, K.D.; McIntyre, T.M.; McEver, R.P.; Prescott, S.M.; Zimmerman, G.A. *J. Cell Biol.*, **1991**, *115*, 223.
- [61] Prescott, S.M.; Zimmerman, G.A.; McIntyre, T.M. *Proc. Natl. Acad. Sci. USA*, **1984**, *81*, 3534.
- [62] Kuijpers, T.W.; Hakkert, B.C.; Hart, M.H.L.; Roos, D. *J. Cell Biol.*, **1992**, *117*, 565.
- [63] Murakami, M.; Matsumoto, R.; Austen, K.F.; Arm, J.P. *J. Biol. Chem.*, **1994**, *269*, 22269.
- [64] Bingham, C.O.; Murakami, M.; Fujishima, H.; Hunt, J.E.; Austen, K.F.; Arm, J.P. *J. Biol. Chem.*, **1996**, *271*, 25936.
- [65] Reddy, S.T.; Herschman, H.R. *J. Biol. Chem.*, **1997**, *272*, 3231.
- [66] Kuwata, H.; Nakatani, Y.; Murakami, M.; Kudo, I. *J. Biol. Chem.*, **1998**, *273*, 1733.
- [67] Naraba, H.; Murakami, M.; Matsumoto, H.; Shimbara, S.; Ueno, A.; Kudo, I.; Ohishi, S. *J. Immunol.*, **1998**, *160*, 2974.
- [68] Fujishima, H.; Sanchez Mejia, R.O.; Bingham, C.O.; Lam, B.K.; Sapirstein, A.; Bonventre, J.V.; Austen, K.F.; Arm, J.P. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 4803.
- [69] Morita, I.; Schindler, M.; Reiger, M.K.; Otto, J.C.; Hori, T.; De Witt, D.L.; Smith, W.L. *J. Biol. Chem.*, **1995**, *270*, 10902.
- [70] Roshak, A.; Sathe, G.; Marshall, L.A. *J. Biol. Chem.*, **1994**, *269*, 25999.
- [71] Gargalovic, P.; Dory, L. *J. Biol. Chem.*, **2001**, *276*, 26164.
- [72] Parolini, I.; Sargiacomo, M.; Galbiati, F.; Rizzo, G.; Grignani, F.; Engleman, J.A.; Okamoto, T.; Ikezu, T.; Scherer, P.E.; Mora, R.; Rodriguez-Boulan, E.; Peschler, C.; Lisanti, M.P. *J. Biol. Chem.*, **1999**, *274*, 25718.
- [73] Karmazyn, M.; Dhalla, N.S. *Can. J. Physiol. Pharmacol.*, **1983**, *61*, 1207.
- [74] Tay, H.K.; Melendez, A.J. *J. Biol. Chem.*, **2004**, *279*, 22505.
- [75] Murakami, M.; Kambe, T.; Shimbara, S.; Kudo, I. *J. Biol. Chem.*, **1999**, *274*, 3103.
- [76] Zoeller, R.A.; Morand, O.H.; Raetz, C.R.H. *J. Biol. Chem.*, **1988**, *263*, 11590.
- [77] Zoeller, R.A.; Lake, A.C.; Nagan, N.; Gaposchkin, D.P.; Legner, M.A.; Lieberthal, W. *Biochem. J.*, **1999**, *338*, 769.
- [78] Nigam, S.; Schewe, T. *Biochim. Biophys. Acta.*, **2000**, *1488*, 167.
- [79] Kell, P.J.; Creer, M.H.; Crown, K.N.; Wirsig, K.; McHowat, J. *J. Pharm. Exp. Ther.*, **2003**, *307*, 1163.
- [80] Roshak, A.K.; Capper, E.A.; Stevenson, C.; Eichman, C.; Marshall, L.A. *J. Biol. Chem.*, **2000**, *275*, 35692.
- [81] Tibes, U.; Rohr, S.P.; Scheuer, W.; Amandi-Burgermeister, E.; Litters, A. *Adv. Exp. Med. Biol.*, **1999**, *469*, 199.
- [82] Balsinde, J.; Shinohara, H.; Lefkowitz, L.J.; Johnson, C.A.; Balboa, M.A.; Dennis, E.A. *J. Biol. Chem.*, **1999**, *274*, 25967.
- [83] Akiba, S.; Mizunaga, S.; Kume, K.; Hayama, M.; Sato, T. *J. Biol. Chem.*, **1999**, *274*, 19906.
- [84] Shinohara, H.; Balboa, M.A.; Johnson, C.A.; Balsinde, J.; Dennis, E.A. *J. Biol. Chem.*, **1999**, *274*, 19906.
- [85] Meyer, M.C.; Rastogi, P.; Beckett, C.S.; McHowat, J. *Curr. Pharm. Design.*, **2005**, *11*, 1301.