Type II secretory phospholipase A2 in cardiovascular disease: a mediator in atherosclerosis and ischemic damage to cardiomyocytes?

Hans W.M. Niessen\textsuperscript{a,b,*}, Paul A.J. Krijnen\textsuperscript{a,b,1}, Cees A. Visser\textsuperscript{a,c}, Chris J.L.M. Meijer\textsuperscript{b}, C. Erik Hack\textsuperscript{a,d,e}

\textsuperscript{a}ICaR-VU, VU Medical Center, Amsterdam, The Netherlands
\textsuperscript{b}Department of Pathology, VU Medical Center, Amsterdam, The Netherlands
\textsuperscript{c}Department of Cardiology, VU Medical Center, Amsterdam, The Netherlands
\textsuperscript{d}Department of Clinical Chemistry, VU Medical Center, Amsterdam, The Netherlands
\textsuperscript{e}Department of Immunopathology, Sanquin Research at CLB, Amsterdam, The Netherlands

Accepted 14 February 2003

Abstract

Inflammatory reactions contribute to the pathogenesis of cardiovascular conditions such as atherosclerosis and ischemic damage in acute myocardial infarction (AMI). Among the mediators involved in inflammation are secretory phospholipase A2 group II (sPLA2-II) enzymes. Though some cells constitutively express sPLA2-II, the synthesis by cells such as hepatocytes is typical for an acute-phase reactant. Recent literature suggests multiple roles for sPLA2-II in cardiovascular disease. In this review we discuss the role of sPLA2-II in various in vivo and in vitro models of atherosclerosis or AMI, including the therapeutic perspective of sPLA2-II inhibitors. It was concluded that sPLA2-II appears to be an important inflammatory mediator of cardiovascular disease.

Keywords: Phospholipases; Ischemia; Infection/inflammation; Artherosclerosis

1. Introduction

Atherosclerosis and one of its major consequences, acute myocardial infarction (AMI), are major cardiovascular conditions in the Western World. Traditionally, these conditions are considered to result from lipid abnormalities, and other risk factors such as hypertension, smoking, hyperhomocysteinemia and diabetes. However, evidence is now accumulating that in addition to these inflammatory reactions in the atherosclerotic lesions as well as in the infarcted myocardium have a major impact on incidence and outcome of atherosclerosis and AMI. For example, a number of studies during the last decade have shown that plasma levels of C-reactive protein (CRP), a typical inflammation marker in humans, constitute an independent risk marker for cardiovascular events both in apparently healthy individuals as well as in patients with established cardiovascular disease such as stable angina pectoris [1]. The beneficial effects of statins, originally developed because of their cholesterol-lowering properties, may at least in part be related to their anti-inflammatory properties [2]. Also, many of the processes ensuing in atherosclerotic vessels during the various stages of the lesions, such as the influx and activation patterns of blood mononuclear cells, show remarkable similarities to processes occurring in established immune or inflammatory diseases [3]. This growing appreciation of a role for inflammation in atherosclerosis has even led some to speculate that this condition may result from or be enhanced by microbial infections in the vessel wall, involving organisms such as \textit{Chlamydiae pneumoniae} or viruses [4]. Pathological studies of the hearts of persons

\*Corresponding author. VU Medical Center, Department of Pathology, Room nr. OE16, De Boelelaan 1117, 1007 MB Amsterdam, The Netherlands. Tel.: +31-20-444-4003; fax: +31-20-444-2964.
E-mail address: jwm.niessen@vumc.nl (H.W.M. Niessen).

\textsuperscript{1}Hans Niessen and Paul Krijnen contributed equally to this review.

\textsuperscript{2}Time for primary review 28 days.

0008-6363/03 – see front matter © 2003 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.
doi:10.1016/S0008-6363(03)00324-9
dying from AMI have provided compelling evidence that inflammatory reactions ensue in the ischemic myocardium starting hours after the onset of complaints. Numerous studies in animals have shown that anti-inflammatory agents under some conditions can reduce infarction size substantially in AMI [5–7]. Initial clinical studies with a naturally occurring complement inhibitor, C1-inhibitor, have yielded promising results [8].

Inflammatory reactions are mediated by an array of so-called inflammatory mediators which amongst others include cytokines, complement and many more. Type II secretory phospholipase A2 (sPLA2-II) constitutes one of the inflammatory mediators possibly involved in the pathogenesis of atherosclerosis and of AMI. A role for this enzyme in either disease is suggested by observations that its circulating levels are high in atherosclerosis and AMI.

In addition, sPLA2-II has been shown to be deposited in atherosclerotic arteries as well as on ischemic cardiomyocytes. Here we discuss the idea that sPLA2-II enhances atherosclerosis and exerts detrimental effects on ischemic cardiomyocytes in infarcted myocardium, and that the enzyme should be considered as an inflammatory mediator in cardiovascular disease rather than being an epiphenomenon.

2. Phospholipase A2 (PLA2) enzymes

PLA2 are widely distributed enzymes that exist in many isozymes, including the 14-kDa secretory phospholipase A2 (sPLA2) and the 85-kDa cytosolic phospholipase A2 (cPLA2) [9]. sPLA2 comprises a small group of homologous proteins with five to eight disulfide bridges. The location of these disulfide bonds in the protein backbone provides the basis for a classification into several subgroups [10].

sPLA2-I is known as pancreatic PLA2, since it is expressed at high levels in the pancreas, to be secreted into the intestine where it hydrolyzes food-derived phospholipids.

Significant levels of sPLA2-I mRNA are also found in spleen, lung and prostate. sPLA2-II was initially discovered in synovial fluid of patients with rheumatoid arthritis (RA) [11], and subsequently found to be an acute phase protein, of which circulating levels may increase more then 100-fold during acute phase reaction. Whereas sPLA2-I and -II are extracellular proteins that are secreted by cells, cPLA2 is an intracellular cytoplasmic protein, of which mRNA has been found in many tissues including heart, spleen, lung and kidney [10].

PLA2 enzymes hydrolyze phospholipids at the sn-2 position to generate free fatty acids and lysolipids, which is the rate-limiting step in the biosynthesis of eicosanoids and platelet-activating factor (PAF) (Fig. 1) [12]. These substances are potent mediators of inflammation, apoptosis and tumorigenesis [12]. This important biological activity explains why inhibitors of PLA2 enzymes have received considerable attention by pharmaceutical companies as drug targets. Hydrolysis of phospholipids in membranes by PLA2 requires binding of the enzyme to the membrane, the binding of which is dependent on electrostatic (charge–charge) interactions amongst others [12].

3. Secretory PLA2-II (sPLA2-II)

sPLA2-II accumulates in inflammatory fluids [13]. Being an acute phase protein, plasma levels strongly increase during inflammatory diseases such as infections, septic shock and RA. However, processes such as carcinogenesis and atherosclerosis may also be accompanied by increased circulating levels [14]. sPLA2-II is constitutively expressed in organs dealing with inflammatory response, such as spleen, thymus, intestines, liver and bone marrow, but also in lung, kidney and ovary. Furthermore, many cell types are able to secrete sPLA2-II such as endothelial cells, smooth muscle cells, platelets, mast cells, neutrophils, macrophages and hepatic cells. All these cells may contribute to the elevation of circulating levels during acute phase responses, as the synthesis of sPLA2-II by these cells is stimulated by proinflammatory cytokines [15,16]. The expression of the sPLA2-II gene is stimulated by factors as diverse as hypoxia, cytokines and phorbol esters [17], and is amongst others regulated by transcription factors including NF-κB, PPAR and C/EBP [18]. NF-κB is an essential component of the cytokine signaling pathways inducing sPLA2-II expression. PPARs control the regulation of genes involved in lipid metabolism in general. While the human sPLA2-II promoter is controlled by C/EBP factors [18]. The activation of these transcription factors is under the control of distinct signaling pathways, like PKC and cAMP [18].

Interaction of sPLA2-II with cellular phospholipids depends in part on the nature of these phospholipids [16]. The enzyme binds with a higher affinity to phosphatatic acid (PA) than to phosphatidylyethanolamine (PE), phosphatidylserine (PS) and phosphatidylcholine (PC) [15,19]. This binding specificity results from non-covalent interactions of the amino acid backbone of the protein with the phospholipids, interactions of which are apparently stronger in the case of PA as compared to PE, PS and PC [19]. sPLA2-II has only a low affinity for PC as a substrate, which is part explained by the presence of a critical valine residue, rather than a tryptophan residue, which is found in PLA2 enzymes with a higher affinity for PC [20]. The presence of the amphiphilic indole side chain in tryptophan allows a better penetration of PLA-2 into the lipid interface of membranes [20]. A V3W mutant of human sPLA2-II, in which this critical valine residue was replaced by tryptophan, showed strong (250-fold) enhanced enzymatic activity for small unilamellar vesicles of
Fig. 1. Schematic presentation of the activation mechanisms and localization of activity of sPLA₂-II and cPLA₂. Phosphorylation of Serine⁵⁰⁵ by a kinase leads to catalytic activity of cPLA₂. Ca⁺⁺ binds to the N-terminal calcium-dependent phospholipid-binding domain (CaLB) of cPLA₂ and thereby promotes translocation to membranes. sPLA₂ is also dependent upon Ca⁺⁺, which binds to sPLA₂-II via a specific calcium-loop. Also, sPLA₂-II can only bind flip-flopped membranes, i.e. when negatively loaded phospholipids PE and PS (depicted as = or -=) are present in the outer membrane leaflet.

PC, whereas wild-type human sPLA₂-II hardly hydrolyzed this substrate [20].

Notably, some biological effects of sPLA₂-II are independent of its catalytic function [21], suggesting it may interact with specific receptors. Indeed the enzyme has been shown to bind to a high affinity receptor (K₅=7.5 pM) of 180 kDa, present on rabbit skeletal muscle [22]. This so-called M-type receptor mediates some of the physiological effects of mammalian sPLA₂-II, including vascular smooth muscle cell (SMC) contraction and cell proliferation. In addition, binding of sPLA₂-II to this receptor may induce internalization of sPLA₂-II [23]. Also, murine sPLA₂-II is a high affinity ligand for murine M-type receptor [24]. However, human sPLA₂-II does not seem to be a physiological ligand for the human M-type receptor [24]. Thus, the function of this putative high affinity sPLA₂-II receptor is likely to be species-dependent.

4. Effector functions of sPLA₂-II

Under normal conditions, the composition of phospholipids in inner and outer leaflet is not similar. The outer leaflet of the membrane contains mainly hydrophobic phospholipids, whereas the inner contains anionic phospholipids such as PS, as well. Due to their hydrophobic nature, the phospholipids in the outer leaflet of normal, vital cells are tightly packed together, and are not hydrolyzed by sPLA₂-II. Under some conditions, the phospholipids of the inner and outer leaflet become rearranged leading to the presence of PS in the outer leaflet. This process is known as membrane ‘flip-flop’. Thus, a translocation of negatively charged phospholipids (PS, and to a lesser extent PE) from the inner layer of the membrane to the outer layer occurs during a membrane flip-flop [25]. In contrast to that of normal membranes, the outer leaflet of flip-flopped membranes is easily hydrolyzed by sPLA₂-II. This affinity of sPLA₂-II for flip-flopped membranes is explained by the presence of a number of cationic residues forming patches on the putative interfacial binding surface of the enzyme [26].

sPLA₂-II generates various lysophospholipids, such as lysoPC, lysoPE, lysoPS and lysoPA [13]. Interestingly, lyso-PA is a messenger acting through G protein-coupled receptors [27], and can stimulate platelet aggregation, cell proliferation and SMC contraction [19]. sPLA₂-II indeed has been described to induce signal-transduction in inflammatory cells. For example, in combination with PAF, the enzyme induces exocytosis of human neutrophils [28]. Notably, the biological function of sPLA₂-II in some cell types is independent of its catalytic function [22].

sPLA₂-II not only hydrolyzes phospholipids in the cell membrane of cells, but also those in bacterial cell walls, leading to impaired integrity of the membranes [15], and providing the enzyme with bactericidal properties [29]. It
has been shown that the assembly of membrane-attack complex of complement increases the susceptibility of the cell membrane of Gram-negative bacteria for sPLA2-II by producing sublethal alterations of the outer envelope of the bacteria [29]. sPLA2-II is also a primary bactericidal agent for Gram-positive bacteria [29].

5. Crosstalk between cPLA2 and sPLA2-II

In mast cells sPLA2-II was found to mediate the selective release of arachidonic acid (AA) by binding to cell surface receptors, followed by induction of signal transduction events that lead to cPLA2 activation [30]. This possibly involves the activation of the PKC/Raf-1/MAPK signalling pathway [31]. Furthermore, in rat fibroblastic 3Y1 cells cPLA2 and its downstream 12- or 15-lipoxygenase pathway were shown to enhance sPLA2-II gene expression. cPLA2 may also alter membrane susceptibility for sPLA2-II [32]. For example, in human embryonic kidney cells, prior production of AA by cPLA2 in cytokine-stimulated cells destabilized the cellular membranes, and rendered them more susceptible to subsequent hydrolysis by sPLA2-II [33]. In contrast, from a study in S49 cells, it was concluded that cPLA2 reduces susceptibility of membranes to sPLA2 during apoptosis [34]. cPLA2 as well as sPLA2-II have partially overlapping functions. For example, in neutrophils stimulated with fMLP both cPLA2 and sPLA2-II were found to contribute to AA release [35]. Moreover, in the human keratinocyte cell line HaCaT, inhibition of both cPLA2 and sPLA2-II blocked TNF-induced activation of NF-κB and subsequent expression of ICAM-1 [36]. cPLA2 and sPLA2-II may be activated by similar stimuli, as has been shown in human natural killer cells, in which both sPLA2-II and cPLA2 were rapidly activated upon CD16 cross-linking [37].

6. Interactions of sPLA2-II with lipoproteins

Among the first events in atherogenesis is modification of low density lipoprotein (LDL) particles in the arterial wall, and subsequent aggregation and fusion of lipid droplets that are relatively depleted in PC [38]. High levels of small dense LDL in plasma are associated with an increased risk for cardiovascular disease [39]. Small dense LDL particles are formed by reduction of the phospholipid content in the surface monolayer LDL, which causes an enhanced interaction with proteoglycans [39]. sPLA2-II may help to reduce the phospholipid content of LDL [39]. Also, 15-lipoxygenase-induced LDL oxidation was enhanced by sPLA2-II [40]. In vitro it was shown that sPLA2-II liberated polysaturated free fatty acids from phospholipids. These fatty acids increase the formation of bioactive phospholipids in LDL, resulting in an enhanced ability to stimulate monocyte-endothelial interactions [41]. However, native LDL proved to be a poor substrate for sPLA2-II, whereas after mild oxidation, which occurs in atherosclerosis, the susceptibility of LDL to phospholipid hydrolysis is increased [42]. In another study it was shown that sPLA2-II induced only little lipolytic modification of LDL, while a strong lipolytic activity was found for the subtype sPLA2-X [43]. Hence, likely subtle differences in composition, oxidation, and possibly other aspects, may alter the susceptibility of LDL for sPLA2-II and other PLA2 enzymes.

Normal high density lipoprotein (HDL) reduces activity of sPLA2-II [44]. In a co-culture system of human endothelial cells and SMCs, HDL from sPLA2-II-transgenic mice or human HDL treated with recombinant sPLA2-II, failed to protect against the formation of biologically active phospholipids in LDL [41]. Furthermore, it has been shown that acute phase HDL containing 27% serum amyloid A (SAA), an acute phase protein, enhanced sPLA2-II activity [44]. This interaction between HDL, SAA and sPLA2-II may explain the alterations in lipoprotein metabolism during acute phase reactions [44]. sPLA2-II may even hydrolyze the phospholipid monolayers of HDL [15]. Therefore, transgenic mice overexpressing sPLA2-II have lower HDL levels, whereas levels of LDL are increased [45]. Furthermore, sPLA2-II caused a decrease in plasma HDL cholesterol in response to inflammatory stimuli in mice, via a direct interaction on HDL particle size and composition [46].

7. sPLA2-II interactions with cells of the vessel wall

Incubation of macrophages with sPLA2-II-treated LDL and HDL results in increased intracellular lipid accumulation, and triggers the formation of foam cells [15]. sPLA2-II itself also activates macrophages via induction of the secretion of enzymes and cytokines [47], while in the presence of LPS, sPLA2-II stimulates iNOS expression and nitrite production in macrophages [48]. In primary cultures of rat endothelial cells and in two different rat endothelial cell lines (SVAREC and RBE4), IL-1β-induced sPLA2-II gene expression and secretion of the enzyme in a dose- and time-dependent manner [49]. In contrast, LPS treatment of cultured primary endothelial cells caused an increase of cPLA2 mRNA, but not of sPLA2-II mRNA [50]. Whether sPLA2 contributes to AA release by endothelial cells is not clear. At least in bovine aortic endothelial cells, an inhibitor of sPLA2-II (CGP-43187) did not affect AA release induced by the non-selective activator of heterotrimeric guanine nucleotide-binding proteins, NaF [51]. Furthermore, VEGF-enhanced PAF synthesis in HUVEC was mediated by sPLA2-V, but not by sPLA2-II [52].

In human vascular SMC, IL-1 did not affect sPLA2-II mRNA levels, while it increased cPLA2-mRNA [53]. In rabbit aortic vascular SMC, norepinephrine enhanced
release of AA via activation of cPLA2 but not sPLA2-II [54].

A main process induced by activated endothelial cells or macrophages is coagulation. sPLA2-II inhibits blood coagulation independent of its lipolytic action [55]. The enzyme inhibits prothrombinase, which is the enzyme complex that catalyzes the formation of thrombin from prothrombin, and which consists of activated factors Xa and Va assembled onto a membrane surface in the presence of calcium [56,57]. Whether this anti-coagulant effect is relevant for the in vivo situation is not clear. From ellipsometric studies (an optical technique measuring the changes in surface-bound mass), it was concluded that plasma sPLA2-II concentrations in inflammatory disease will not be sufficiently elevated to inhibit coagulation efficiently [58].

8. Relation between sPLA2-II and atherosclerosis in vivo

Rheumatoid arthritis (RA) patients have an increased risk for cardiovascular events. sPLA2-II in RA patients significantly correlates with CRP, soluble ICAM and LDL-I, while it inversely correlates with HDL [59]. This would suggest that sPLA-II is a cardiovascular risk marker. Indeed, circulating levels of sPLA2-II constitute an independent risk marker for coronary artery disease and predict cardiovascular events [60]. An increase in circulating levels of sPLA2-II also predicted clinical coronary events independently of other risk factors in patients with unstable angina [60]. Finally, elevated blood levels of sPLA2-II predict restenosis in patients undergoing coronary angioplasty [61].

Atherosclerotic plaques exhibit a series of features that are similar to those of chronic inflammation [62]. In humans sPLA2-II is present in the intima, media and adventitia of the atherosclerotic wall, not only in mature lesions but also at a very early stage of atherosclerosis [15]. sPLA2-II was found in foam cells, calcifications sites, areas of cell necrosis and in the extracellular matrix [62]. In the abdominal human aorta, sPLA2-II was present in all advanced atherosclerotic lesions, but only in some preatheromas and early lesions of atherosclerosis [63]. In contrast, in the thoracic aorta, sPLA2-II was found at a similar frequency in all stages of atherosclerotic lesions, in all three layers of the vessel wall [63]. In another study of human carotid arteries, sPLA2-II was found throughout the media in atherosclerotic but also in normal artery specimens [64]. In contrast, in another study, sPLA2-II did not stain normal human arteries without thickened intima [62]. The presence of sPLA2-II in atherosclerotic lesions is not unique for humans. The enzyme was also present in atherosclerotic lesions of transgenic mice overexpressing sPLA2-II [45]. Interestingly, the presence of DNA of Chlamydia pneumonia, HSV-1 and CMV was found to be associated with sPLA2-II expression and other signs of local inflammation in human atherosclerosis [15].

9. sPLA2-II in the heart

We have described the presence of sPLA2-II in infarcted myocardium [65] suggesting the enzyme to be involved in the inflammatory reactions ensuing in heart infarcts. This sPLA2-II may originate from the blood or be produced locally by cardiomyocytes. Indeed, Northern blotting revealed the presence of mRNA of sPLA2-II in the rat heart [66]. Cytokines such as TNF-α stimulate transcription levels of sPLA2-II in isolated neonatal rat cardiomyocytes [67]. Interestingly, the borderzone of the infarcts in humans contained cells that had bound sPLA2-II in spite of normal morphology [65]. In contrast, normal cardiomyocytes in non-ischemic sites did not bind sPLA2-II. Thus, binding of sPLA2-II may be one of the earliest phenomena on the outside of cells during ischemia. In vitro, we have identified a flip-flopped, but caspase-3-negative population of H9c2 cells, and a rat cardiomyocyte cell line, upon exposure to metabolic inhibitors. These cells were killed upon subsequent incubation with sPLA2-II (manuscript submitted by Nymeyer et al.). Similar results were obtained with adult rabbit cardiomyocytes. These findings together suggest that sPLA2-II may induce cardiomyocyte cell death in human infarction. Previously, we have hypothesized that sPLA2-II may also indirectly damage reversibly injured cardiomyocytes by generating binding sites for CRP [68], which upon binding may activate complement. Indeed, in human myocardial infarction, sPLA2-II depositions precede those of CRP [65]. Interestingly, there is evidence that CRP can regulate sPLA2-II activity in a substrate-concentration dependent fashion [69].

The studies discussed above suggest a role for sPLA2-II in the inflammatory damage ensuing in infarcted myocardium. However, from a study in hearts from wild-type and sPLA2-II deficient mice it was concluded that sPLA2-II does not play a prominent role in the development of irreversible cell damage in the ischemic-reperfused murine myocardium [70]. Notably, in this study the role of sPLA2-II was not analyzed during acute phase reactions, since Langendorf perfusion of isolated hearts was used. As we did not find sPLA2-II depositions in human myocardial infarction until at least 6–12 h after the onset of symptoms, i.e. at which time blood levels of sPLA2-II in patients with AMI start to increase [65], acute phase concentrations of sPLA2-II are likely to be required to mediate inflammatory damage in the heart. Moreover, mouse blood hardly contains CRP, and hence CRP-dependent damage induced by sPLA2-II may have been missed in the Langendorf perfusion model.
10. Studies with sPLA2-II inhibitors

In the placenta, sPLA2-II specific antisense oligonucleotides A and B inhibited the expression and activity of sPLA2-II [71]. Another potent inhibitor is LY315920 ([3-(aminooxoacetyl)-2-ethyl-1-(phenylmethyl)-1H-indol-4-yl]oxyacetate), a pharmacological drug that functions as a selective, stoichiometric inhibitor of the catalytic activity of sPLA2-II. LY315920 inhibited sPLA2-II serum activity after intravenous or oral administration to transgenic mice expressing the human sPLA2-II [72]. Other novel sPLA2-II inhibitors, petrosaspongiolide M (isolated from the Caledonian marine sponge *Petrosaspongia nigra*), and cacospongiolide B (isolated from the sponge *Fasciospongia cavernosa*), had anti-inflammatory properties in mice and rats with respect to arthritis and post-inflammatory edema [73,74]. Unfortunately, no studies with these inhibitors have been performed in animal models for myocardial infarction. So it remains to be established whether sPLA2-II inhibitors are useful in the treatment of AMI.

Some drugs developed because of other biological properties, also appeared to inhibit sPLA2-II activity or sPLA2-II-induced phenomena. Alminoprofen, a NSAID (a member of the phenylpropionic acid class of drugs), and aspirin inhibit sPLA2-II activity [75,76], as does heparin [77]. Another NSAID, indomethacín, inhibits sPLA2-induced aggregation of platelets [75]. Chemically modified tetracyclines, devoid of antimicrobial properties, not only inhibit sPLA2-II but also some matrix metalloproteinases, that play an important role in the process of atherosclerosis [78]. Furthermore, in patients with hypercholesterolemia, both simvastatin and atorvastatin caused a reduction in sPLA2-II [2]. Whether the beneficial effects on cardiovascular disease of the drugs discussed in this section are due to their effects on sPLA2-II remains to be established yet.

11. Discussion

Inflammation plays an important role in both atherosclerosis and acute myocardial infarction (AMI), although via different mechanisms. Inflammation in atherosclerosis likely contributes to recruitment of inflammatory cells in the lesions and the formation of foam cells, and also to the formation of unstable plaques or plaque ruptures, which are the main processes that elicit (cardio)vascular events. In AMI, local inflammatory reactions in the ischemic myocardium contribute to myocardial damage and infarction size and play a role in tissue remodelling [65].

Various inflammatory mediators have been identified to play a role in both atherosclerosis and AMI, including cytokines, adhesion molecules, complement and acute phase proteins. Among the latter is type-II secretory phospholipase A2 (sPLA2-II) [1]. All current evidence suggests a role for sPLA2-II in cardiovascular pathology. In atherosclerosis, sPLA2-II not only plays a role in macrophage activation and foam cell formation [15,47], but it also hydrolyzes LDL [39] and HDL [15], resulting in increase numbers of pro-atherogenic small LDL particles, and an impaired function of anti-atherogenic HDL. Although sPLA2-II depositions have been found locally in atherosclerotic lesions [62,63], and clinical studies have revealed a correlation between sPLA2-II and vascular complications [39,60], until now the role of sPLA2-II in the induction of local complications of atherosclerotic plaques themselves or subsequent vascular complications has not been clarified. Also, the relevance of the anti-coagulant function of sPLA2-II remains to be established [56]. With regard to the latter, one may speculate that the enzyme induces prolonged bleeding in a complicated atherosclerotic plaque, which can result in vascular complications. Also, the potential antibacterial effects of sPLA2-II within atherosclerotic plaques, is not well established [15,29].

With respect to putative therapeutic options in atherosclerosis, it has been shown that sPLA2-II activity in patients with hypercholesterolemia who receive statins, is inhibited [2]. Also, aspirin is able to reduce sPLA2-II levels [75,76]. Yet, at the moment it can only be speculated that the beneficial effects of either drug is due to their effect on circulating sPLA2-II. Yet, except for a potential anti-coagulant effect, all the evidence to date points to a detrimental role for sPLA2-II in the development of atherosclerotic lesions. Thus, although the role of sPLA2-II in atherosclerosis remains a matter of speculation, studies with specific sPLA2-II inhibitors in animal models for atherosclerosis seem to be warranted. Also, in AMI the function of sPLA2-II seems to be detrimental in that it promotes inflammation. Hence, for the treatment of AMI, specific inhibitors of the enzyme also constitute an attractive option. The time-window for the start of therapy with sPLA2-II inhibitors in patients is likely to be 6–24 h after the onset of complaints, as blood levels start to increase in this period, which is accompanied with deposition of the enzyme in the ischemic myocardium. To our knowledge, binding of sPLA2-II to jeopardized cardiomyocytes seems to be among the first events in the processes that ultimately lead to the death of these cells [65]. We speculate that a major event in these processes is the binding of CRP, which is facilitated by the effects of sPLA2-II on membrane phospholipids in the outer leaflet of injured cells. Complement and neutrophils are also among the inflammatory mediators triggered by sPLA2-II and CRP in the ischemic myocardium [1,65]. To what extent sPLA2-II locally produced by cardiomyocytes contribute to the inflammation in the heart is not yet clear, although the time-relationship between increasing plasma levels and the deposition in the heart suggest most of the enzyme is derived from the blood [70]. Nevertheless, current understanding of the role of sPLA2-II in patients with AMI thus
far supports the use of specific sPLA2-II inhibitors in these patients.

In conclusion sPLA2-II plays an important pathophysiological role in both atherosclerosis and AMI (Fig. 2). Although the local effect of sPLA2-II in atherosclerosis has to be studied in more detail, the results of the studies discussed above make it worthwhile to start in vivo animal studies with sPLA2-II inhibitors with respect to

![Diagram](image-url)

**Fig. 2.** Overview of the role of sPLA2-II in atherosclerosis and in post-AMI damage to cardiomyocytes. In blood vessels sPLA2-II promotes atherosclerosis. sPLA2 has been found in atherosclerotic plaques where it induces (small dense) LDL increase, HDL decrease and it triggers macrophages to express certain enzymes and to transform into foam cells. Atherosclerosis may lead to AMI. sPLA2-II induces damage to cardiomyocytes during the inflammatory reactions ensuing in the ischemic myocardium. It can bind to flip-flopped membranes [PE and PS (+/-) in outer leaflet] promoting cell death through the activation of caspase 3, which ultimately leads to secondary necrosis. sPLA2-II also promotes the binding of CRP and the subsequent activation of the complement system, leading to secondary necrosis.
atherosclerosis development and the treatment of AMI, and eventually also in humans.

Acknowledgements

Dr Niessen is a recipient of the dr. E. Dekker program of the Netherlands Heart Foundation (D99025).

References


