

The secretory phospholipase A₂ group IIA: a missing link between inflammation, activated renin-angiotensin system, and atherogenesis?

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Abstract: Inflammation, lipid peroxidation and chronic activation of the renin-angiotensin system (RAS) are hallmarks of the development of atherosclerosis. Recent studies have suggested the involvement of the pro-inflammatory secretory phospholipase A₂ (sPLA₂)-IIA in atherogenesis. This enzyme is produced by different cell types through stimulation by pro-inflammatory cytokines. It is detectable in the intima and in media smooth muscle cells, not only in atherosclerotic lesions but also in the very early stages of atherogenesis. sPLA₂-IIA can hydrolyse the phospholipid monolayers of low density lipoproteins (LDL). Such modified LDL show increased affinity to proteoglycans. The modified particles have a greater tendency to aggregate and an enhanced ability to insert cholesterol into cells. This modification may promote macrophage LDL uptake leading to the formation of foam cells. Furthermore, sPLA₂-IIA is not only a mediator for localized inflammation but may be also used as an independent predictor of adverse outcomes in patients with stable coronary artery disease or acute coronary syndromes. An interaction between activated RAS and phospholipases has been indicated by observations showing that inhibitors of sPLA₂ decrease angiotensin (Ang) II-induced macrophage lipid peroxidation. Meanwhile, various interactions between Ang II and oxLDL have been demonstrated suggesting a central role of sPLA₂-IIA in these processes and offering a possible target for treatment. The role of sPLA₂-IIA in the perpetuation of atherosclerosis appears to be the missing link between inflammation, activated RAS and lipidperoxidation.

Keywords: secretory phospholipase A₂, lipoproteins, renin-angiotensin system, inflammation, atherosclerosis

Introduction

Atherosclerosis with its associated cardiovascular events, myocardial infarction (MI), sudden cardiac death or stroke, is one of the leading causes of death in the western countries (Ross 1999). Inflammation and lipid peroxidation, as well as chronic activation of the renin-angiotensin system (RAS), are hallmarks of atherogenesis. Activated RAS, inflammatory processes and lipid peroxidation products contribute to the initiation, progression and rupture of atherosclerotic plaques (Neaton et al 1992; Lowe et al 1998; Thomas et al 2002). Therefore reducing pro-inflammatory mediators, and inhibiting the modulation of their releasing pathways, may be important both for the stability of atherosclerotic lesions and the perpetuation of atherosclerotic plaques (Taniguchi et al 2005).

In addition to this link, more direct interactions have been suggested between a chronically activated RAS, and elevated pro-inflammatory cytokines and lipoproteins, which may also promote atherogenesis (Nickenig et al 2000). An increasing amount of evidence indicates that secretory phospholipase A₂ (sPLA₂) enzymes present in the vessel wall have localized effects that promote these processes (Kovanen et al 2000;

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Hurt-Camejo et al 2001; Niessen et al 2003; Tietge et al 2005; Menschikowski et al 2006). Three members (group IIA, group V and group X) of the various sPLA₂ isozymes have been detected in murine or human atherosclerotic lesions (Murakami et al 2004; Rosengreen et al 2004; Wootton-Kee et al 2004); expression and localization of secretory phospholipase A₂ group IIA (sPLA₂-IIA) in human atherosclerotic tissue have been the best documented until now (Six et al 2000; Kudo et al 2002; Jaross et al 2002). In this context, recent investigations have suggested the involvement of sPLA₂-IIA as part of a systemic and localized acute-phase-reaction in the development of atherosclerosis, not only as a specific marker of inflammation but probably as a central link between activated RAS and lipid peroxidation (Keidar et al 1997; Hayek et al 2000; Luchtefeld et al 2006; Divchev et al pers comm).

This study explains the importance of sPLA₂-IIA as a key enzyme of inflammation-based atherosclerotic development, and a prognostic marker of cardiovascular events, based on current literature and the experimental findings of our group.

What are phospholipases (in general sPLA₂s) good for?

Phospholipases are enzymes that play a crucial role in the metabolism of phospholipids. Various groups and a remarkable number of subgroups have been identified, but specific functions for only some of these phospholipases have been established in humans. Their classification is related to the site of action on the phospholipid molecules. In this context, phospholipases may act as acylhydrolases (PLAs and PLB), lysophospholipases or phosphodiesterases (PLC and PLD) (Six et al 2000; Kudo et al 2002). For example, phospholipases A₂ (PLA₂s) catalyze the sn-2 ester bonds of glycerophospholipids and, more importantly, appear to be the most influential subfamily in pro-inflammatory processes and thereby in inflammation-initiated diseases.

This subfamily can be characterized as a group of calcium-dependent lipolytic enzymes with a preserved calcium-binding loop and a His-Asp diad at the catalytic site. More than 13 groups and up to 20 different subgroups have been described so far (Six et al 2000; Kudo et al 2002; Jaross et al 2002). Nonsecretory PLA₂s include the Ca²⁺-sensitive arachidonoyl-selective 85-kDa group IV α cytosolic PLA₂ (cPLA₂ α) (Leslie 1997; Bonventre 1999), paralogs of this enzyme (Pickard et al 1999), and several Ca²⁺-independent PLA₂s (iPLA₂s) (Balsinde et al 1997).

Many cell types can secrete sPLA₂s, especially sPLA₂-IIA such as mesangial cells (Pfeilschifter et al 1989; Schalkwijk

et al 1991), vascular smooth muscle (Nakano et al 1990; Kurihara et al 1991), endothelial cells (Murakami et al 1993), platelets (Hayakawa et al 1988), mast cells (Foneth et al 1994; Reddy et al 1996), neutrophils (Wright et al 1990), macrophages (Hidi et al 1993; Brabour et al 1993; Vial et al 1995), and hepatic cells (Crowl et al 1991).

Several pro-inflammatory stimuli may lead to secretion of diverse enzymes including phospholipases as part of a host defense mechanism. Such stimuli could be microbial pathogens as well as chemical irritants, allergens or physical stress factors. cPLA₂ α as well as the sPLA₂s have been involved in various physiological and pathological functions such as release of pro-inflammatory mediators, cell proliferation, ischemic injury, inflammatory and allergic disease, lipid modification, cancer, and antibacterial defense (Bonventre 1999; Valentin et al 1999; Sapirstein et al 2000; Granata et al 2003). The presence of sPLA₂-IIA activity in tears or seminal plasma, which may come into contact with bacterial pathogens, also suggests primary antibacterial properties of these enzymes. In this sense, sPLA₂-IIA is able to destroy the membranes of Gram-positive bacteria. Interestingly, in Gram-negative bacteria sPLA₂-IIA cannot directly attack the phospholipids of the intact cell membranes, but needs the cooperative action of neutrophils which produce bactericidal permeability-increasing protein (BPI) to support this function (Wright et al 1990; Laine et al 1999). Phospholipases could therefore act in the frame of combined inflammation associated humoral and cellular responses.

SPLA₂s do not exhibit acyl chain specificity, whereas a preferential effect on arachidonic acid (AA)-containing membrane phospholipids, compared with those containing other fatty acids, could be demonstrated for cPLA₂ α . The cPLA₂ α , group IIA, and group V PLA₂s are the primary PLA₂s responsible for producing AA and its metabolites in endothelial, fibroblastic mast and macrophage cell lines (Balboa et al 1996; Reddy et al 1997; Pruzanski et al 1998). A profound cross-talk between cytosolic phospholipase A₂ (cPLA₂) and sPLA₂-IIA with regard to free radical release has been confirmed by some investigations (Han et al 2003). Immunohistochemistry studies have further demonstrated the presence of group IIA, group V and group X secretory phospholipase A₂ in murine and human atherosclerotic lesions (Murakami et al 2004; Rosengren et al 2004; Wootton-Kee et al 2004; Webb 2005).

On the other hand, it could be shown that there are changes in serum cholesterol levels with decreased concentrations of high density (HDL) and low density lipoproteins (LDL) in patients suffering from generalized infectious diseases

such as sepsis. Moreover, a frequent finding in patients with persisting infections, metastatic tumors or chronic inflammatory diseases is hypocholesterolemia (Green et al 1991; Vadas et al 1993). The serum concentration of sPLA₂-IIA, especially the activity of the enzyme, is markedly increased in these diseases, acting as an acute-phase-reactant (de Beer et al 1997; Ivandic et al 1999; Menschikowski et al 2000).

In acute or chronic inflammatory states, serum levels of pro-inflammatory cytokines, such as interleukin (IL)-1 β , IL-6, tumor necrosis factor (TNF)- α , and interferon (IFN)- γ are also increased and may induce sPLA₂-IIA (Peilot et al 2000; Menschikowski et al 2000; Antonio et al 2002). These pro-inflammatory cytokines can also be released at an increased rate by the liver and locally by other cells especially within the atherosclerotic plaque. Such increased PLA₂-activity in serum is thought to lead to a more efficient clearance of lipoproteins mainly through the liver, thereby resulting in hypocholesterolemia (de Beer et al 1997; Ivandic et al 1999; Menschikowski et al 2000; Jaross et al 2002).

Therefore, early evidence suggested the existence of a central link between systemic as well as local inflammatory processes and lipid metabolism, and that this link could be sPLA₂-IIA.

The secretory phospholipase A₂, group IIA role in atherogenesis

The key role played by sPLA₂-IIA in the development of atherosclerosis, not merely as a mediator for localized inflammation, has been indicated by a number of recent in-vitro and in-vivo studies as follows. The enzyme seems to play an important role as an integral modulator at several stages of atherogenesis.

In the arterial wall, sPLA₂, especially sPLA₂-IIA, may exert proatherogenic effects in multiple steps (Hurt-Camejo et al 2001). The isoenzyme sPLA₂-IIA is located mainly in vascular smooth muscle cells of normal human arteries (Romano et al 1998; Sartipy et al 1998). In contrast, in atherosclerotic plaques the enzyme is detectable within the lipid cores, in macrophage-rich regions and in the extracellular matrices of the affected intima. Furthermore, sPLA₂-IIA has a close spatial relationship to collagen fibres (Romano et al 1998). As mentioned above, the expression of sPLA₂-IIA in these cells is assumed to be up-regulated in vitro by several cytokines present in human atherosclerotic lesions, including IL-1 β , TNF- α and IFN (Menschikowski et al 2000; Peilot et al 2000; Antonio et al 2002).

One of the first important features in atherogenesis is the retention of LDL particles in the subendothelial space.

The modification of LDL mediated by sPLA₂-IIA can change the structural organisation of the LDL particle leading to more pronounced lipid accumulation in the vessel wall. Such sPLA₂-IIA modified LDL are characterized through an increased affinity for proteoglycans.

As a result of the hydrolytic activity, sPLA₂-IIA releases free fatty acids and lyso-phosphatidylcholine(PC) (Yuan et al 1995; Arbibe et al 1998; Fourcade et al 1998; Hurt-Camejo et al 2001; Hurt-Camejo et al 2001). This action may affect the functions and properties of vascular endothelial and smooth muscle cells, and of macrophages, at sites of LDL accumulation (Sparrow et al 1988; Leitinger et al 1999). It should be noted that sPLA₂-IIA prefers phosphatidic acid (PA) as substrate over other phospholipids found in plasma membranes including phosphatidylserine (PS), phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Sintko and colleagues reported the following order of substrate preference: PA > PE approximately PS > PC (Snitko et al 1997).

The ability of sPLA₂-IIA to hydrolyze PC seems to be weak, but several conditions may amplify that function. sPLA₂-IIA acts on oxidized (ox-)LDL more efficiently by transforming LDL to a more atherogenic form. This action is based on the fact that oxidative modification of phospholipids increases their sPLA₂-IIA susceptibility (Eckey et al 1997). Furthermore, the closed spatial contact of sPLA₂-IIA with matrix proteoglycans (in general decorin, biglycan and versican) may increase the hydrolytic activity of sPLA₂-IIA toward PC in LDL (Sartipy et al 2000).

Moreover, sPLA₂-IIA modified proteoglycan-bound lipoproteins show a greater tendency to aggregation and fusion. The result is a progressive deposition of lipids within the extracellular matrices of the arterial intima (Hakala et al 2001). In addition to enhancing the retention of LDL particles in the vessel wall, there is also evidence that sPLA₂ modification may promote macrophage LDL uptake, leading to foam cell formation (Heinecke et al 1991; Aviram et al 1992).

It should be noted that other sPLA isoenzymes detectable in circulation and human atherosclerotic lesions, especially of group V and group X, have more potent actions on lipoproteins compared with sPLA₂-IIA (Bezzine et al 2000; Hanasaki et al 2002; Gesquiere et al 2002). Obviously, the sole potency of sPLA₂-IIA in hydrolyzing the primary substrates phosphatidylethanolamine and phosphatidylserine may not be the only important feature in atherosclerotic plaque perpetuation, especially in transforming LDL into more atherogenic forms. Interestingly, sPLA₂-V has a preferential action in hydrolysis of HDL compared with LDL, as reviewed by Murakami and Kudo (2003). Similar to

sPLA₂-V, sPLA₂-X definitely shows more pronounced PC-hydrolysing activity. On the other hand, sPLA₂-X modified LDL particles have a lower tendency to aggregate (Hanasaki et al 2002; Murakami et al 2003). Nevertheless, we can suppose that sPLA₂ isoenzymes may act synergistically under certain conditions (Shoda et al 1999).

Together, HDL and apolipoprotein A-I (apoA-I) are positive prognostic factors in preventing perpetuation of arteriosclerotic plaque formation; there is increasing evidence that sPLA₂-IIA may transform these particles to pro-atherogenic and pro-inflammatory reactants. As shown by de Beer and colleagues, sPLA₂-IIA dependent hydrolysis of HDL results in a more efficient scavenger receptor class B type I cholesteryl ester uptake, leading to an accelerated HDL catabolism in the kidney during inflammatory states (deBeer et al 2000). Moreover, HDL- and ApoA-I-catabolism as well as HDL-cholesteryl ester tissue uptake were significantly enhanced under conditions of sPLA₂-IIA overexpression even in the absence of inflammatory preconditions (Tietge et al 2000).

The above mentioned effects promoted by sPLA₂-IIA – in general the release of AA – are primarily based on increased enzyme activity. However, the noteworthy non-enzymatic properties of sPLA₂-IIA can also contribute to atherogenesis. In this way, catalytic inactive sPLA₂-IIA was shown to promote COX-2 expression in mast cells (Tada et al 1998). Further effects may be mediated by the sPLA₂ M-type receptor on smooth muscle cells independent of enzyme activity (Silliman et al 2002) and by activating signaling pathways such as ERK (Han et al 2003) MAP kinases or protein kinase C (Hernandez et al 1998). Further research is needed to make clear whether these effects are present in atherosclerotic lesions, and how far they may contribute to atherogenesis.

sPLA₂-IIA as a prognostic marker in coronary artery disease

In a similar way to chronic inflammatory diseases, plasma levels of sPLA₂-IIA were shown to be elevated in patients with coronary artery disease (CAD) and to predict coronary events in asymptomatic patients or patients with stable angina (Kugiyama et al 1999). Moreover, increased plasma levels of sPLA₂ in stable patients undergoing percutaneous coronary angioplasty (PCI) also provide independent prognostic information over other classic cardiovascular risk factors and clinical covariables (Liu et al 2003). The prognostic value of sPLA₂-IIA in patients with acute coronary syndromes (ACS) was first described in a study of patients with unstable angina by Kugiyama and colleagues (2000). Increased plasma

levels of sPLA₂-IIA predicted recurrent coronary events, mainly revascularization procedures, independently of other established risk factors (Kugiyama et al 2000). Mallat and colleagues (2007) also evaluated the prognostic importance of plasma sPLA₂-IIA levels and enzyme activity across the entire spectrum of ACS by recruiting patients from the Global Registry of Acute Coronary Events (GRACE). In this impressive study, they showed that plasma sPLA₂ activity, in contrast to CRP or IL-18, was a major independent predictor of death and new or recurrent myocardial infarction (MI) in patients with ACS. Interestingly, total sPLA₂-activity had a better prognostic value than the sPLA₂-IIA antigen level.

However the study's most important finding was the fact that a single determination of sPLA₂-activity, obtained during the two days after the onset of ischemic symptoms, provides powerful prognostic information in patients with ACS. The association between sPLA₂ activity and the risk of subsequent death or MI was independent of the other known predictors of major adverse outcomes in patients with ACS, including the presence or absence of a history of MI or signs of heart failure at admission (Mallat et al 2005).

Additional evidence to confirm the importance of catalytic activity, as well as an elevated sPLA₂-IIA antigen level, was gathered by Korotaeva and colleagues (2005). They demonstrated that sPLA₂-IIA is involved in the development of restenosis after PTCA in human coronary arteries and in the generation of atherogenic LDL (Korotaeva et al 2005).

Interestingly, sPLA₂-IIA also seems to play an important role in predicting risk for coronary artery disease and adverse coronary events in healthy subjects, especially when combined with other pro-inflammatory risk factors like CRP (Boekholdt et al 2005; Mallat et al 2007).

Thus sPLA₂-IIA can be shown to be not only a mediator for localized inflammation but also a powerful independent predictor of adverse outcomes in patients with stable CAD, or in acute coronary syndromes.

Angiotensin, LDL-peroxidation, and atherogenesis

Early evidence of a potential role for angiotensin (Ang) II in LDL oxidation was gathered by Keidar (1998) who reported that the LDL of hypertensive patients was more responsive to oxidative modification than the LDL of normotensive subjects. Meanwhile, various interactions between Ang II and oxLDL have been demonstrated especially by the Keidar group. For example, Ang-II injection into apolipoprotein E-deficient (E₀) mice led to increased cellular oxLDL uptake by their peritoneal macrophages (MPMs) via proteoglycan

macrophage stimulation pathway, a process mediated by II-6 (Keidar et al 1995, 2001). In human coronary endothelial cells, Ang-II enhanced the oxLDL-uptake by up-regulating the lectin-like receptor for oxLDL (LOX-1). This effect could be blocked by the angiotensin receptor blockers (ARBs) Losartan and Candesartan (Mehta et al 2001, 2002). Ang-II increased the cellular lipid peroxidation of MPMs by 50%–100%. Moreover, Ang-II enhanced scavenger receptor affinity to oxLDL on macrophages (Keidar 1998).

The effects of Ang-II are predominantly mediated via activation of the AT₁-receptor which is coupled through guanine nucleotide binding proteins (G-proteins), adenylate cyclase and cyclic adenosine monophosphate (cAMP) as second messenger, as well as phospholipases A₂, C and D and their products, arachidonic acid (AA), inositol triphosphate (IP₃), or diacylglycerol (DG).

The mechanism of Ang-II-mediated cellular lipid peroxidation involved Ang-II binding to its cellular receptor underlined by the fact that preincubation of the cells with the Ang-II antagonist, saralasin, completely inhibited this effect. Inhibitors of phospholipase A₂, C and D also substantially reduced Ang-II-induced macrophage lipid peroxidation (Keidar et al 1995).

Interestingly, AT₁-receptor blockade with Losartan inhibited LDL oxidation and macrophage cholesterol biosynthesis (Keidar et al 1999), and attenuated atherosclerosis in E₀ mice (Keidar et al 1997). In a trial by Hayek and colleagues (2000), losartan therapy for a period of 4 weeks did not significantly affect the degradation of native LDL monocyte-derived macrophages (HMDM) derived from patients. However, Losartan therapy significantly reduced HMDM uptake of oxLDL as shown by a 78% reduction in oxLDL cell-association and a 21% reduction in oxLDL degradation (Hayek et al 2000), a finding which has been also confirmed for other ARBs (Metha et al 2001, 2002).

All these findings suggested an integral link between angiotensin II (Ang-II) and its receptor mediated effects, and activation of pro-inflammatory and pro-atherogenic pathways which could further promote lipid peroxidation.

sPLA₂-IIA, the possible link between activated RAS, inflammation, and lipidperoxidation: a target for treatment?

As mentioned above, angiotensin II type 1 (AT₁)-receptor blockade may reduce LDL-modification and atherosclerotic plaque formation in animal models of atherosclerosis.

Furthermore, Cassis and colleagues (2007) demonstrated that AT₁-mediated atherogenesis is dependent on AT₁ expression in the vessel wall, and that the presence of this receptor in resident tissues is required to initiate AngII-induced atherosclerosis. Because of increasing evidence for involvement of sPLA₂-IIA in modifying LDL, our group assessed whether ANG II, via its AT₁-receptor, enhances sPLA₂-IIA-dependent lipid peroxidation in vitro and in patients with CAD. Therefore, rat aortic smooth muscle cells were stimulated with ANG II. This stimulation resulted in enhanced sPLA₂-IIA protein expression and sPLA₂-IIA activity. LDL-peroxidation was consequently enhanced by ANG II. Interestingly, all these effects could be reduced by AT₁-receptor blockade with losartan. Furthermore, ANG II-induced sPLA₂ activity and LDL-peroxidation were prevented by the sPLA₂-IIA activity inhibitor LY311727 (Luchtefeld et al 2006).

These findings suggested that sPLA₂-IIA activation could be the supposed link between activated RAS and lipidperoxidation, and that ANG II may elicit pro-atherosclerotic effects via sPLA₂-IIA-dependent LDL-modification.

In order to evaluate potential clinical implications of these findings, a small number of patients with angiographically documented CAD were treated with the AT₁-receptor blocker irbesartan (300 mg/d) for 12 weeks. Blood samples were obtained from patients pre- and post-treatment and from healthy volunteers. sPLA₂-IIA serum level and activity, circulating antibodies against oxidized LDL (oxLDL) and oxLDL, were determined in patients and found to be significantly increased compared to healthy volunteers. Irbesartan therapy reduced these markers of inflammation, whereas total cholesterol, HDL- and LDL-fractions remained unchanged (Luchtefeld et al 2006).

Based on these findings we investigated further the potential effect of a combined treatment with pravastatin and irbesartan on oxLDL and sPLA₂-IIA in a group of patients with CAD. We postulated that the addition of an AT₁-receptor antagonist to the standard secondary prevention therapy with a statin might exert additional effects on LDL oxidation and sPLA₂-IIA. We demonstrated that both treatment regimens comparably influenced LDL-cholesterol levels. However, sPLA₂ activity was reduced only in patients treated with the adjunction of pravastatin and irbesartan. Consequently, only the combined treatment resulted in a significant reduction of ox-LDL levels independent of changes in LDL cholesterol (Divchev et al pers comm).

All these findings suggest a possible role for AT₁-receptor blockade in reducing LDL-peroxidation. Whether these

effects can reduce the perpetuation of atherosclerosis and especially adverse cardiovascular events in patient with CAD remains to be elucidated in further studies.

Conclusion

In conclusion, secretory phospholipases A₂ (in general sPLA₂-IIA) were shown to play an important role in atherogenesis. Furthermore, the activity of sPLA₂-IIA is a powerful independent predictor of adverse events in stable patients with CAD and in ACS. Focusing on recent data, expression and activation of sPLA₂-IIA in atherosclerotic lesions seemed to be one of the links between activated RAS, inflammation and the processes of lipid accumulation and lipid peroxidation in the vessel wall as main features of atherosclerosis. Therefore, direct inhibition of sPLA₂-IIA through specific antagonists, or indirectly through AT₁-receptor blockade, might be a new therapeutic option used to intervene in this detrimental process of plaque development. The confirmation of these therapeutic effects and their relevance in human atherosclerosis remain the foremost challenges.

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