SECRETORY PHOSPHOLIPASE A2 OF GROUP IIA: PATHOGENIC OR PROTECTIVE FACTOR DURING ATHEROSCLEROSIS?

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Introduction

One of the best-characterized types of the structurally heterogeneous superfamily of phospholipase A2 enzymes is the secretory phospholipase A2 of group IIA (sPLA2-IIA) [1,2], which was initially isolated from rheumatoid arthritic synovial fluid and platelets and which was originally designated as non-pancreatic or synovial-specific PLA2 [3-5]. Large amounts of this enzyme have been found at various inflamed sites and in the serum of patients with severe inflammations such as sepsis, septic shock, and polytrauma. The level of enzyme expression correlated strongly with the degree of the disorders [6-9]. These data suggest that sPLA2-IIA plays a pivotal role in inflammation. Based on these findings there exists some considerable hope that with the help of specific inhibitors of sPLA2-IIA new forms of therapy for severe inflammatory diseases can be established. The specific biological functions of sPLA2-IIA, however, are not completely understood. In addition to cell injury, sPLA2-IIA may be involved, similar to other phospholipases A2, in cell signaling, apoptosis, and remodelling of cell membranes [10-12]. Beyond this, new in vivo investigations have shown that an efficient bactericidal agent is available in sPLA2-IIA [13,14]. Because of this the question arises as to how far a suppression of sPLA2-IIA, especially in bacterially caused inflammatory diseases, can be considered really beneficial at each stage of the disease’s progression.

This question also arises because of recently performed in vitro studies showing that statins lead to a potentiation of the IFN-γ-mediated sPLA2-IIA induction in HASMC and HepG2 cells [15] instead of a reduction, as we had at first assumed based on the published antiinflammatory properties of statins [16-18]. Similar effects of statins in IL1β-stimulated rat mesangial cells have been described by Petry et al. [19]. Given the assumption that sPLA2-IIA acts proinflammatory and proatherogenic, this effect stands in contradiction of the benefit of statins in primary and secondary prevention of coronary heart diseases [20-23]. One simple explanation could be that the presumed proinflammatory and proatherogenic effects of sPLA2-IIA upregulation are negligible because of the other cardioprotective effects of statins such as inhibition of cholesterol biosynthesis or increased synthesis of nitric oxide [24-26]. On the other hand, it is known that activation of sPLA2-IIA produces multiple but controverting effects in different tissues [27]. There is evidence, for example, that sPLA2-IIA, in addition to its bactericidal properties, exhibits also antithrombotic and antiinflammatory properties as well, from which it could be concluded that sPLA2-IIA expression, with its pathogenic effects, is indeed associated with protective functions.

Possible Pathogenic Functions of Spla2-IIA during Atherogenesis

We are interested in the role of this enzyme in atherogenesis because of the finding that in atherosclerotic lesions, but not in normal nonatherosclerotic arteries without signs of active
inflammatory reactions, the sPLA₂-IIA is expressed [28,29]. Numerous in vitro and in vivo studies suggest that the sPLA₂-IIA upregulation is connected with proatherogenic effects [30].

First, macrophages exposed to sPLA₂-modified lipoproteins exhibit excessive cellular lipid accumulations and transform into foam-like cells [31,32]. Although native lipoproteins are poor substrates for sPLA₂-IIA-mediated hydrolysis, investigations in vitro have shown that after mild oxidation induced by copper ions or 2,2-azobis (2-amidininopropane), the susceptibility of LDL to sPLA₂-IIA-mediated phospholipid hydrolysis increased [33]. It can be assumed that during inflammatory reactions strongly oxidatively modified lipoproteins emerge in the vessel wall, which for its part represents a suitable substrate for the sPLA₂-IIA expressed within the wall.

Second, studies on transgenic mice documented that in vivo a sPLA₂-IIA expression in the vessel wall is accompanied by an intensified formation of atherosclerotic lesions [34]. In this way, following a 12-week high-fat, high-cholesterol diet, increased fatty streak lesions in the aortic sinus of sPLA₂-IIA-transgenic mice along with sPLA₂-IIA-positive immunostainings could be demonstrated [34]. That an altered lipoprotein metabolism need not be a prerequisite for intensified lesion formation, has been shown by investigations on LDL receptor knock-out mice (LDLR⁻/⁻), reconstituted with bone marrow cells expressing human sPLA₂-IIA [35]. Thus, after 12 weeks of maintaining these animals on a cholesterol-rich diet, the LDLR⁻/⁻/sPLA₂-IIA mice developed significantly larger lesions in the aortic arch and aortic sinus without exhibiting any changes in serum lipoprotein concentrations by comparison to those of control mice with wild-type bone marrow cells. By using the myeloid-specific CD11b promoter instead of the sPLA₂-IIA’s own promoter fused with the human genomic sPLA₂-IIA gene, a macrophage human sPLA₂-IIA overexpression model was generated [36]. Bone marrow from this model was then translated to LDLR⁻/⁻ mice and the effect of a local macrophage-specific vascular sPLA₂-IIA expression was investigated. After a 10-week high-fat diet the analysis of the aortic root, near the heart valve, indicated that the sPLA₂-IIA expressed in macrophages resulted in a 2.3-fold increase in the lesion size [36]. Finally, a macrophage-specific overexpression of human sPLA₂-IIA was found to increase the atherogenesis by directly modulating oxidative stress in vivo [37].

Third, the serum level of sPLA₂-IIA could be identified as a prognostic parameter for developing coronary events independent of other risk factors in prospective studies of patients with CHD [38-40]. Although the source of the serum-sPLA₂-IIA in the patients analyzed in the studies is still unclear, the inflamed sites of the vessel wall itself and the liver come under discussion. If the sPLA₂-IIA in serum really originates from the affected vessel areas, the level of serum sPLA₂-IIA could represent an important diagnostic aid for the estimation of the activity of inflammatory reactions and, in connection with that, the vulnerability of atherosclerotic plaques. Hence, it is assumed that the plaque formation, because of an amplified synthesis and secretion of hydrolases through activated inflammatory cells, become unstable and in turn leads, through the contact of plasma components with subendothelial and procoagulative structures, to a sudden occlusion of a blood vessel [41].

Possible Protective Effects of Spla₂-IIA Upregulation during Atherosclerosis

A protective effect of the sPLA₂-IIA against gram-positive and gram-negative bacteria could be verified, in addition to in vitro studies also in vivo through investigations of sPLA₂-IIA-transgenic mice. In this way, the transgenic mice when compared with the control animals showed a significantly higher resistance to Staphylococcus aureus [13]. While the majority of
the transgenic animals showed only minor symptoms of sepsis and remained completely alive after having received an i.p. injection of *S. aureus*, after 24 hours 85% of the non-transgenic control animals had already died. The deceased animals exhibited severe congestion of the lung, liver, and kidneys and accumulation of hemorrhagic exudate in the pleural and peritoneal cavities. Later, the same group could also verify a higher resistance of the sPLA2-IIA transgenic animals to gram-negative *E. coli* bacteria in comparison to the control animals [14]. The investigations on the transgenic mice underline the notion that sPLA2-IIA expression leads to an improved resistance to bacteriological infections. If the potential bactericidal characteristics of sPLA2-IIA are viewed in connection with atherosclerosis, then a mechanism could exist here as well to explain the benefit of statin treatment. As a series of studies showed, the incidence of myocardial infarction and stroke is elevated by frequent infections [42-44]. In recent years along with viral infections, bacteriological infections as well have attracted interest as a possible cause of atherosclerosis [45-50].

Just as for a number of snake-venom-sPLA2 anticoagulant, activities have also been described for mammalian sPLA2-IIA [51-56]. Investigations on venom sPLA2 illustrated that sPLA2 exerts its anticoagulant effect by means of protein-protein rather than protein-phospholipid interactions [57]. Therefore, it has been concluded that the phospholipase A2 enzyme inhibits the formation of the normal Xa-Va complex by competing with factor Va for binding to factor Xa or replacing bound factor Va from the complex. Similar behaviours have been found in case of sPLA2-IIA [58]. Indeed, human sPLA2-IIA exhibited significant anticoagulant activity that did not require its enzymatic activity. The inhibitory effect of sPLA2-IIA on the prothrombinase activity of FXa, FV, phospholipids, and Ca$^{2+}$ complex was enhanced upon the preincubation of sPLA2-IIA with FXa, but not with FV [58]. Taken together, even though verification *in vivo* is still not available, that sPLA2-IIA in humans as well acts as an anticoagulatory agent, here a further mechanism could exist to explain the benefits of statins in the prevention and treatment of CHD, in that in the presence of IFN-γ, through the stimulation of sPLA2-IIA synthesis and secretion into the bloodstream, statins may inhibit the formation of thrombin. The formation of a thrombus after the rupture of atherosclerotic plaques is viewed as a sudden and fatal incident in CHD as well.

Along with its bactericidal and antithrombotic properties, the ability of sPLA2-IIA to be able to “distinguish” between native and oxidative modified lipoproteins could suggest a further positive effect in the upregulation of sPLA2-IIA. If one considers that during inflammation not only cytokines and sPLA2-IIA are released at a higher rate but that also increased amounts of oxidatively modified lipoproteins are very probably produced, a beneficial mechanism could exist, which consists in the notion that oxidatively modified lipoproteins are removed from the bloodstream by organs with high sPLA2-IIA expression, such as the liver. Speaking for this assumption is a number of data gathered on sPLA2-IIA-transgenic mice. First, the liver of transgenic mice exhibited the highest sPLA2-IIA expression [59,60]. Second, in sPLA2-IIA-transgenic mice, an elevated level of oxidized phospholipids was found in the liver [61]. Third, the livers of transgenic mice were found to contain significantly increased concentrations of free and esterified cholesterol by comparison to the livers of their nontransgenic littermates [62].

In humans also it is presumed that the formation of sPLA2-IIA by the liver is elevated during inflammation as an acute-phase reactant. In addition to the liver, Tietge et al. [63] also found in the adrenals of sPLA2-IIA-transgenic mice a significantly higher uptake of HDL cholesteryl esters in comparison to that of control animals. The extent to which the sPLA2-IIA-mediated hepatic and adrenal uptake of modified lipoproteins during inflammation applies also
to humans is still the subject of speculation. It is conceivable, however, that for the induced synthesis of stress hormones during the acute-phase reaction, precursors are necessary in greater amounts, as initially hypothesized by Groen et al. [64]. HDL cholesteryl esters are known as a source of cholesterol for steroid hormone synthesis [65]. With that, this mechanism would likewise be viewed as protective, since (i) the increasing accumulation of oxidatively modified lipoproteins, which has a cell-toxic effect, is filtered out of the bloodstream during inflammation and (ii) the adrenals, supplied with sufficient precursors during inflammation, boosts corticoid synthesis.

The role played by sPLA₂-IIA during apoptosis is hence still unexplained. In baby hamster kidney cells there is evidence of sPLA₂-IIA-generated antiapoptotic survival signals, suggesting that high levels of sPLA₂-IIA accumulated at inflammatory sites might impact the final effect of inflammation by triggering cell-protective machinery [66]. Similarly, enhanced survival of mast cells has been demonstrated by pancreatic sPLA₂ and bee venom sPLA₂ isotypes [67]. Since apoptosis is seen as an essential cause for the instability of atherosclerotic plaques an increased synthesis of sPLA₂-IIA and with that an increased build up of antiapoptotic survival signals, could represent a positive effect here as well.

Conclusions

Numerous new studies demonstrated that sPLA₂-IIA is a multipotent enzyme which, in addition to its pathogenic effects, may have also protective functions during inflammation. Particularly the efficient bactericidal properties of sPLA₂-IIA demonstrated in recent years put the function of sPLA₂-IIA in another light. Even though the bactericidal properties of sPLA₂-IIA have been demonstrated in vitro and in vivo cannot be uncritically attributed to its effect in humans, it is tempting to speculate that a statin therapy for patients with bacterial infections characterized by an heightened formation of IFN-γ and IL-1β, in comparison to patients who have not taken statins, leads to an increased expression of sPLA₂-IIA. With a statin therapy patients could be more resistent to bacterial infection, thereby explaining the possible benefits of statins in the prevention and therapy of sepsis [68]. Along with antibacterial characteristics the antithrombotic properties of sPLA₂-IIA have been described, such that here a further explanation of the benefits of statins could exist, in that through the induced sPLA₂-IIA expression the formation of clots is hampered.

The extent to which an expression of sPLA₂-IIA has pathogenic or protective functions with respect to atherosclerosis depends possibly on whether expression of the enzyme as the consequence of an inflammatory reaction is induced locally in the vessel wall or systemically as the result of an acute-phase reaction. In the former case the investigations on transgenic mice speak for the notion that sPLA₂-IIA expression is connected with an increased susceptibility to atherosclerosis. By comparison, in a systemic expression the possibility exists that a protective effect grows out of this in that with inflammation the increased number of oxidatively modified lipoproteins are removed from the bloodstream via the liver and to a lesser extent via the adrenals.

In summary, despite intense efforts the question cannot be answered conclusively as to whether the pathogenic or protective effects of an sPLA₂-IIA upregulation will finally prevail. Therefore, further investigation is necessary to evaluate, for example, of whether in vivo also a synergism on the sPLA₂-IIA expression between proinflammatory cytokines and statins can be found.
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