Touqui L *et al* / Acta Pharmacol Sin 2003 Dec; 24 (12): 1292-1296

Interaction of secreted phospholipase A2 and pulmonary surfactant and its pathophysiological relevance in acute respiratory distress syndrome

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INTRODUCTION

Phospholipases A_2 (PLA_2s) belong to a family of enzymes that hydrolyze phospholipids at the sn-2 position leading to the liberation of fatty acids and lysophospholipids (Fig 1). Mammalian PLA_2s are divided into two major classes according to their location: high molecular mass intracellular PLA_2 and low molecular mass secreted PLA_2 ($sPLA_2$) (Fig 2). There have been substantial progresses in understanding the regulation of $sPLA_2$ expression, especially the type-IIA $sPLA_2$ ($sPLA_2$-IIA), the most known enzyme of this group. The bacterial endotoxin lipopolysaccharide (LPS) induces the synthesis and secretion of $sPLA_2$–IIA either directly or via paracrine/autocrine processes involving certain cytokines. This enzyme has been shown to be regulated at the transcriptional level by the transcription factor NF-κB.

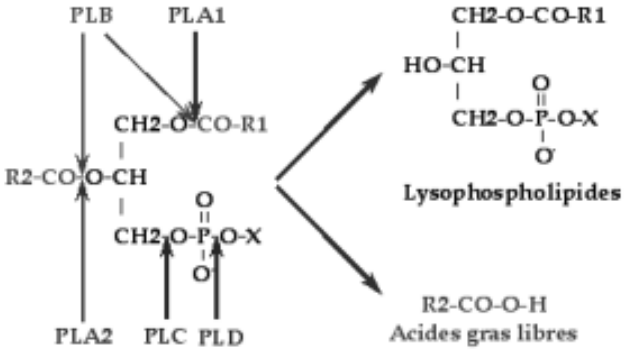

Fig 1. Mechanisms of action of various phospholipases.

$sPLA_2$-IIA plays a major role in the pathogenesis of various inflammatory diseases such as acute respiratory distress syndrome (ARDS). The latter is characterized by arterial hypoxemia, noncardiogenic pulmonary edema and an alteration of pulmonary surfactant which increases surface tension at the air-liquid interface.

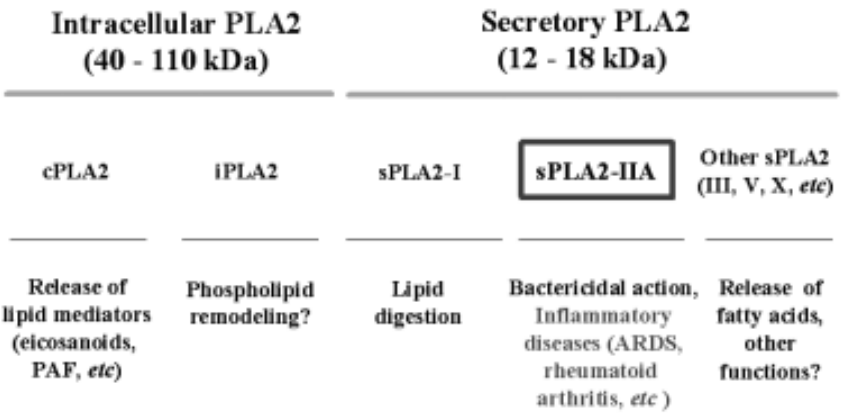

Fig 2. Classfication of mammalian Phospholipase A2.

$sPLA_2$-IIA plays a key role in the hydrolysis of surfactant phospholipids a process that may contribute to alveolar collapse and impairment of gas exchange. Conversely, the expression of $sPLA_2$-IIA is inhibited by surfactant in a guinea pig model of ARDS and in alveolar macrophages (AM), the main source of $sPLA_2$-IIA in this model. This inhibition is mainly due to an alteration of NF-κB activation, a process in which surfactant protein A (SP-A) and surfactant phospholipid phosphatidylglycerol play a major role. Therefore, in pathological situations, such as ARDS, in which surfactant is altered, $sPLA_2$-IIA production may be exacerbated leading to further surfactant alteration and the establishment of a vicious circle.

MECHANISMS INVOLVED IN THE REGULATION OF sPLA2 EXPRESSION

Effects of bacterial endotoxin and cytokines and their pathophysiological significance High levels of $sPLA_2$-IIA have been associated with different inflammatory or infectious diseases, such as allergic rhinitis, rheumatoid arthritis, septic shock or ARDS (Murakami M, *et al* 1997; Touqui L and Arbibe L 1999). A clinical trial showed increased levels of an enzyme identical to $sPLA_2$-IIA in the bronchoalveolar lavage fluids (BALF) of patients with ARDS (Kim DK *et al* 1995). On the other hand, it is generally recognized that endotoxin, the lipopolysacharide (LPS) portion of the cell wall of the Gram negative bacteria, is the major bacterial factor responsible of the induction of $sPLA_2$-IIA expression. LPS induces the synthesis and secretion of $sPLA_2$-IIA in various cells and tissues in different experimental models of inflammatory diseases (Murakami M *et al*). Several evidences suggest that cytokines such as TNF-α mediates the LPS-induced $sPLA_2$-IIA synthesis. Indeed, we showed that intratracheal instillation of LPS to guinea pig induced an increased secretion of TNF-α in the alveolar space preceding that of $sPLA_2$-IIA. In this ARDS model, incubation of alveolar macrophages (AM) with anti-TNF-α antibody, suppresses the LPS-induced $sPLA_2$-IIA synthesis (Arbibe L *et al* 1997).

Subsequent studies showed that $sPLA_2$-IIA played a key role in the hydrolysis of surfactant phospholipids in various animal models of ARDS (see below). This enzyme is widely distributed in several human tissues including heart, liver, skeletal muscle, colon, ovary and small intestine whereas $sPLA_2$-V is preferentially expressed in the heart and at a much lower extent in the lung and liver. *In situ* hybridization of human lung biopsies showed that $sPLA_2$-V and $sPLA_2$-X were uniquely expressed in airway epithelium (Seeds MC *et al* 2000). The studies of Sawada *et al*. (1999) showed that in the rat, LPS induced $sPLA_2$-IIA expression preferentially to $sPLA_2$-V, whereas the latter is more widely distributed than $sPLA_2$-IIA in the mouse in which the expression of $sPLA_2$-V is enhanced by LPS. More careful investigations on various mouse strains revealed that $sPLA_2$-IIA was preferentially localized in the intestine. However, this enzyme is absent in all tissues in the C57BL/6J strain because of a natural disruption of its gene (Kennedy BP *et al* 1995) without significant abnormalities during the life span of this strain, suggesting a possible compensation of $sPLA_2$-IIA by other $sPLA_2$. More recent studies revealed the existence of human and mouse $sPLA_2$ subtypes of $sPLA_2$-II (Valentin E *et al* 1999; Valentin E *et al* 1999; Suzuki N *et al* 2000) named $sPLA_2$-IIC, $sPLA_2$-IID, $sPLA_2$-IIE and $sPLA_2$-IIF. The expression of $sPLA_2$-IIE mRNA in human is restricted to the brain, heart, lung and placenta. In mouse, $sPLA_2$-IIE is highly expressed in uterus, and at lower levels in other tissues. In $sPLA_2$-IIA-deficent mice (C57BL/6J), the expression of $sPLA_2$-IIE is increased in the lung and small intestine upon LPS challenge more markedly than in mice strains producing $sPLA_2$-IIA. This suggests the existence of an interregulation between $sPLA_2$-IIA and $sPLA_2$-IIE expressions.

The signaling pathways involved in the regulation of $sPLA_2$-IIA expression: the role of protein kinases and transcription factors Cyclic AMP, known to activate protein kinase A (PKA), was first reported to mediate $sPLA_2$-IIA expression in vascular smooth muscle cells (VSMC) and renal mesangial cells (Suzuki N *et al* 2000). Indeed, treatments of these cells with agents that increase intracellular cAMP concentration (such as prostaglandin E_2 (PGE_2), forskolin, cholera toxin or salbutamol) lead to the induction of $sPLA_2$-IIA synthesis. However, these findings contrast with those reported with guinea pig AM in which cAMP has been shown to suppress the expression of $sPLA_2$-IIA (Pfeilschifter J *et al* 1991).

In rat astrocytes, protein kinase C (PKC) plays a role in the induction of $sPLA_2$-IIA expression since phorbol ester enhances the level of $sPLA_2$-IIA mRNA, whereas PKC inhibitors reduce this level (Vial D *et al* 1998). However, recent studies showed that activation

of PKC in rat mesangial cells leads to the inhibition of IL-1β-induced $sPLA_2$-IIA expression (Oka S and Arita H 1991). In these studies, PKC-ε, a particular subtype of PKC, is the most likely candidate mediating the down-regulation of cytokine-induced $sPLA_2$-IIA synthesis.

The earlier pharmacological investigations showed that NF-κB activation was an essential component of the cytokine signaling pathways inducing $sPLA_2$-IIA expression. Indeed, pyrrolidine dithiocarbamate (PDTC), a potent NF-κB inhibitor, suppressed IL-1β and TNF-α-induced $sPLA_2$-IIA expression in rat mesangial cells (Scholz K *et al* 1999). Recently, studies from our laboratory showed that inhibitors of NF-κB activation down-regulated $sPLA_2$-IIA expression induced by LPS in guinea pig AM (Walker G *et al* 1995).

More recent studies established the existence of binding sites for transcription factors including NF-κB, C/EBP and PPAR. Other factors like CTF/NF1 and Sp1 might be involved in the regulation of the $sPLA_2$-IIA promoter. The PPAR factors also play a role in the modulation on $sPLA_2$-IIA gene expression in rat VSMC, and probably in other cell types and animal species. Indeed, PPARs represent a family of transcription factors, which control the regulation of genes involved in lipid metabolism. The activity of these factors is regulated by several fatty acids and their derivatives such as 15-dPGJ2 and 9-HODE (Aaloui EL and Azher M *et al* 2002).

In summary, it seems clear that the effect of PKC and PKA on $sPLA_2$-IIA expression varies with the cell type considered. The mode of cross-communication between PKC/PKA and the transcription factors involved in the regulation of $sPLA_2$ gene expression needs to be investigated. It seems likely that the induction (or repression) of $sPLA_2$-IIA promoter activity results from the cooperation between different elements including nuclear receptors, transcription factors and co-activators organized as an enhanceosome. However, it should kept in the mind that the type of transcription factors involved in the induction of sPLA2-IIA gene expression varies with animal species.

Effect of glucocorticoids Glucocorticoids (GC) are with no doubt the most potent inhibitors of $sPLA_2$-IIA synthesis and/or activity in various cell types and animal models of inflammatory diseases. Earlier studies have demonstrated that GC induced the synthesis of a protein named lipocortin with anti- PLA_2 properties (Andreani M *et al* 2000). However, subsequent studies attributed this anti-PLA_2 activity to the ability of lipocortin to sequestrate phospholipid substrates (Hirata F and Hirata A 1990).

More recent studies established that the inhibitory effect of GC was mainly due to their ability to prevent PLA_2 synthesis. In agreement with this finding, it has been shown that subcutaneous administration of dexamethasone, a synthetic GC, reduce circulating levels of $sPLA_2$-IIA in endotoxemic guinea pigs (Davidson FF *et al* 1990). Dexamethasone also inhibits the expression of $sPLA_2$-IIA in various tissues from rats with endotoxic shock (De Castro CM *et al* 1995). These *in vivo* observations are in agreement with the results of *in vitro* studies using different cell types. Indeed, dexamethasone suppresses the synthesis of $sPLA_2$-IIA in rat VSMC, astrocytes and mesangial cells (Valentin E *et al* 1999). Dexamethasone has also been shown to inhibit $sPLA_2$-IIA synthesis induced by cell adherence in guinea pig AM (Nakano T and Arita H 1990; Hidi R *et al* 1993). Recent study showed that, at concentration that effectively reduces the synthesis of $sPLA_2$-IIA, dexamethasone fails to interfere with the expression of sPLA2-V, thus suggesting that this drug acts selectively on $sPLA_2$-IIA gene expression (Hidi R *et al* 1993).

Effect of growth factors Transforming growth factor-β (TGF-β) is a macrophage-derived peptide, which exhibits diverse biological activities ranging from growth stimulation or inhibition to immunomodulation of many cell types. TGF- β2 and TGF-β1 suppress, respectively, the synthesis and the secretion of $sPLA_2$-IIA by mesangial and by elicited guinea pig peritoneal macrophages (Van der Helm HA *et al* 2000). In contrast, this growth factor stimulates the synthesis and activity of $cPLA_2$, a process leading to an enhanced release of arachidonic acid (AA). Subsequent studies suggested that the inhibitory effect of TGF-β2 on $sPLA_2$-IIA expression was mediated by PGE_2, a major cyclo-oxygenase (COX)-derived metabolite of AA.

Other growth factors have been involved in the inhibition of $sPLA_2$-IIA expression in various cell types. Indeed, platelet-derived growth factor (PDGF) has been shown to inhibit both IL-1β- and forskolin-induced $sPLA_2$-IIA expression in rat mesangial cells (Pfeilschifter J *et al* 1990). This inhibitory effect appears to be mediated by tyrosine phosphorylation since it was reversed by the tyrosine kinase inhibitor genistein (Pfeilschifter J *et al* 1990 and Bolognese B *et al* 1995). Finally, recent report showed that the Insulin like growth factor-1 (IGF-1) down-regulated the IL-1β-induced $sPLA_2$-IIA synthesis in rabbit chondrocytes (Muhl H *et al* 1991).

INTERACTION OF SECRETORY PLA2 WITH PULMONARY SURFACTANT

Hydrolysis of surfactant phospholipids by $sPLA_2$-IIA in animal models of ARDS Surfactant is a lipid-protein complex surrounding AM and lowering surface tension along the alveolar epithelium, thereby promoting alveolar stability. It is composed of 10 % protein and 90 % lipid, and has a high proportion of dipalmitoyl phosphatidylcholine (DPPC) (Konieczkowski M and Sedor JR 1993). Alteration of pulmonary surfactant increases surface tension at the air-liquid interface thus contributing to alveolar collapse and impairment of gas exchange. Hydrolysis of surfactant phospholipids may represent one of the mechanisms responsible of the alteration of surfactant observed in ARDS. Indeed, hydrolysis of DPPC is an early physiopathological event in ARDS, that leads to an accumulation of lyso-phosphatidylcholine (lyso-PC) (Jacques C *et al* 1997). Sepsis secondary to peritonitis in rats lead to lung injury which is accompanied by increased $sPLA_2$ activity, reduced phosphatidylcholine (PC) content, and increased levels of lyso-PC (Lewis JF and Lobe A (1993). More recently, a clinical trial showed an increase in the levels of an unidentified $sPLA_2$ in the BALF of patients with ARDS. However, the role of this enzyme in the hydrolysis of surfactant phospholipids in human ARDS has not been investigated (Gregory TJ *et al* 1991).

Earlier studies showed that *in vitro* incubation of $sPLA_2$ from *Naja Naja* snake venom with calf lung surfactant lead to a decrease in the amounts of PC and a parallel increase in the those of lyso-PC (Von Wichert P *et al* 1981). On the other hand, intra-tracheal instillation of this enzyme to guinea pigs induces an acute lung injury (Kim D *et al* 1995). However, it should be kept in the mind that these results must be interpreted with caution, because venom $sPLA_2$s exhibit a much higher ability than mammalian $sPLA_2$ to hydrolyze phospholipids on organized structures such as surfactant (Holm B *et al* 1991).

Taken together these considerations led us to examine the role of $sPLA_2$ in the hydrolysis of surfactant phospholipids in animal models of ARDS. Our recent studies showed that $sPLA_2$-IIA was a crucial enzyme involved in the hydrolysis of surfactant phospholipids in a guinea-pig model of ARDS induced by intratracheal instillation of LPS (Edelson JD *et al* 1991) and in a rat model of ARDS induced by intranasal instillation of *Pseudomonas aeruginosa* (Verheij HM *et al* 1980).

Regulation of sPLA2-IIA expression and/or activity by surfactant

Action at transcriptional level: Inhibition of sPLA2-IIA expression by various surfactant components Besides its mechanical properties, surfactant also has a role in host defense functions and has been shown to inhibit a number of metabolic processes involved in lung inflammation, such as the release of inflammatory mediators (Arbibe L *et al* 1998). Our earlier studies showed that isolated guinea pig AM synthesized and secreted $sPLA_2$-IIA after prolonged *in vitro* incubation whereas freshly collected AM had no detectable $sPLA_2$-IIA expression (Hidi R *et al* 1993). This led us to suggest that the *in vivo* synthesis of $sPLA_2$-IIA may be repressed by pulmonary factor(s). Accumulating evidences from the literature have suggested that pulmonary surfactant may be one of these factors. We have shown that Curosurf®, a semi-natural surfactant used in surfactant replacement therapy in premature new borns with respiratory failures, down-regulates the synthesis of $sPLA_2$-IIA by blocking its expression at the transcriptional level in cultured AM (Attalah HL *et al*) (Fig 3). Phospholipid components of surfactant, especially phosphatidylglycerol, play a major role in this inhibition, which may be explained at least in part by the impairment of TNF-α secretion (Wright JR 1997). More recently, we showed that the intratracheal instillation of Curosurf® inhibited the pulmonary expression of $sPLA_2$-IIA in LPS-treated guinea pigs (Hidi R *et al* 1997) and that signaling pathways linked to NF-κB activation are involved in this inhibition (Berge A *et al* 1999). These findings are in agreement with the previous studies showing that exogenous surfactant suppresses NF-κB activation in human monocytic cells (Wu YZ *et al* 2002).

Action at postranslational level: Inhibition of sPLA2-IIA activity by SP-A SP-A is the major protein of lung surfactant which belongs to the collectin subgroup of the mammalian C-type lectin family (WU YZ *et al* 2003). The collectins form multimeric structures having collagenous amino-terminal domains that tether a globular carboxyl-terminal, carbohydrate recognition domain (CRD). Previous clinical studies have shown an increased levels of lyso-PC associated with a reduced concentrations of SP-A in the BALF of ARDS patients (Gregory TJ *et al* 1991). We showed that SP-A acted as an inhibitor of $sPLA_2$-IIA activity through a direct protein-protein interaction (Arbibe L *et al* 1998).

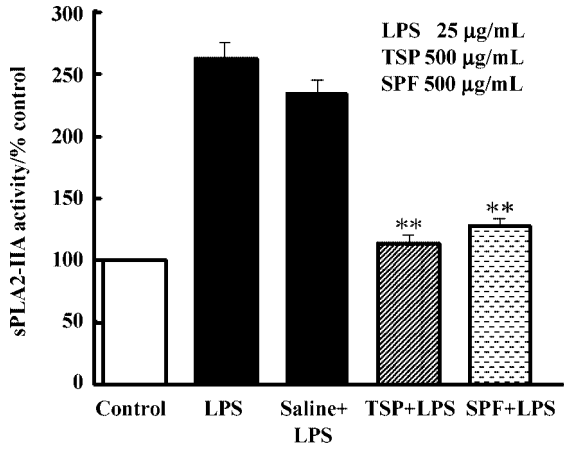

Fig 3. Effect of surfactant preparations on sPLA2-IIA expression in AM (for details see Wu YZ, *et al*. Am J Respir Crit Care Med 2003).

The CRD of SP-A shares sequence homology with the two $sPLA_2$ inhibitors (PLI-A and B) purified from the plasma of the Habu snake *Trimeresurus flavoridis* $sPLA_2$ suggesting that this region could be involved in $sPLA_2$-IIA binding (Antal JM *et al* 1996).

We demonstrated recently that $sPLA_2$-V and $sPLA_2$-X also efficiently hydrolyzed surfactant phospholipids, *in vitro*, in contrast to $sPLA_2$-IIC, IID, IIE and IIF which have no effect. We therefore investigated the *in vitro* effect of SP-A of these $sPLA_2$ and the consequences of $sPLA_2$-IIA inhibition by SP-A on surfactant phospholipid hydrolysis. We observed that SP-A inhibited $sPLA_2$-X activity but failed to interfere with that of $sPLA_2$-V. Intratracheal administration of $sPLA_2$-IIA to mice causes hydrolysis of surfactant phospholipids accompanied by a respiratory distress in SP-A gene-targeted more markedly than in wild type mice (Crough E 1998).

The inhibition of $sPLA_2$-IIA activity by SP-A may be of great interest in a pathophysiological point of view. Indeed, several clinical studies demonstrated that a pronounced decrease of SP-A levels was observed in the BALF of ARDS patients, probably due to its proteolytic degradation by the neutrophil elastase (Inoue S *et al* 1991). Hence, the excessive catabolism of surfactant phospholipids by $sPLA_2$-IIA might be explained by the marked defect in SP-A content observed in ARDS.

CONCLUDING REMARKS

Animal models of human inflammatory diseases such as ARDS, have been extensively used to investigate the potential pathophysiological roles of $sPLA_2$-IIA in these diseases. ARDS is a major cause of mortality and morbidity in critically ill patients. Despite intensive research during the last decade, the processes involved in the development of ARDS remains still unclear, and no specific therapy has proven effective in either preventing or reversing the development of the disease. Identification of factors involved in the pathogenesis of ARDS might open up a new area for the treatment of this disease. Indeed, hydrolysis of surfactant phospholipids by $sPLA_2$–IIA may increase surface tension at the air-liquid interface and also leads to the generation of a cytotoxic lyso-PC which seriously injures alveolo-capillary barrier.

On the other hand, alteration of surfactant would result in the removal of its inhibitory effect on $sPLA_2$-IIA thus leading to the accumulation of this enzyme within alveoli. This led us to hypothesize that a vicious circle may occur in pathophysiological conditions leading to increased synthesis of $sPLA_2$-IIA in lung tissues. Under conditions, the hydrolysis of surfactant phospholipids would result in the removal of their inhibitory effect on $sPLA_2$-IIA synthesis and an accumulation of this enzyme in the alveolar space, which in turn leads to further surfactant alteration, and to the installation of a vicious circle.

Recent clinical trials showed that instillation of exogenous surfactant preparations failed to improve the clinical symptoms of patients with ARDS (Pison U *et al* 1989). Two hypotheses can be proposed to explain this failure : i) surfactant alteration in ARDS is only one factor in the complex process of lung injury; ii) these clinical trial have used artificial surfactant preparations lacking proteins that might have altered the properties of surfactant, iii) the administered surfactant preparations are susceptible to attack by sPLA2-IIA present in the alveolar space of ARDS patients, which may lead to alteration of these preparations.

In the light of our studies we can speculate that the absence of SP-A from these surfactant preparations might have lowered their resistance to hydrolysis by $sPLA_2$-IIA. Thus, the use of surfactant preparations in combination with inhibitors of $sPLA_2$-IIA could represent a promising strategy for the treatment of ARDS.

ACKNOWLEDGEMENTS We thank Dr Michel CHIGNARD for reading and correcting the manuscript.