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

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INTRODUCTION

Phospholipases A₂ (PLA₂s) 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 PLA₂s are divided into two major classes according to their location: high molecular mass intracellular PLA₂ and low molecular mass secreted PLA₂ (sPLA₂) (Fig 2). There have been substantial progresses in understanding the regulation of sPLA₂ expression, especially the type-IIA sPLA₂ (sPLA₂-IIA), the most known enzyme of this group. The bacterial endotoxin lipopolysaccharide (LPS) induces the synthesis and secretion of sPLA₂-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.

sPLA₂-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.
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-$\kappa$B 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 lipopolysaccharide (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-$\alpha$ 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-$\alpha$ in the alveolar space preceding that of sPLA$_{2}$-IIA. In this ARDS model, incubation of alveolar macrophages (AM) with anti-TNF-$\alpha$ 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$_{2}$-IIF 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-deficient 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 sPLA2-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 sPLA2-IIA synthesis.

The earlier pharmacological investigations showed that NF-κB activation was an essential component of the cytokine signaling pathways inducing sPLA2-IIA expression. Indeed, pyrrolidine dithiocarbamate (PDTC), a potent NF-κB inhibitor, suppressed IL-1β and TNF-α-induced sPLA2-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 sPLA2-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 sPLA2-IIA promoter. The PPAR factors also play a role in the modulation on sPLA2-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 sPLA2-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 sPLA2 gene expression needs to be investigated. It seems likely that the induction (or repression) of sPLA2-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 be kept in 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 sPLA2-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-PLA2 properties (Andreani M et al 2000). However, subsequent studies attributed this anti-PLA2 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 PLA2 synthesis. In agreement with this finding, it has been shown that subcutaneous administration of dexamethasone, a synthetic GC, reduce circulating levels of sPLA2-IIA in endotoxemic guinea pigs (Davidson FF et al 1990). Dexamethasone also inhibits the expression of sPLA2-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 sPLA2-IIA in rat VSMC, astrocytes and mesangial cells (Valentin E et al 1999). Dexamethasone has also been shown to inhibit sPLA2-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 sPLA2-IIA, dexamethasone fails to interfere with the expression of sPLA2-V, thus suggesting that this drug acts selectively on sPLA2-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 sPLA2-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 cPLA2, a process leading to an enhanced release of arachidonic acid (AA). Subsequent studies suggested that the inhibitory effect of TGF-β2 on sPLA2-IIA expression was mediated by PGE2, a major cyclo-oxygenase (COX)-derived metabolite of AA.

Other growth factors have been involved in the inhibition of sPLA2-IIA expression in various cell types. Indeed, platelet-derived growth factor (PDGF) has been shown to inhibit both IL-1β- and forskolin-induced sPLA2-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 sPLA2-IIA synthesis in rabbit chondrocytes (Muhl H et al 1991).
INTERACTION OF SECRETORY PLA2 WITH PULMONARY SURFACTANT

Hydrolysis of surfactant phospholipids by sPLA2-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 1981). On the other hand, intra-tracheal 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 sPLA2-IIA after prolonged in vitro incubation whereas freshly collected AM had no detectable sPLA2-IIA expression (Hidi R et al 1993). This led us to suggest that the in vivo synthesis of sPLA2-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 sPLA2-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 sPLA2-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 monocyteic 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 collagenuous 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 sPLA2-IIA activity through a direct protein-protein interaction (Arbibe L et al 1998).
The CRD of SP-A shares sequence homology with the two sPLA2 inhibitors (PLI-A and B) purified from the plasma of the Habu snake *Trimeresurus flavoridis* sPLA2 suggesting that this region could be involved in sPLA2-IIA binding (Antal JM et al 1996). We demonstrated recently that sPLA2-V and sPLA2-X also efficiently hydrolyzed surfactant phospholipids, *in vitro*, in contrast to sPLA2-IIC, IID, IIE and IIF which have no effect. We therefore investigated the *in vitro* effect of SP-A of these sPLA2 and the consequences of sPLA2-IIA inhibition by SP-A on surfactant phospholipid hydrolysis. We observed that SP-A inhibited sPLA2-X activity but failed to interfere with that of sPLA2-V. Intratracheal administration of sPLA2-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 sPLA2-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 sPLA2-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 sPLA2-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 sPLA2-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 sPLA2-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 sPLA2-IIA in lung tissues. Under conditions, the hydrolysis of surfactant phospholipids would result in the removal of their inhibitory effect on sPLA2-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 sPLA2-IIA. Thus, the use of surfactant preparations in combination with inhibitors of sPLA2-IIA could represent a promising strategy for the treatment of ARDS.

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