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SHORT COMMUNICATION

# Inhibition by Pulmonary Surfactant Curosurf of Secretory Phospholipase A2 Expression in Guinea-Pig Alveolar Macrophages

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**ABSTRACT.** Replacement therapy with exogenous surfactant has been proven successful in animal models of acute respiratory distress syndrome (ARDS). Here, we investigated the effect of seminatural surfactant Curosurf® on the expression of secretory phospholipase A2 (sPLA2) in guinea-pig alveolar macrophages (AM). The latter produced an sPLA2 activity whose level was markedly reduced when culture medium was supplemented with Curosurf®. This effect was concentration-dependent and was accompanied by a decrease in sPLA2 mRNA levels. By contrast, when AM were first cultured for 20 hr and then incubated with Curosurf®, no significant change was observed in their sPLA2 activity. Finally, f-Met-Leu-Phe (FMLP)-induced thromboxane $B_2$ release from AM was not altered by Curosurf®, indicating that the inhibition of sPLA2 expression cannot be attributed to a nonspecific membraneous effect of Curosurf®. These findings show that pulmonary surfactant modulates the expression of sPLA2 in AM and suggest that this effect may account for the clinical efficacy of surfactant replacement therapy in ARDS. BIOCHEM PHARMACOL **54**;9:1055–1058, 1997. © 1997 Elsevier Science Inc.

**KEY WORDS.** PLA2; macrophages; surfactant; ARDS; guinea-pig

The physiopathological changes in lung during acute respiratory distress syndrome ARDS§ [1] may be caused, at least in part, by the release of inflammatory lipid mediators, including eicosanoids, lysophospholipids and platelet activating factor PAF [2]. An early metabolic step in the production of these mediators is catalyzed by phospholipase A2 (PLA2, phosphatide 2-acylhydrolase, EC 3.1.1.4), a widely distributed enzyme catalyzing the hydrolysis of ester bounds at the sn-2 position of membrane phospholipids [3]. Mammalian PLA2s characterized thus far can be classified as group I (pancreatic PLA2), group II (nonpancreatic PLA2, sPLA2) and cytosolic PLA2 (cPLA2), based on their primary structure [3].

The levels of sPLA2 were elevated (500–1000 times above basal values) in biological fluids from various inflammatory diseases [4]. A clinical trial demonstrated an increase of sPLA2 activity in bronchoalveolar lavage fluids (BALF) of patients with ARDS whose levels correlated positively with the severity of the disease [5]. Recent studies from our laboratory showed that endotoxin induced a marked expression of sPLA2 in a guinea-pig model of acute lung injury and that alveolar macrophages (AM) are the main source of this enzyme [6]. Taken together, these observations suggest that sPLA2 may play a role in the development and progression of ARDS. Replacement therapy with surfactant has been shown to improve the clinical outcome in animal models of ARDS [7]. Seminatural surfactant Curosurf® has been reported to reduce mortality in premature infants with respiratory distress syndrome [8]. The benefical effect of surfactant is mainly due to its biophysical properties (i.e., reduction of surface tension in the lung), but little attention has been paid to its antiinflammatory properties. Here, we show that Curosurf® is able to inhibit the synthesis of sPLA2 by isolated AM and suggest that this effect may be an additional or alternative mechanism accounting for the clinical efficacy of surfactant replacement therapy.

## MATERIALS AND METHODS

### *Materials*

Male Hartley guinea-pigs were obtained from Elevages Lebeau. Chemicals and reagents were from Sigma. 1-hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3-phosphoglycerol (PG) was from Interchim. [$^3$H]-arachidonic acid ([$^3$H]-AA, 80-135 Ci/mmol) was from Amersham. Seminatural surfactant (Curosurf®) was prepared by Chiesi Laboratories from

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§ *Abbreviations:* AA, arachidonic acid; AM, alveolar macrophage; ARDS, acute respiratory distress syndrome; BALF, bronchoalveolar lavage fluid; FMLP, f-Met-Leu-Phe; LDH, lactate dehydrogenase; sPLA2, type-II secretory phospholipase A2; $TxB_2$, thromboxane $B_2$; TNF, tumor necrosis factor.

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porcine lungs as described [9] and was provided by Laboratoires Serono.

### *Macrophage Isolation and Incubation Procedure*

Guinea-pigs weighting 600–1000 g were anesthetized by the intravenous injection of sodium pentobarbital (20 mg/kg) and bronchoalveolar lavages were performed as previously described [10]. AM were adjusted to $3 \times 10^6$ cells/mL and allowed to adhere in 35 mm culture dishes for 1 hr at 37° in 5% $CO_2$/95% air. The plates were then washed three times and incubated with serum-free RPMI 1640, in the presence or absence of Curosurf® for different time intervals (1 to 20 hr). Culture medium was then removed and the cells were washed before sPLA2 assay or RNA extraction. The effect of surfactant on cell viability was verified by the trypan blue dye exclusion test and the release of actate dehydrogenase (LDH) in the medium.

### *Measurement of $sPLA_2$ Activity and Thromboxane $B_2$ Release*

At the end of the incubations, the culture dishes were kept in an ice bath and supernatants were removed. Macrophage homogenates were prepared as previously decribed [10], and the measurement of sPLA2 activity was carried out according to Radvanyi et al. [11] using the fluorescent phosphatidylglycerol (PG) as substrate. After 20 hr incubation with or without Curosurf®, AM were washed twice and then stimulated with f-Met-Leu-Phe (FMLP, 1 μM) for 15 min. The release of thromboxane $B_2$ ($TxB_2$) was measured by radioimmunoassay as previously decribed [10].

### *Analysis of $sPLA_2$ mRNA Expression*

Total RNA was extracted and electrophoresed (10 μg/lane) on a 1% agarose gel and then transferred onto nylon membranes. The blots were hybridized at 68° overnight with guinea-pig sPLA2-II cDNA as described by Vial et al. [12].

## RESULTS AND DISCUSSION

The level of sPLA2 activity was very low in freshly collected AM, but increased in a time-dependent manner to reach maximal values within 16–20 hr of culture (Fig. 1). No sPLA2 activity was detected in the supernatant of AM under our experimental conditions (i.e., culture in serum-free medium) (data not shown). Our results showed that incubation of AM with Curosurf® markedly reduced the increase in sPLA2 activity (Fig. 1). This effect was concentration-dependent with maximal inhibition being observed at 375 μg/mL of Curosurf® (Fig. 2). These concentrations are similar to those of synthetic surfactant Exosurf® used in other studies [13] and had no effect on cell viability as verified by the trypan blue dye exclusion test and the release of LDH in the medium (data not shown).

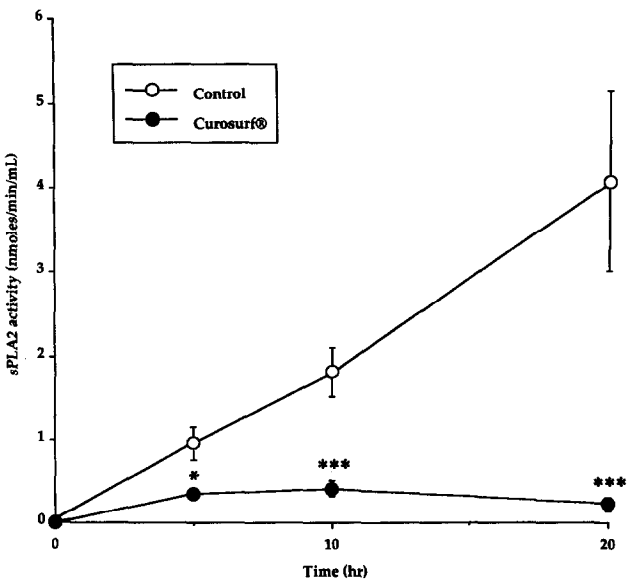


**FIG. 1. Effect of Curosurf® on the evolution of sPLA2 activity in AM. Isolated AM were incubated with Curosurf® at 500 μg/mL (final concentration). At the indicated time intervals, the plates were washed twice and sPLA2 activity was evaluated in the AM homogenates. The results show sPLA2 activity in control (○) and Curosurf®-treated AM (●) and are expressed in nmoles of hydrolysed phospholipids/min/mL (mean ± SE, N = 6). *P < 0.05; ***P < 0.001 (Student's *t*-test).**

Since Curosurf® is mainly composed of phospholipids, which are the substrates of PLA2, we examined whether the inhibition of sPLA2 activity observed in AM was due to an interference of Curosurf® with the PLA2 assay. Aliquots (50 μL) of AM homogenates were added to fluorescent substrate suspension preallably mixed with 25 μg/mL of Curosurf®. The use of this concentration, which represents 1/20 of the maximal concentration of Curosurf® (500 μg/mL) in the Fig. 1, was based on the fact that 50 μL of cell homogenates were added to 1 mL substrate suspension for the measurement of PLA2 activity. This showed that the presence of Curosurf® did not interfere with the measurement of sPLA2 activity in AM homogenates (4.6 ± 0.3 and 5.5 ± 1.0 nmoles/mL/min, in the absence and presence of Curosurf®, respectively, N = 3). On the other hand, to verify whether the attenuation of sPLA2 production by AM is due to a nonspecific membraneous action of Curosurf®, we examined the effect of the latter on FMLP-induced $TxB_2$ release, a receptor-coupled process linked to G-protein/phospholipase C activation [14]. When AM were incubated for 20 hr with 500 μg/mL of Curosurf®, washed and then stimulated with FMLP for 15 min, no significant effect on the release of thromboxane $B_2$ was observed (42.4 ± 8.4 and 45.4 ± 15.1 ng/mL, in control and Curosurf®-treated AM, respectively; N = 5).

Finally, AM were first cultured for 20 hr (a period after which the plateau of sPLA2 synthesis was reached) and then incubated for an additional 2 hr with Curosurf®. Under these conditions, no significant change in the sPLA2 activity was observed as compared to untreated AM

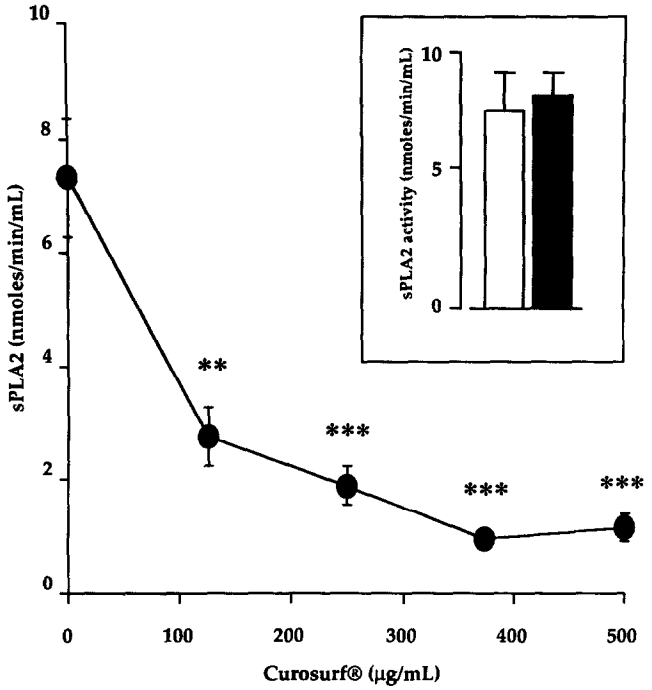


FIG. 2. Concentration-dependent effect of Curosurf® on sPLA2 activity in AM. AM were incubated for 20 hr with the indicated concentrations of Curosurf® and sPLA2 activity was then measured in AM homogenates. Insert: AM were allowed to adhere for 20 hr, washed and incubated without (□) or with (■) 500 µg/mL of Curosurf® for 2 additional h before the measurement of sPLA2 activity. The results are expressed in nmol/min/mL (mean ± SE, N = 6). **P < 0.01; ***P < 0.001 (Student's *t*-test).

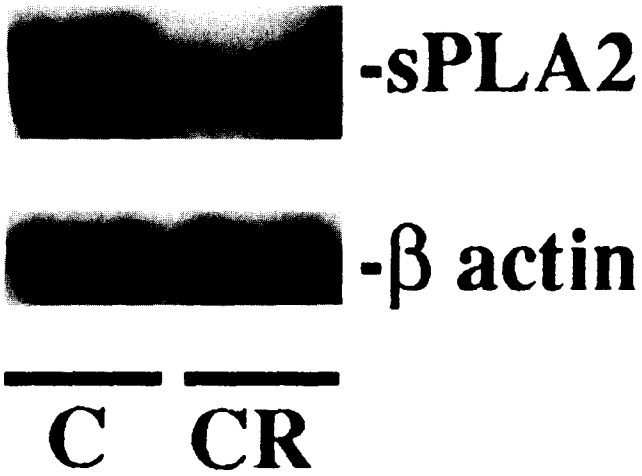


FIG. 3. Effect of Curosurf® on sPLA2 mRNA expression. AM were incubated with 500 µg/mL of Curosurf® for 20 hr. The cells were then washed and total RNA extracted. The expression of sPLA2 mRNA was analyzed as decribed previously [12]. The figure shows sPLA2 mRNA expression in control (C) and Curosurf®-treated AM (CR) and is representative of two independent experiments.

(Fig. 2, insert). This suggests that the ability of Curosurf® to reduce the level of sPLA2 activity is not due to a direct interaction between sPLA2 and Curosurf® but to the inhibition of the synthesis of this enzyme. Indeed, preincubation of AM with Curosurf® markedly reduced the level of sPLA2 mRNA (Fig. 3).

Our studies show that Curosurf® attenuates the synthesis of sPLA2 by AM and that this effect is due to the inhibition of sPLA2 gene expression. Although the mechanisms involved in this inhibition are still unclear, we hypothesize that the suppression of tumor necrosis factor-α (TNF-α) secretion by Curosurf® may be one of the mechanisms involved in this process. Indeed, a recent report showed that the secretion of TNF-α by human AM is inhibited by synthetic surfactant Exosurf® [13]. In addition, recent studies in our laboratory demonstrated that induction of sPLA2 synthesis in guinea-pig AM is mediated by TNF-α through an autocrine/paracrine process [6]. Thus, it is likely that Curosurf® inhibits the expression of sPLA2 by interfering with the release of TNF-α by AM. The fact that AM are able to phagocytize pulmonary surfactant and to metabolize its components [15] led us to postulate that some products of this metabolism are probably involved in the inhibition of sPLA2 expression. The investigation of the effect of various components (proteins and phospholipids) of Curosurf® on the expression of sPLA2 would help us to understand the mechanisms involved in its down-regulation.

Since sPLA2 has a potential inflammatory role in ARDS, we suggest that the inhibition of its synthesis by Curosurf® may be an additional or alternative mechanism that could account for the clinical benefit of surfactant therapy in respiratory distress syndromes.

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