Changes in the inflammatory response during and after cardiac surgery
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Citation for published version (APA):

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Biphasic release of secretory phospholipase A2 during and after cardiopulmonary bypass

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Introduction

Recently Nakamura and colleagues\(^1\) reported on the enhancement of secretory phospholipase A\(_2\) (sPLA\(_2\)) activity during cardiac operations. sPLA\(_2\) activity increased after systemic heparinization, before the patient was connected to the extracorporeal circuit, and was partially reduced after protamine administration. PLA2 hydrolyses the sn-2-ester bond of phospholipids and catalyzes the rate-limiting step in the formation of eicosanoids from membranes. Consistent herewith, Nakamura and colleagues\(^1\) showed that sPLA\(_2\) activity correlated with the generation of circulating 6-keto-prostaglandin F\(_{1\alpha}\) during cardiac surgery and concluded that systemic heparinization stimulates eicosanoid production, which contributes to the vasodilating and anticoagulant action of heparin during cardiopulmonary bypass (CPB). However, cytokines such as interleukin-1\(\beta\), interleukin-6 and tumour necrosis factor-\(\alpha\) also stimulate the production of sPLA\(_2\) by cells including neutrophils, platelets, endothelial cells and liver cells. The regulatory sequences of the sPLA\(_2\) gene contain a putative interleukin-6 responsive element, homologous to that found in several acute phase genes\(^2\). Hence sPLA\(_2\) may be an acute phase protein. The acute phase response is a sequential series of events that are induced by proinflammatory cytokines. An acute phase response is clinically marked by leukocytosis, fever, metabolic changes, and increased (but also decreased) synthesis of various liver proteins. The physiological importance of this response is unknown. The first acute phase proteins to rise during the acute phase response are sPLA\(_2\) and C-reactive protein (CRP). We studied the release of sPLA\(_2\) not only during CPB but also during the acute phase reaction induced by this procedure.

Methods

We studied the release of sPLA\(_2\) and CRP in ten patients, seven man and three women aged 58 years (median, range 54 to 67 years) and weighing 82 kg (range 79 to 91 kg) scheduled to undergo elective coronary artery bypass grafting. The study was approved by the Medical Ethics Committee of the Academic Medical Centre of Amsterdam and written informed consent was obtained from each patient. Anesthesia and surgical procedure were standardized and identical for all patients. In brief, on the morning of operation, patients received their usual dose of antianginal drugs and lorazepam (2 to 4 mg) as premedication. Anesthesia was induced intravenously with etomidate (0.2 mg/kg), fentanyl (50 \(\mu\)g/kg), pancuronium bromide (0.1 mg/kg), and midazolam (0.1 mg/kg), and maintained by
supplemental doses. After endotracheal intubation, patients were ventilated to normocapnia with oxygen and air mixture. Cefamandole (2 gm) was given intravenously for infection prophylaxis. A radial artery catheter and a flow-directed pulmonary artery catheter (Swan-Ganz, Baxter/ American Edwards Laboratories, Santa Ana, Calif.) were inserted for hemodynamic measurements and collection of blood samples. Volume was supplemented and further deviations from pressures or vascular resistances were treated with appropriate vasoactive medication. The extracorporeal circuit consisted of a hollow-fiber oxygenator with integrated heat exchanger (Univox, Baxter Healthcare Corp., Irvine, Calif.) and roller pump. CPB duration was 93 minutes (80 to 111 minutes). Patients received 250 IU heparin/kg body weight. (Leo Pharmaceutical Products, Weesp, The Netherlands). Blood samples were collected from the radial artery catheter or the arterial site of the extracorporeal circuit and by venous puncture after removal of the radial artery catheter. Platelet-poor plasma was prepared by centrifugation immediately after collection and stored at -70°C. Circulating concentrations of sPLA₂ and CRP were determined with enzyme-linked immunosorbent assays as previously described.

Results

Consistent with the data of Nakamura and coworkers,¹ we found significant increases, that is, up to threefold the baseline levels, of sPLA₂ during CPB (Fig. 1). However, levels increased tremendously, that is, up to thirty times baseline levels, on the first and second postoperative days. To demonstrate that sPLA₂ release during CPB is induced by systemic heparin, we incubated 0.2 mL of whole blood of a healthy volunteer in phosphate-buffered saline solution containing increasing heparin (Leo Pharmaceutical Products) concentrations for 15 minutes at room temperature on a shaker (200 rpm). Heparin at a concentration of 3 U/mL, comparable with that during systemic heparinization, caused a 2.5-fold increase (± 0.55, standard deviation) of sPLA₂ from blood cells, and reached a maximal 4.1-fold increase (± 0.81) at 25 U/mL (Fig. 2). These in vitro data also suggest that the elevations of sPLA₂ during CPB are mainly explained by heparinization of the patient.

Comment

The elevation on the postoperative days likely reflected that sPLA₂ is an acute phase protein, as stated previously²-⁴. Its production and release continued for several days while preceding the release of the prototype of acute phase proteins in man,
**Figure 1** Release of sPLA₂ and CRP during and after cardiac surgery. During heparinization, a minor release of sPLA₂ of threefold baseline levels is found, which is partly antagonized by the administration of protamine sulfate. During the acute phase response, a second and major release of sPLA₂ occurs, reaching levels of thirty times baseline levels. Data presented are medians with interquartile range. * p< 0.01 compared with baseline, Wilcoxon rank-sum test.

**Figure 2** Effect of heparin on the release of secretory phospholipase A₂ levels in whole blood. Whole blood was incubated with various concentrations of heparin (U/mL) for 15 minutes at room temperature. Data presented are medians with range (n=3). * p< 0.01 compared with baseline, Wilcoxon rank-sum test.
CRP. The role of sPLA$_2$ in the acute phase response in general and after cardiac surgery more specifically is not clear. It has been hypothesized that sPLA$_2$ and CRP, in a combined effort, promote phagocytosis of injured cells and tissue debris by neutrophils.

The molecular base for this hypothesis is that sPLA$_2$ preferentially hydrolyzes phospholipids of cells that have undergone a flip-flop (i.e. an exchange of phospholipids of the inner and outer leaflet of the plasma membrane, for example, as a consequence of ischemia) to generate binding sites for CRP. Bound CRP in turn may induce complement activation via the classical pathway and so promote phagocytosis of metabolically compromised cells that are generated during inflammation or ischemia$^4$. During cardiac surgery, the tissues of heart and lungs especially are deprived of oxygen during aortic crossclamping and may therefore be the primary target for sPLA$_2$ activity, subsequent CRP deposition, and finally complement- and neutrophil-mediated damage$^5$. Alternatively, sPLA$_2$ release during the first postoperative day may further contribute to the generation of proinflammatory prostaglandins and leukotrienes by the mechanism described by Nakamura and associates$^1$. Most typical, one in three patients have rhythm disturbances, mainly atrial fibrillation with fast ventricular rate, but all patients have leukocytosis and fever on the first, second or third postoperative day$^5$. Our data suggest that sPLA$_2$ may be involved in the pathogenesis of these complications.

References

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