Circulating Secretory Phospholipase A_2 in Critical Illness - The Importance of the Intestine

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ABSTRACT

Objective: *To review the role of secretory phospholipase A_2 in the pathogenesis of multiple organ failure in the critically ill patient.*

Data sources: *Relevant articles and published reviews on secretory phospholipase A_2 in critical illness.*

Summary of review: *Secretory phospholipase A_2 ($sPLA_2$) has an important role in inflammation and in antimicrobial defence. However, excessive activity of $sPLA_2$ has been shown to result in tissue damage and has been implicated as a mediator of organ failure associated with critical illness. Gastrointestinal release of secretory phospholipase A_2 from Paneth cells increases during intestinal ischaemia and may be an important factor in the pathogenesis of the multiple organ dysfunction syndrome. In experimental models, specific PLA_2 inhibitors reduce organ failure associated with sPLA infusion and may play an important role in reducing organ failure in the management of the critically ill patient.*

Conclusions: *Intestinal ischaemia may play an important role in the pathogenesis of the multiple organ dysfunction syndrome in the critically ill patient. In patients with sepsis, specific PLA_2 inhibitors have the potential to reduce organ failure and improve morbidity and mortality.* (**Critical Care and Resuscitation 2001; 3: 244-249**)

Key words: Secretory phospholipase A_2, multiple organ dysfunction syndrome, critical illness

The ubiquitous enzyme phospholipase A_2 (PLA_2) is pivotal in the generation of a spectrum of biologically active lipids. For example, the PLA_2 catalysed hydrolysis of phospholipid substrates releases arachidonic acid for subsequent metabolism to biologically active eicosanoids, particularly prostaglandins and leukotrienes (via the cyclooxygenase or lipooxygenase pathways). Concomitant release of lysophospholipid activates white cells directly and yield precursors of platelet activating factor (PAF) which is also an important inflammatory mediator.[1] PLA_2's are now classified into subtypes according to their function, localisation, mechanism and structure.

Identification of secretory phospholipases

Secretory phospholipase type A_2 ($sPLA_2$) isoenzymes are small (13 - 15 kDa and approximately 125 amino acids in length), basic, calcium dependent enzymes that specifically hydrolyse the sn-2 acyl bond of phospholipids. The resulting hydrolysis products provide precursors for inflammation.

All $sPLA_2$ isoenzymes have a catalytic histidine-aspartate dyad and are extremely stable proteins as a consequence of six disulphide bonds in their structure. Secretory PLA_2 iso-enzymes form a major component of snake and bee venoms (hundreds of iso-forms have been identified in snake venom alone[2]) and have long been recognised to be an important mediator of inflammation. However, $sPLA_2$ enzymes have other functions including digestion, enhancement of phagocytosis, membrane remodeling (removal of peroxidised or senescent phospholipids) and signal transduction, although the precise role of phospholipases in some systems remains unclear. The magnitude of the upregulation of $sPLA_2$ during infectious or inflammatory episodes is consistent with an important role for these enzymes in host defense.

In general, $sPLA_2$ enzymes bind tightly to anionic

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phospholipid containing interfaces but have little activity on neutral membranes. The external monolayer of mammalian cells is made up almost entirely of neutral zwitterionic phospholipids, mainly phosphatidylcholine, with little phosphatidylethanolamine,[3-5] while the inner layer contains anionic phospholipids (phosphatidylserine, phosphatidylglycerol and phosphatidylinositol) together with the majority of the phosphatidylethanolamine. When intact, the neutral outer membrane of mammalian cells permits negligible binding and hydrolysis by $sPLA_2$.[6] In contrast, the bacterial membrane is composed almost entirely of anionic phospholipids and consequently represents a preferred substrate for the cationic $sPLA_2$ molecule. These observations have led to a suggestion that $sPLA_2$ plays an important role in microbial defence. Further experimental observations support this concept. Transgenic mice expressing human $sPLA_2$ have been shown to resist experimental *Staphylococcus aureus* infection and improve survival by reducing numbers of live bacteria in their tissues.[7] A similar effect is observed with experimental *Escherichia coli* infection.[7] Secretory phospholipase A_2 is markedly more active toward *E. coli* in the presence of bactericidal permeability-increasing protein as this disrupts the phospholipid structure of the membrane, causing these two factors to operate synergistically.[8]

In the 24 hr following injection of a lethal dose of *E. coli* into baboons, the plasma $sPLA_2$ level rises more than 100-fold. Serum collected 24 hr after bacterial challenge exhibits potent bactericidal activity (in contrast to serum from unchallenged animals). This bactericidal activity is almost completely blocked by a monoclonal antibody to human $sPLA_2$, while addition of purified human $sPLA_2$ to pre-challenge serum confers potent antibacterial activity against staphy-lococci, equal to that of the serum from challenged animals.[9]

Secretory PLA_2 has been demonstrated to promote phagocytosis of injured cells and tissue debris,[10,11] an action which is predicted by the observation that $sPLA_2$ has activity against disrupted mammalian cell membranes but minimal activity against intact membranes. These data suggest that $sPLA_2$ may also have an important role in removal of damaged cells.

In the early nineties three forms of PLA_2 isoenzymes were recognised.[12] In mammals two secretory isoenzymes were described, $sPLA_2$ type I from pancreatic juice and $sPLA_2$ type IIa from synovial fluid; $sPLA_2$ type IIa was also identified in the plasma of patients with sepsis or inflammation. The third PLA_2 enzyme recognised was the larger (85 kDa) intracellular or cytosolic PLA_2 (designated $cPLA_2$ or $iPLA_2$). This hydrolyses the same bond of phospholipids but, unlike secreted forms, is not calcium dependent.

Over the last few years the number of recognised $sPLA_2$ isoenzymes has expanded considerably. Ten distinct types of secretory phospholipase have been described in mammalian tissues so far including type IB, IIA, IID, IIE, V and X.[13] Most have been defined only in the last few years and precise tissue localisation, physiological function and pathological activity remain to be clarified.[1]

In general the enzymatic activity of the newly described PLA_2 isoenzymes are similar to those of type IIa-$sPLA_2$, features of which are well described.[14] However, type V $sPLA_2$ catalyses the hydrolysis of phosphatidylcholine phospholipid more effectively than does type IIa-$sPLA_2$, by virtue of a much higher binding affinity and activity for compactly packed phosphatidylcholine bi-layers.[15] Type V-PLA_2 also has a much-reduced thermal stability compared with type IIa-PLA_2.[16]

Many antibodies used in immunoassays which were thought specific for $sPLA_2$-type IIa have recently been shown to cross-react with $sPLA_2$ - type V.[17,18] Consequently much tissue and circulating phospholipase activity currently attributed to type IIa PLA_2 may be revealed to be the result of other $sPLA_2$ isoenzymes as further data become available. Certainly the greater activity on intact mammalian (neutral zwitterionic phospholipid) cell membranes by type V-$sPLA_2$ would be expected to result in greater tissue damage.

Serum phospholipase A_2 levels in sick patients

Serum $sPLA_2$ levels are elevated in critically ill patients, particularly in those with sepsis.[19,20] Higher levels of $sPLA_2$ have been reported in septic patients who died compared with those who survived.[21,22] Confirmation of the relationship between sepsis and increased plasma $sPLA_2$ concentration has been provided by Pruzanski *et al*,[23] who demonstrated a marked increase in the activity of circulating PLA_2 within 3 hr of endotoxin infusion in humans. Peak levels occurred at 24 hr and were more than 20 times greater than baseline values.[23]

PLA_2 may mediate some of the clinical features of septic shock, as parenteral administration of purified exogenous $sPLA_2$ produces hypotension in experimental models, and $sPLA_2$ levels have been found to correlate directly with the magnitude and duration of circulatory collapse.[24] Increased serum type IIa $sPLA_2$ concentration have also been found to occur in diseases involving tissue destruction[25] and after elective operations (e.g. aortic surgery and coronary artery bypass surgery[26,27]).

The source of phospholipase activity in the blood

The cellular source of the $sPLA_2$ in serum is unclear. In normal subjects, without an inflammatory stimulus,

$sPLA_2$ is present in large amounts in cartilage, Paneth cells, prostatic tissue, seminal fluid and lacrimal fluid (tears).[28] However, $sPLA_2$ is not found (using immuno-histochemical or immunochemical methods) in resting (i.e. unstimulated) inflammatory cells.[29] Many cells synthesise $sPLA_2$ when challenged by endotoxin, including hepatic Kupffer cells[30] and neutrophils. However, stimulated neutrophils exhibit low $sPLA_2$ activity,[31] suggesting that they may not be an important source of the high serum levels found in critically ill patients. Vascular smooth muscle cells have been shown to secrete significant amounts of $sPLA_2$ in response to stimulation by pro-inflammatory interleuk-ins or endotoxin and may be a major source of $sPLA_2$ seen in septic patients.[32]

In the critically ill patient, the small intestine appears to be a major site of $sPLA_2$ synthesis. This $sPLA_2$ has been reported to be identical to that isolated from synovial fluid ($sPLA_2$ sub-type IIa). However as recent advances in molecular biology techniques have identified the existence of many more $sPLA_2$ iso-enzymes, it is likely that one or more of these newly described isoenzymes is secreted by cells in the small intestine.

Paneth cells and secretory phospholipase A_2

Within the small bowel, $sPLA_2$ mRNA is found in Paneth cells, but not in any other cell type of the intestinal tract.[33] Paneth cells are situated at the base of crypts on the small intestine. The granules contain a number of proteins associated with roles in host defense, including lysozyme, secretory phospholipase A_2, and alpha-defensins, (termed cryptdins).[34] The Paneth cell $sPLA_2$ may play a role in digestion of dietary phospholipids[35] or may help to maintain sterility in crypts by virtue of an antibacterial action.[36] In terms of overall quantity of phospholipase activity, the small intestine represents the predominant proportion of the body's resting $sPLA_2$ activity.

Secretory phospholipase A_2 release from Paneth cells

Significant $sPLA_2$ is secreted from rat intestine, harvested for transplantation and preserved in University of Wisconsin solution, during the first few hours of preservation. These elevations in $sPLA_2$ activity appear to be related to active secretion rather than to leakage from dying cells.[37,38] This observation lends strong support to the concept that $sPLA_2$ secretion might be an important mediator of local intestinal damage and distant organ damage in clinical bowel ischaemia.

Secretory phospholipase A_2 has recently been implicated as a key enzyme of local inflammation after gut ischemia-reperfusion and a major mediator of the associated distant organ injury.[39] Both PAF and endotoxin have been shown to up-regulate Paneth cell $sPLA_2$ expression at both transcriptional and post-transcriptional levels, significantly increasing enzyme activity of intestinal $sPLA_2$.[40]

It has been reported that the secretory phospholipase A_2 found in the gut is type IIa and identical to the type IIa found in synovial fluid.[29,33] However, immunoassays at the time were unable to differentiate type IIa from type V (or other iso-enzymes). $sPLA_2$ has been isolated from both pig[41] and human ileum.[42] Both were considered to be identical to synovial type IIa PLA_2. In the case of the human isolate the NH_2-terminal amino acid sequence, amino acid composition, molecular weight, and elution behavior were found to be identical to those of human group II PLA_2 purified from synovial fluid or spleen.[42]

However, other authors have found unusual phospholipase activities in the extracts from bowel. Mansbach, *et al* found significant PLA_2 activity in rat gut using phosphatidycholine as a substrate (against which synovial type PLA_2 shows almost no activity),[35] and a preference for plasmenylcholine rather than plasmenyl-ethanolamine was also found in extracts of rat small intestine by Fukishima and Serrero.[43]

Additional evidence of unique phospholipase activity from an intestinal origin is suggested by Minami *et al* who examined sera from patients with inflammatory bowel disease. When this sera was subjected to reverse phase high performance liquid chromatography, the phospholipase activity was resolved into two peaks and the authors concluded that two forms of phospholipase A_2 immunochemically related to group II enzyme were present in these patients.[44]

Phospholipase as a mediator of organ failure

Systemic release of $sPLA_2$ occurs in states of profound illness including sepsis, shock, severe injury and pancreatitis, all of which are linked to the development of acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS). Experimental and clinical evidence suggests that $sPLA_2$ may serve a primary role in the development of organ dysfunction in these conditions.[45-48]

Direct instillation of $sPLA_2$ into lungs has been shown to result in significant injury.[49]

Early intestinal infarction, with necrosis limited to the mucosa, is associated with hyperaemia and focal necrosis in the liver, while more profound intestinal infarction with necrosis extending into muscular layers is associated with massive necrosis or centrilobular necrosis of the liver, despite hepatic blood flow being uninterrupted.[50] Such remote injury is presumably mediated by factors released from injured bowel, of which $sPLA_2$ has been demonstrated to be a major

component.

Bowel ischaemia during critical illness

Haemorrhagic shock produces depression in microvascular blood flow, which persists despite fluid resuscitation.[51] This concept that the gut might suffer disproportionate ischaemia in shock states together with the observation that the ischaemic bowel is a potent source of cytokines and phospholipase has led to the suggestion that the gut may be a significant 'driver' of the MODS.[52,53]

In a study to evaluate the association of intestinal damage with systemic inflammatory response syndrome (SIRS) in a surgical intensive care unit, Gollin *et al*[54] found a high incidence of intestinal injury. In this study intestinal mucosal viability was assessed by serial measurement of serum and urine intestinal fatty acid binding protein (iFABP) which is a sensitive and specific marker for intestinal mucosal injury. They found that 75% of their patients with raised serum iFABP died or developed severe adult respiratory distress syndrome, while 82% of those without detectable serum iFABP recovered without major morbidity.

Potential target of biological modification

Inhibition of $sPLA_2$ activity could result in the suppression of several important classes of inflammatory lipid mediators (prostaglandins, leukotrienes, platelet activating factor, lysophospholipid). Consequently, clinical use of $sPLA_2$ inhibitors seems attractive.

Non-selective inhibition of phospholipase activity impairs essential phospholipid metabolism and cell viability. However, secretory PLA_2 appears to be the important mediator of inflammatory injury and selective inhibition of $sPLA_2$, by linking the inhibitor to a molecule which does not permit intracellular passage of the inhibitor, appears to effectively inhibit secretory phospholipase activity without associated cellular toxicity. Such an agent may have the potential to enter clinical practice.

Blocking of phospholipase A_2 activity in intestine preserved prior to grafting appears to greatly improve intestinal preservation.[37,38] Also phospholipase inhibition has been shown to limit the effects of intestinal ischaemia/reperfusion in vivo.[55-57] These observations suggest the potential clinical utility of $sPLA_2$ inhibition in clinical practice.

Current investigation into the pathological role of the recently described iso-enzymes of $sPLA_2$ promises to greatly increase our understanding of phospholipase activity in clinical illness. Clearer definition of the isoenzymes released from the intestine in shock and in regional hypoperfusion/reperfusion is likely to provide insights into the mechanisms of injury and may provide a mechanism for improved diagnosis and/or treatment.

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