

Secretory type II phospholipase A₂ binds to ischemic myocardium during myocardial infarction in humans

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Abstract

Objective: An increase of circulating secretory Phospholipase A₂ (sPLA₂) is a risk factor for coronary artery disease. We hypothesized that this reflects participation of sPLA₂ in local inflammatory reactions ensuing in ischemic myocardium. Therefore, we studied the course of circulating sPLA₂ in patients with acute myocardial infarction (AMI) or unstable angina pectoris (UAP), and investigated the presence of sPLA₂ in infarcted myocardial tissue. **Methods:** Plasma samples of 107 patients with AMI or UAP, collected on admission and at varying intervals thereafter, were tested for the presence of sPLA₂ and C-reactive protein (CRP). Cumulative release values of these parameters were calculated, which allowed for comparison of the results rearranged in time according to the onset of symptoms. By immunohistochemistry we studied the presence of sPLA₂ and CRP in myocardial tissue of 30 patients who died subsequent to AMI. **Results:** Levels of sPLA₂ became elevated during the disease course in 66 of the 87 patients with AMI, and were higher than those of the patients with UAP of whom 8 of the 20 had elevated levels. By immunohistochemistry sPLA₂ was found to be localized in the infarcted myocardium, particularly in its borderzone, from 12 h after the onset of AMI. Positive staining for sPLA₂ was more extensive than that for CRP. **Conclusions:** The localization pattern of sPLA₂ in infarcted myocardium as well as its plasma course, in relation to those of CRP, are in line with a supposed pro-inflammatory role during AMI for sPLA₂ as a generator of lysophospholipids serving as ligands for CRP. © 2002 Published by Elsevier Science B.V.

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1. Introduction

Impaired perfusion of the myocardium induces a local inflammatory response [1,2] comprising a complicated interaction of ischemic cardiomyocytes with inflammatory cells, cytokines, complement factors and acute phase proteins.

Although animal studies have shown that local inflammatory reactions may contribute significantly (up to 65%) to infarct size [1–3], the contribution of inflammation to the extent of tissue damage following myocardial ischemia and infarction in humans is poorly understood. Circulating levels of parameters that reflect inflammation, like C-reactive protein (CRP) and serum Amyloid A, may behave as cardiovascular risk factors [4–7]. Secretory phospholipase A₂ (sPLA₂), a known pro-inflammatory mediator, was

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recently found to be such a cardiovascular risk factor, as an increase in circulating levels of sPLA₂ in patients with unstable angina can predict the outcome of clinical coronary events [8]. sPLA₂ hydrolyses the sn-2-ester bond of phospholipids to produce free fatty acids and lysophospholipids [9]. In doing so these enzymes catalyse the rate limiting step in the formation of several pro-inflammatory lipid mediators like prostaglandins, leukotrienes, lipoxins and platelet-activating factor [10]. Excessive and uncontrolled sPLA₂ activity, has long been postulated to be a contributing factor to the tissue damage and organ dysfunction that occurs in a variety of human acute and chronic inflammatory diseases [11]. Its crucial role in the pro-inflammatory cascade makes it an interesting target for novel anti-inflammatory therapies. Indeed, several potent inhibitors, tested *in vitro* and in an animal sepsis model have been developed [12,13]. To what extent sPLA₂ participates in the inflammatory cascade in ischemic myocardium however is unknown and therefore the aim of this study.

Recently we have shown that CRP localizes in infarcted myocardium together with activated complement fragments, suggesting CRP contributes to inflammatory changes ensuing locally in ischemic myocardium [14]. Indeed, in a recent study it was shown that human CRP enhances myocardial damage in a rat model for acute myocardial infarction [15]. CRP has the ability to bind a number of substances, among which are lysophospholipids [16,17]. These are products of the hydrolysis of the phospholipids of the cell membrane by activity of sPLA₂. We hypothesized that sPLA₂ may be involved in the formation of suitable ligands for binding of CRP to infarcted myocardial muscle cells [18]. Therefore, in this study, we also examined the course of sPLA₂ in relation to that of CRP in patients with AMI, and studied its presence in ischemic myocardium.

2. Methods

2.1. Patients

From June 1994 to June 1996 patients were included in the study when they full-filled criteria for acute myocardial infarction (AMI) or unstable angina pectoris (UAP) and had given informed consent. Criteria for AMI were: clinical evidence of AMI (i.e. typical chest pain and ST-segment elevation of ≥ 2 mV in two contiguous leads of a standard ECG), and a diagnostic rise in cardiac enzymes during the hospitalisation period (serum lactate dehydrogenase (LD), creatine kinase (CK) or creatine kinase MB-fraction (CK-MB)). UAP was defined as angina at rest occurring during at least two episodes in the previous 48 h or in one episode lasting at least 15 min, and not alleviated by sublingual nitrates, and, in case of ECG recordings, accompanied by ST-segment deviations at rest,

in the absence of a rise in cardiac enzymes (LD, CK or CK-MB) on admission or during the first days thereafter. Patients with (auto)immune disorders, infectious disease, malignancy or pregnancy were excluded from the study.

For immunohistochemical studies, patients, referred to the Department of Pathology for autopsy, were included, when at autopsy they showed signs of a recently developed AMI, i.e. decreased Lactate Dehydrogenase (LD) staining of the affected myocardium. Some of the patients participated in earlier studies on the involvement of CRP, complement, ICAM-1 and CD66B in infarcted myocardium [14,19].

The study was approved by the ethics committee of the VU Medical Center Amsterdam and was performed according the institutional guidelines. Furthermore, the investigation conforms with the principles outlined in the Declaration of Helsinki.

2.2. Collection of plasma samples and laboratory investigations

sPLA₂ and CRP were assessed in venous blood samples of 5 ml that were obtained on admission and 2, 6, 12, 24, 48 and 72 h thereafter. These samples were collected in Venoject tubes (Terumo, Rome, Italy) containing 10 mM benzamidine 100 μ g per ml soy bean trypsin inhibitor and 10 mM EDTA (final concentrations) to prevent *in vitro* activation. After centrifugation for 10 min at 1300 g the plasma was collected and stored at -70°C , until assays were performed. Venous samples for determination of CK-(MB) and LD were collected every 4 h after admission during the first 24 h and at 36, 48 and 72 h thereafter. CK-(MB) and LD were determined directly by spectrophotometer at 30°C , using a centrifugal analyser (BM/Hitachi 747) and commercial test kits (both from Boehringer Mannheim, Mannheim, Germany). sPLA₂ and CRP were measured with ELISAs as previously described [20,21]. Two different monoclonal antibodies (4A1 and 10B2) against human sPLA₂ (kindly provided by Dr F.B. Taylor Jr., Oklahoma Medical Research Foundation, Oklahoma City, OK) were used as coating and catching antibodies, respectively. Results were compared with those obtained with cultured medium from Hep G2 cells stimulated with human IL-6 as this medium contains a significant amount of sPLA₂. The amount of sPLA₂ in this cultured medium was assessed by comparison with purified recombinant human sPLA₂ (kindly provided by Prof. H.M. Verheijen, CMLE, Utrecht, the Netherlands). In a previous study we took blood samples of 19 healthy volunteers to obtain normal ranges. These had an average amount of 3.4 ng/ml sPLA₂ (S.D. ± 1.3). As a cut-off point 5 ng/ml was considered to be elevated, as the 95th percentile of the healthy volunteers samples was 4.9 [20]. The detection limit of the test was 1.0 ng/ml sPLA₂ (S.D. ± 0.8) and the Inter Calibration Variance of the test was 7.4% (S.D. $\pm 1.4\%$).

2.3. Calculation of enzyme- and sPLA₂ release during AMI

Cumulative release values (Q) of LD, between the onset of symptoms ($t=0$) and time t , indicated as $Q(t)$, were calculated as described [22,23] from the equation:

$$Q(t) = C(t) + \text{TER} \cdot \int_0^t e^{\text{ERR}(\tau-t)} \cdot C(\tau) d\tau + \text{FCR} \cdot \int_0^t C(\tau) d\tau$$

Where $C(t)$ is the plasma activity of the variable, TER the fractional transcappillary escape rate constant, ERR the fractional extra vascular return rate constant and FCR the fractional catabolic rate constant for the elimination of the variable from plasma. Values of $C(t)$ were obtained by subtraction of the normal steady-state plasma activity, C_s , from the actual measured activities. Values used for TER, ERR and FCR were, respectively, 0.014, 0.018 and 0.015 h. Infarct size was defined as $Q(t)$ calculated over 72 h for LD, that is $Q(72)$. $Q(72)$ was divided by the normal LD content of human myocardium, which is 220 U per gram of wet weight [24], to obtain infarct size in gram-equivalents of myocardium per litre of plasma (greq. l⁻¹). Following the same approach cumulative release of sPLA₂ was calculated from plasma sPLA₂ concentrations (ng l⁻¹). Values used for TER, ERR and FCR of sPLA₂ were 1.9, 0.94 and 14.1 h⁻¹, respectively, as estimated for equally sized and renally cleared proteins [25].

2.4. Processing of tissue specimens

Myocardial tissue specimens were obtained, within 12 h after the patient had deceased, from the infarcted as well as from the adjacent sites. These latter sites showed normal LD-staining patterns and were studied as internal controls. A total of 30 patients with signs of AMI at macroscopic evaluation (abnormal LD-staining pattern) were included; 20 males (average age of death 70.7 years) and 10 females (average age of death 75.6 years). Four patients without signs of AMI (normal LD-staining patterns at macroscopic evaluation) were also included. Before being prepared as cryo-sections, the tissue specimens were stored at -196°C (liquid N₂). The glass slides used for microscopy were pre-treated with 0.1% poly-L-lysine (Sigma Chemical Company, St. Louis, MO) to enhance adherence of the frozen tissue sections.

2.5. Antibodies and immunohistochemistry

Monoclonal antibody 5G4 (IgG-1 subclass) against CRP, has been previously used for immunohistochemical studies [14]. Monoclonal antibody 4A1 (IgG-1 subclass) is described above. A total of 4 μm thick frozen sections were mounted onto glass slides, dried for 1 h by exposure to air and fixed in acetone ('Baker Analysed Reagent', Mallinckrodt Baker BV, Deventer, Holland).

The slides were incubated at room temperature for 10 min with normal rabbit serum (Dakopatts A/S, Glostrup, Denmark) 1–50 diluted in phosphate buffered saline, pH 7.4, containing 1%, w/v, BSA (PBS-BSA) after a rinse in PBS.

Incubation of the slides with specific antibody solutions was performed for 60 min at room temperature (4A1 diluted 1:100 in PBS-BSA; 5G4 1:500 in PBS-BSA). In control experiments, similar incubations were performed with irrelevant monoclonal antibodies: IgG1 and mouse myeloma protein, MOPC (Cappel, Organon Teknika, Turnhout, Belgium). The slides incubated with antibodies against CRP and sPLA₂ were washed for 30 min with PBS and incubated with horseradish-peroxidase conjugated rabbit-anti-mouse immunoglobulins (RaM-HRP, Dakopatts A/S, Glostrup, Denmark), 1–25 diluted in PBS-BSA. Thereafter, the slides were washed again in PBS and incubated for 4 min in 0.5 mg/ml 3,3'-diamine-Benzidine-tetrahydrochloride (DAB, Sigma) in PBS containing 0.01%, v/v, H₂O₂, washed again, counterstained with hematoxylin for 40 s, dehydrated, cleared and finally mounted.

Microscopic criteria were used to estimate infarct duration in all myocardial tissue specimens [26,27]. General markers between 0 and 12 h after onset of AMI were beginning coagulation necrosis. General markers between 12 and 24 h after onset of AMI were, beginning PMN infiltration, between 1 and 3 days, massive interstitial PMN infiltration and coagulation necrosis. Between 3 and 5 days after onset of AMI, massive decay of PMN. A marker for the episode of 5–9 days after onset of AMI was the presence of a cell rich infiltrate of lymphocytes and especially macrophages. And between 9 and 14 days after onset of AMI the presence of prominent granulation tissue. Damaged fibres were characterized by the intensity of eosinophilic staining of involved myofibers, loss of nuclei and cross striation, infiltration of polymorphonuclear cells and lymphocytes, and presence of fibrosis. As morphological judgement is more reliable in paraffin slides, corresponding paraffin tissue slides were also made to confirm the ratio of jeopardized vs. non-jeopardized tissue. The proportion of cardiomyocytes positive for CRP and sPLA₂ was related to the area of decreased LD staining of affected myocardium.

Three independent investigators (H.W.M.N., W.K.L., R.N.) each judged and scored all slides for infarct duration and anatomical localization of specific antibody as visualized by immunohistochemical staining. For the final scoring results, consensus was achieved by the three investigators.

2.6. Comparative statistics

Data analysis was performed with SPSS for Windows version 9.0. As the data were not normally distributed, median and 25th–75th percentiles were used in the tables,

Table 1
sPLA₂ plasma levels (ng/ml) in patients with AMI or UAP

Time ^a	AMI (n=87) ^c			UAP (n=20) ^c			Odds ratio	95% CI
	Median	(25th–75th percentile)	No. of patients ^b	Median	(25th–27th percentile)	No. of patients ^b		
0	2.71	(1.50–4.70)	21	2.40	(1.20–2.80)	1	6.05	(0.76–47.67)
2	3.50	(1.80–7.10)	30	2.40	(1.55–3.05)	2	4.73	(1.02–21.78)
6	3.20	(1.20–6.30)	27	2.12	(1.04–2.71)	2	1.13	(0.96–1.33)
12	3.90	(1.90–7.40)	38	2.45	(1.68–3.20)	3	4.39	(1.11–16.09)
24	6.00	(2.60–12.00)	52	2.50	(1.69–3.94)	4	1.13	(1.00–1.278)
48	9.14	(4.14–19.00)	62	2.70	(1.71–5.30)	7	1.20	(1.09–1.37)
72	8.14	(3.85–16.50)	55	2.80	(1.57–6.30)	5	1.21	(1.04–1.48)

^a Time refers to hour after admission.

^b Refers to number of patients with an elevated level of sPLA₂ (>5 ng/ml), compared to the control group of healthy volunteers.

^c AMI Acute Myocardial Infarction, UAP Unstable Angina Pectoris.

and in graphs mean±S.E.M. to represent the data. To represent data distribution over the different groups Odd's ratios (25th–75th percentiles) were calculated. To evaluate differences between the groups Kruskal Wallis tests were performed. Logistic regression analysis was applied to identify factors associated with elevation of sPLA₂ above normal levels. Statistical significance was defined as a value of $P < 0.05$.

3. Results

3.1. Plasma studies

To evaluate its course during AMI, sPLA₂ levels were measured in plasma samples from patients with AMI and compared to those in patients with UAP (Table 1). On

admission, 21 of the 87 AMI patients had elevated sPLA₂ vs. 1 of the 20 patients with UAP. The difference in plasma sPLA₂ between either patient group was significant from $t = 12$ h after admission on.

The time elapsed from the onset of AMI, i.e. the time between the onset of clinical symptoms and time of admission to the hospital varies from one patient to another. Hence, alignment of results to the time of admission will not represent similar phases in AMI. We therefore aligned these results, by calculating in a 2-compartment model, the cumulative release values, according to the onset of the AMI, i.e. the time that patient had noted the first clinical symptoms. The aligned median cumulative levels of sPLA₂ were significantly higher in the AMI group than those in the UAP group. The differences between the groups already were significant from $t = 6$ h, up to $t = 72$ h (Fig. 1). The increase in the cumulative

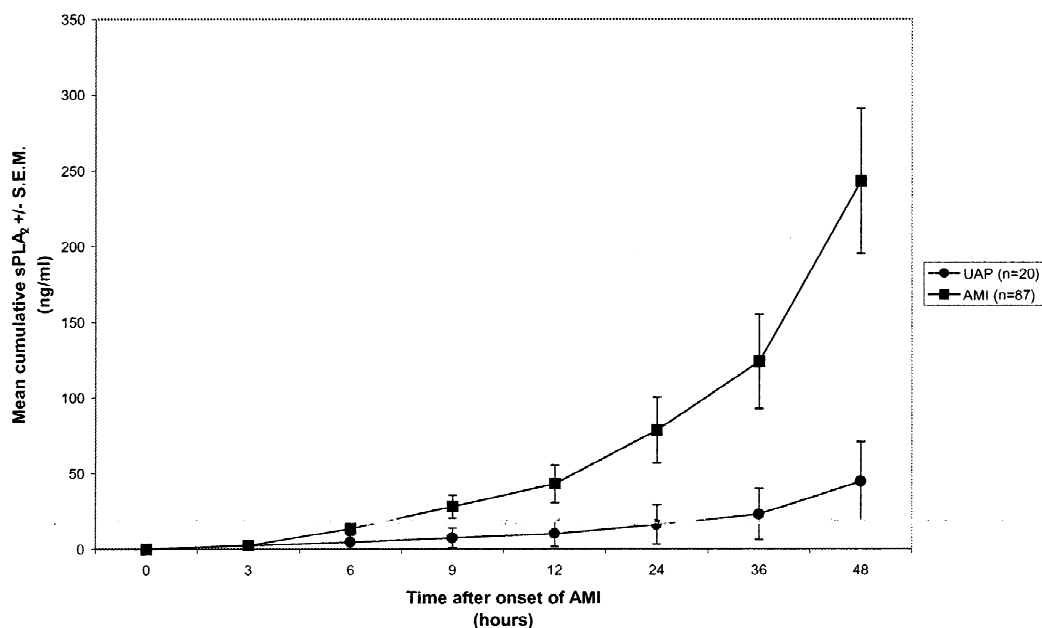


Fig. 1. Mean cumulative sPLA₂ plasma levels in patients with AMI vs. UAP with standard error of mean. The differences between UAP and AMI are significant from 6 h and on ($P < 0.02$).

Table 2

Rise in mean cumulative sPLA₂ in patients with AMI vs. UAP with standard error of mean and significance according to 'Kruskall Wallis'

Time episode (h)	AMI (n=87)		UAP (n=20)		P value
	Mean	S.E.M.	Mean	S.E.M.	
0–12	43.1	12.4	10.2	8.8	0.020
12–24	35.4	9.9	5.9	4.2	0.003
24–36	45.3	10.0	6.9	4.0	0.000
36–48	119.4	18.4	21.7	9.4	0.000
48–72	444.8	302.6	28.6	8.9	0.001

sPLA₂ levels during each period was also significantly higher in the AMI group compared to the UAP group in any of the time episodes (Table 2).

CRP was measured as well in the same plasma samples. A detailed analysis of these data will be described elsewhere (manuscript in preparation). The cumulative levels of CRP in the AMI group were significantly higher than those in the UAP group from $t=12$ ($P<0.04$) on (Fig. 2). The rise of cumulative CRP levels during each period was also higher in the AMI group compared to the UAP group in all evaluated time episodes (Table 3). Comparison of the cumulative plasma sPLA₂ values with those of CRP revealed that the increase of sPLA₂ preceded that of CRP in plasma of patients with AMI (see also Figs. 1 and 2).

By means of multi-variant analysis it was observed that, for both the UAP and AMI group, no significant influence on elevation of sPLA₂ levels was found for the method of therapy (thrombolysis vs. PTCA) after admission, nor for usage of Aspirin, Calcium-antagonists or Nitrate at the moment of admission. In the AMI group usage of β -Blockers (OR 8.1, 95% CI 1.0–64.4) was associated with elevated levels of sPLA₂.

Table 3

Rise in mean cumulative CRP in patients with AMI vs. UAP with standard error of mean and significance according to 'Kruskall Wallis'

Time episode (h)	AMI (n=87)		UAP (n=20)		P value
	Mean	S.E.M.	Mean	S.E.M.	
0–12	9.9	1.9	5.7	2.9	0.004
12–24	25.8	3.2	4.6	1.9	0.000
24–36	35.1	5.4	8.3	2.3	0.000
36–48	32.4	3.9	10.1	2.2	0.001
48–72	59.0	9.8	15.6	3.5	0.011

3.2. Tissue studies

By immunohistochemistry, we found evidence for localisation of sPLA₂ in human infarcted myocardium (Fig. 3). Most intense staining for sPLA₂ was found in the borderzone of the infarcted myocardium. When looking at (sub)-cellular structures in more detail, sPLA₂ was found to bind to the plasma membrane, though the cross striation of the myofibres and foci in the cytoplasm were also partly stained for sPLA₂. Depositions of sPLA₂ was also detected in the smooth muscle cells of intramyocardial arteries. In a

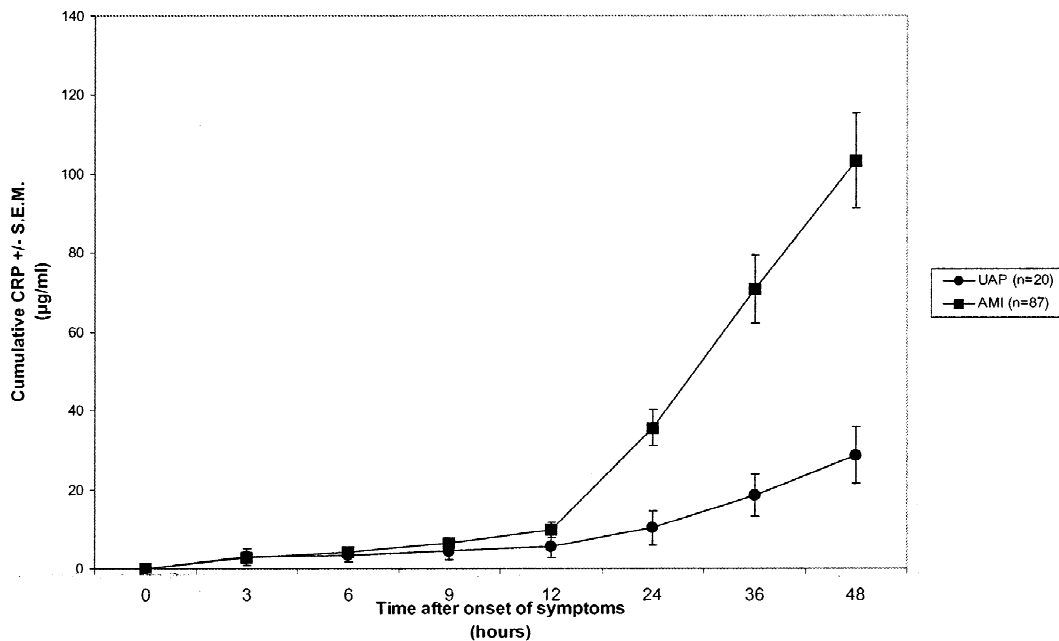


Fig. 2. Mean cumulative CRP plasma levels in patients with AMI vs. UAP with standard error of mean. The differences between UAP and AMI are significant from 12 h and on ($P<0.04$).



Fig. 3. Positive staining of myofiber elements (sarcolemma and cytoplasm) in infarcted myocardium for secretory Phospholipase A₂ (mAb 4A1) (magnification $\times 400$).

previous study we have shown that also CRP binds to infarcted human myocardium [14]. However, in contrast to CRP, sPLA₂ was also found to bind to morphologically normal cardiomyocytes, adjacent to the infarcted area (Fig. 4). The results of the immunohistochemical studies are summarized in Tables 4 and 5. Table 4 shows that 1 out of 14 patients with an infarct age of less than 12 h stained positive for sPLA₂ and CRP. However, the area staining positive in this patient was relatively low being 5–10%. More extensive localization of sPLA₂ was found in two of the three infarcts of 12–24 h. CRP appeared in infarcts of more than 24 h (Table 4). In general, the area of the infarct containing sPLA₂ was larger than that where CRP was localized (Table 5).

Staining of the myocardial tissue specimens with control antibodies yielded negative results. Internal controls, i.e. specimens taken from non-infarcted sites of the myocardium of the same patient — usually the right ventricle wall — did not stain for either CRP nor for sPLA₂. Finally, myocardial tissue of three patients with no signs of AMI did not stain for CRP or sPLA₂, nor did one sample of fetal cardiac tissue, the latter to be regarded as a pure, non-ischemic tissue control. Hence, the results obtained with sPLA₂ and CRP were considered to specifically reflect localisation of these proteins in the infarcted tissue.

4. Discussion

Phospholipase A₂ (PLA₂) hydrolyses the sn-2-ester bond of phospholipids to produce free fatty acids and lysophospholipids [9]. In doing so these enzymes catalyse

the rate limiting step in the formation of several pro-inflammatory lipid mediators [10]. Here we report an early elevation of type II PLA₂ (further denoted as sPLA₂) in plasma of patients suffering from acute myocardial infarction (AMI).

These plasma levels started to increase at 6 h after onset of AMI. Leong et al. and Langton et al. have reported an increase of PLA₂ activity at a later episode in plasma of patients suffering from AMI, respectively at 2–4 days and 24–36 h after onset of symptoms [28,29]. Calculation of the cumulative release values allowing for the alignment of our results to the onset of symptoms of AMI [22,23], presumably explains why we could detect significant elevations of sPLA₂ at an earlier stage. Our results of sPLA₂ in the UAP group concur with those of the study of Kugiyama et al. [8], although Kugiyama did not study elevation of sPLA₂ in AMI patients.

Significant localization of sPLA₂ in the ischemic myocardium started at 12–24 h after the onset of complaints, i.e. at a time that the increase of plasma levels already had begun. These depositions of sPLA₂ together with reported depositions of sPLA₂ in rheumatoid arthritis and atherosclerosis [30,31] point to a role for sPLA₂ in local inflammatory reactions.

Theoretically sPLA₂ fixed to the ischemic myocardium may have originated from cardiomyocytes. Considering the ischemic conditions of these cells we do not consider this explanation likely, the more since sPLA₂ depositions apparently occurred at a time that plasma levels of this enzyme were rising. sPLA₂ also can be synthesized by many other cells including endothelial cells, fibroblasts, smooth muscle cells and HepG2 cells, a hepatocarcinoma

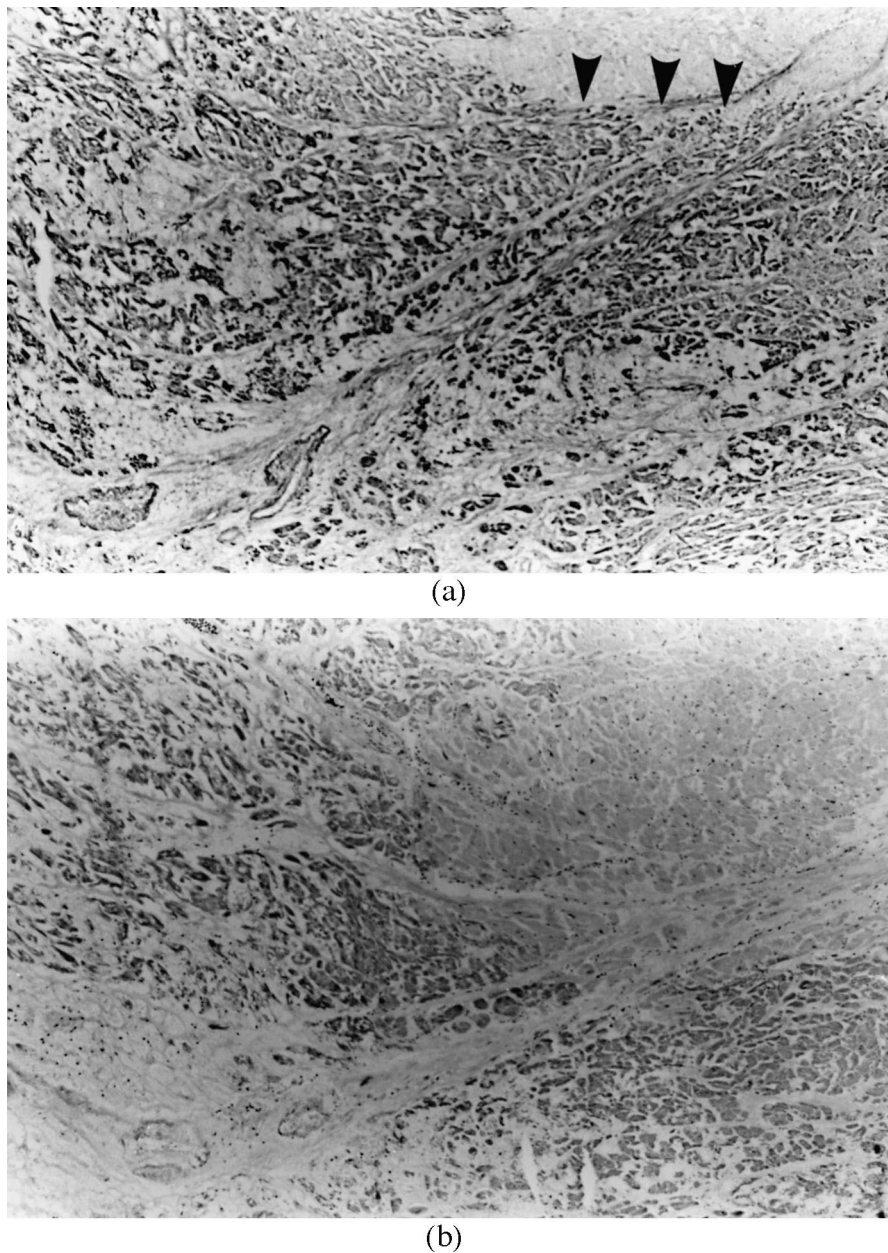


Fig. 4. A/B Localisation of secretory Phospholipase A₂ (A, mAb 4A1) and C-reactive protein (B, mAb 5G4) in infarcted myocardium. Notice that the areas of both stainings overlap, though sPLA₂ occupies a larger area (arrows).

cell-line considered to represent an in-vitro model to study the regulation of acute phase protein synthesis [32–35]. Hence, sPLA₂ in the ischemic myocardium may have originated from the blood that, as a result of the ensuing acute phase response, contained increasing levels of this enzyme or is locally released from blood vessels.

During acute myocardial infarction (AMI) the acute phase response is induced together with the activation of the complement system [1,2]. The acute phase protein CRP is able to activate this complement system after binding to a suitable ligand [21,36,37]. Recently, we found co-localisation of activated complement and CRP in myocardial tissue of patients with AMI, suggesting involvement of

Table 4
Immunohistochemical positive staining for CRP and sPLA₂ in patients with AMI

Infarct age	Total no. of patients each period	No. of patients with positive staining for	
		CRP	sPLA ₂
0–12 h	14	1	1
12–24 h	3	0	2
1–3 days	4	4	4
3–5 days	2	2	2
5–9 days	3	3	3
9–14 days	4	4	4
Total no. of patients	30		

Table 5
Area occupied by CRP in case of positive sPLA₂ staining

Infarct age	Number of patients with staining of CRP and/or sPLA ₂	Amount of patients in which the area of staining by sPLA ₂ is larger (>), equal (=) or smaller (<) than the area of CRP		
		Area sPLA ₂ >	Area sPLA ₂ =	Area sPLA ₂ <
0–12	1	–	1	–
12–24	2	2	–	–
1–3 days	4	3	1	–
3–5 days	2	1	1	–
5–9 days	3	2	1	–
9–12 days	4	3	–	1
Total no. of patients	16	11	4	1

CRP in the activation of complement ensuing locally in the infarcted myocardium [14]. CRP has the ability to bind to products of sPLA₂ activity, namely lysophospholipids [16,17]. The presence of lysophospholipids has been demonstrated in infarcted myocardium [38]. Therefore, we have hypothesized that the presence of lysophospholipids, generated from phospholipids by cPLA₂ or sPLA₂, may be involved in the formation of suitable ligands for CRP in the membranes of reversibly injured cardiomyocytes [18]. Subsequent activation of complement may then trigger inflammatory reactions in the ischemic myocardium. In agreement herewith, in this study we describe a significant elevation of circulating sPLA₂ (present at 6 h after AMI) preceding that of CRP (present at 12 h), in patients with AMI. Furthermore we found depositions of sPLA₂ to be more extensive than those of CRP. sPLA₂ depositions occurred partly in areas adjacent to the infarcted area, which contained cardiomyocytes with a morphology that appeared to be normal, implying that the apparently normal cardiomyocytes of the areas adjacent to the infarction that bound sPLA₂, presumably were not healthy but may represent a reversibly injured population. Altogether, our findings strengthens our hypothesized role of sPLA₂ as an inducer of ligands of CRP, and as an enhancer of inflammation.

In conclusion, we show a rise of circulating levels of sPLA₂ in patients with AMI, and depositions of this enzyme in ischemic myocardium. Hence, we suggest that sPLA₂ participates in the local inflammatory reactions ensuing in infarcted myocardium, for example by generating binding sites on cardiomyocytes for CRP, which subsequently triggers complement activation.

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