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INCREASED SERUM PHOSPHOLIPASE A2 ACTIVITY IN ADVANCED CHRONIC LIVER DISEASE AS AN EXPRESSION OF THE ACUTE PHASE RESPONSE

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SUMMARY

Phospholipase A2 (PLA2) modifications were investigated in patients with acute and chronic liver diseases, PLA2 variations were related to indices of liver function as well as to parameters of the acute phase response. Serum PLA2 activity modifications were fluorimetrically measured in 105 patients affected by acute and chronic liver diseases or extra-hepatic diseases. One-way ANOVA demonstrated a significant difference among groups ($F = 4.53$, $P<0.001$); Bonferroni's test for pairwise comparisons showed that patients with hepatocellular carcinoma had higher mean values than subjects with benign extra-hepatic diseases ($P<0.01$) and mild chronic liver disease ($P<0.05$). Multiple regression analysis, performed choosing PLA2 as the dependent variable and blood urea nitrogen, C-reactive protein, alkaline phosphatase and α1-fetoprotein as predictor variables was significant (multiple $R = 0.7056$, multiple $R2 = 0.4978$, $F = 15.36$, $P = <0.0001$). The standardized regression coefficients found to be significant were those of C-reactive protein, blood urea nitrogen and α1-fetoprotein. In conclusion, in patients with chronic liver disease, serum PLA2 activity increases parallel to disease severity and accompanies the expression of proteins of the acute phase response that, like PLA2 activity, increase in serum while liver synthesis declines.

KEY WORDS Phospholipase A2 Acute phase response Liver disease Hepatocellular carcinoma

INTRODUCTION

Pancreatic phospholipase A2 (PLA2; EC3.1.1.4) is a protein physiologically secreted as a proenzyme by the exocrine pancreas in response to secretagogue stimuli. It is transformed into the active form in the duodenal juice by the action of trypsin (Mansbach, 1990). Its premature activation in the pancreas may play a role in the pathogenesis of acute pancreatitis and in some of its related systemic complications (Das *et al.*, 1987; Nevalainen, 1980). In fact, high levels of this enzyme have been measured in sera of patients with acute pancreatitis and they have been related to a poor outcome (Funakoshi *et al.*, 1991; Kazmierczak *et al.*, 1991; Kitagawa *et al.*, 1991; Mäkelä *et al.*, 1990; Matsuda *et al.*, 1986; Nevalainen *et al.*, 1985).

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Moreover, high serum PLA2 levels have been associated with a variety of clinical conditions, both benign and malignant, and with renal failure (Basso *et al.*, 1990; Funakoshi *et al.*, 1991; Kazmierczak *et al.*, 1991; Kitagawa *et al.*, 1991; Oka *et al.*, 1990). Since PLA2 has a low molecular weight and is ultrafiltered by the renal glomeruli, the high levels measured in renal failure can be easily explained. In other clinical situations, not characterized by abnormality in renal function, some authors hypothesized the presence of sub-clinical pancreatic involvement, as is the case, for example, in colelithiasis (Basso *et al.*, 1990; Kitagawa *et al.*, 1991). However, the ability to synthetise PLA2 is not exclusive to the pancreas and PLA2 liberation occurs from both neoplastic and normal tissues other than the pancreas (Crowl *et al.*, 1991; Grönroos and Nevalainen, 1992). Interestingly, it appears that in cancer patients high serum PLA2 levels can be detected in the absence of evidence of actual production by the tumour and therefore may be part of the array of responses by the organism when cancer develops (Lukas *et al.*, 1992; Oka *et al.*, 1990).

Recent studies indicated that high PLA2 levels may be encountered in patients with chronic liver disease and hepatocellular carcinoma (Funakoshi *et al.*, 1991; Oka *et al.*, 1990). However, there is currently no clear pathophysiological explanation for such findings in the setting of the whole spectrum of liver diseases (including acute hepatitis). The aim of this study was therefore to investigate PLA2 modification in a group of patients with acute and chronic liver diseases, and to relate PLA2 variations to established indices of liver function and to parameters of the acute phase response.

MATERIALS AND METHODS

Patients

We studied a total of 105 patients referred to our institution for complete diagnostic work-ups. Eight patients were affected by acute hepatitis (7 male and 1 female; mean age±standard deviation 52.1±18.7, range 19–70); etiology of the disease was viral (hepatitis B and hepatitis D virus coinfection) in one case, alcoholic in 5, and drug-induced in 2. Twenty-seven patients had mild chronic liver diseases (18 male, 9 female, age 54.2±12.1, range 26–72). In detail: 5 patients had chronic persistent hepatitis related to hepatitis B virus (HBV) infection in one case and to hepatitis C virus (HCV) infection in the others; 15 patients had chronic active hepatitis related to HBV infection in 6 cases, to HCV infection in 8 and cryptogenic in one; 7 patients had liver steatofibrosis: the 4 male patients had a history of elevated ethanol consumption while the 3 female were obese. Twenty-six patients had liver cirrhosis (15 male and 11 female; age 56.7±11.4, range 36–76), that was alcoholic in origin in 18, due to hepatitis virus infection in 7 (1 HBV, 6 HCV), and autoimmune (primary biliary cirrhosis) in 1. Twenty-five had hepatocellular carcinoma (23 male and 2 female; age 61.4±13.5, range 33–79); all of them developed neoplasia on a cirrhotic liver; three patients were HBsAg positive, twelve were anti HCV positive, and ten had a history of chronic alcohol consumption. Six patients had malignant extra-hepatic disease (5 male and 1 female; age 62.7±1.7, range 60–64): 4 had adenocarcinoma of the pancreas, 1 carcinoma of the esophagus,and 1 adenocarcinoma of the colon; three of these patients had metastasis to the liver. Thirteen patients had different benign extra-hepatic disease (8 male and 5 female; age 48.8±20.2, range 15–79): in detail, diagnoses were mesenteric vein thrombosis, benign stenosis of the main bile duct, sideroblastic anemia, aortic valve insufficiency,

cystinuria, extracapillary glomerulonephritis, type I diabetes mellitus, celiac disease, colelithiasis, epilepsy, hyperlipidemia, varicocele, hypertension. Diagnoses of chronic persistent and chronic active hepatitis, liver steatofibrosis, primary biliary cirrhosis and extra-hepatic malignancy were all confirmed histologically and/or at autopsy. Cirrhosis was diagnosed clinically on the basis of evidence of portal hypertension, ascites, hypoalbuminemia, hyergammaglobulinemia and confirmed histologically in the majority of patients. Hepatocellular carcinoma was diagnosed in the presence of raised (>400 ng/ml) serum levels of alpha-1-fetoprotein and/or suggestive radiological imaging; it was always confirmed histologically or at autopsy. Acute hepatitis was diagnosed clinically, and confirmed histologically in seven patients out of eight; extra-hepatic diseases were each diagnosed on the basis of accepted diagnostic criteria.

Biochemical determinations

Serum pancreatic phospholipase A2 activity was measured by means of a fluorimetric method (Thuren *et al.*, 1985) in fasting serum samples, stored at –20°C for a maximum of 4 months. Serum alpha-1-fetoprotein was measured by a radioimmunometric method. Serum alpha-1-antitrypsin was determined nephelometrically. All other biohumoral parameters were measured by means of commercial kits.

Statistical analysis

One-way analysis of variance was applied to detect differences in the population means with regard to PLA2 activity. Bonferroni's test was used for multiple comparisons among the groups. One-way analysis of covariance was used in order to exclude the possibility that variations of renal function (measured by blood urea nitrogen) might exert a decisive influence on PLA2 activity detected in serum. To test the existence of differences among the groups in regard to categorical variables (such as normal or pathological PLA2 activity), Pearson chi-square test was performed. All possible subset regression analysis permitted us to choose, among a set of parameters, the best subset able to predict PLA2 activity modifications; then, multiple regression analysis was utilized to correlate PLA2 activity variations with variations in the parameters so identified. When appropriate, results of PLA2 activity, alpha-1-fetoprotein and C-reactive protein determinations were transformed logarithmically, due to their scattered distribution. All statistical tests were performed by means of the BMDP™ statistical software package (Dixon *et al.*, 1990).

RESULTS

In Table 1 we report mean values ± SD of serum PLA2 activity, C-reactive protein (CRP), albumin, total bilirubin, alkaline phosphatase and blood urea nitrogen (BUN) detected in the study population. Figure 1 shows the logarithmically transformed individual values of PLA2 (LogPLA2) in the various groups of patients studied. The analysis of variance demonstrated a significant difference among groups; patients with hepatocellular carcinoma had higher mean values than patients with benign extra-hepatic diseases and those with mild chronic liver diseases. A value of 18 pmol $min^{-1}ml^{-1}$ (representing the upper limit of roughly 95% of patients with benign extrahepatic diseases and mild forms of chronic liver disease) was chosen as the cut-off limit between normal and pathological PLA2 levels. Chi-square test demonstrated a significant

Table 1. Mean values ± Standard Deviation of PLA2 activity, C-reactive protein (CRP), albumin, alkaline phosphatase (ALP), total bilirubin and blood urea nitrogen (BUN) in the studied population

	PLA2 activity pmol min-^{1} ml-^{1}	CRP mg/l	Albumin g/dl	ALP U/L	Bilirubin mg/dl	BUN mg/dl
Acute Hepatitis	16.6 ± 7.7	12.3 ± 11.8	2.72 ± 0.64	145 ± 33	13.3 ± 9.5	25.7 ± 17.5
Chronic Liver Disease	12.3 ± 3.2	4.7 ± 8.7	4.10 ± 0.47	95 ± 43	0.9 ± 0.3	16.2 ± 4.6
Liver Cirrhosis	16.2 ± 8.0	10.6 ± 8.5	3.39 ± 0.69	174 ± 166	2.4 ± 1.6	18.8 ± 10.0
Hepatocellular Carcinoma	22.2 ± 23.1	33.8 ± 46.1	3.22 ± 0.60	248 ± 233	2.6 ± 2.2	24.9 ± 11.1
Extra-hepatic Malignancies	19.8 ± 8.3	46.9 ± 42.6	3.21 ± 0.73	297 ± 148	3.0 ± 3.1	41.7 ± 28.6
Extra-hepatic Benign Diseases	10.4 ± 5.3	5.1 ± 7.3	3.89 ± 0.53	105 ± 50	0.6 ± 0.2	21.3 ± 9.3

deviation of observed from expected frequency of PLA2 pathological values in the various groups of patients (Pearson chi-square test 16.7, P = 0.005): the highest frequencies of pathological values were found in subjects with malignancies. We found a significant correlation between LogPLA2 and blood urea nitrogen (BUN) (R = 0.389, P<0.001); alpha-1-antitrypsin (R = 0.323, P = 0.012); cholinesterase (R= –0.383, P<0.001); albumin (R = –0.254, P = 0.024); alkaline phosphatase (ALP) (R = 0.263, P = 0.011); and the logarithmically transformed values of alpha-1-fetoprotein (LogAFP) (R = 0.399, P<0.001) and of CRP (LogCRP) (R - –0.514, .P<0.001). No correlation was observed between LogPLA2 and AST, gamma-glutamyltransferase, white blood cell count, erythrocyte sedimentation rate and total bilirubin. By all possible subset regression analyses, we identified among the above mentioned parameters the best subset able to predict PLA2 activity variations: it included BUN, LogCRP, LogAFP and ALP (Mallow's CP = 4.01). Table 2 describes the results of multiple regression analysis performed choosing LogPLA2 as the dependent variable and BUN, ALP, LogCRP and LogAFP as predictor variables. The analysis was highly significant; among the standardized partial regression coefficient, those of LogCRP, LogAFP and BUN were found to be significant. Analysis of covariance (one-way ANCOVA) was performed choosing LogPLA2 as dependent variable and BUN as concomitant variable. The one-way ANCOVA confirmed the significance given by the one-way ANOVA even when PLA2 data were adjusted for the covariate BUN (F = 3.93, P<0.005).

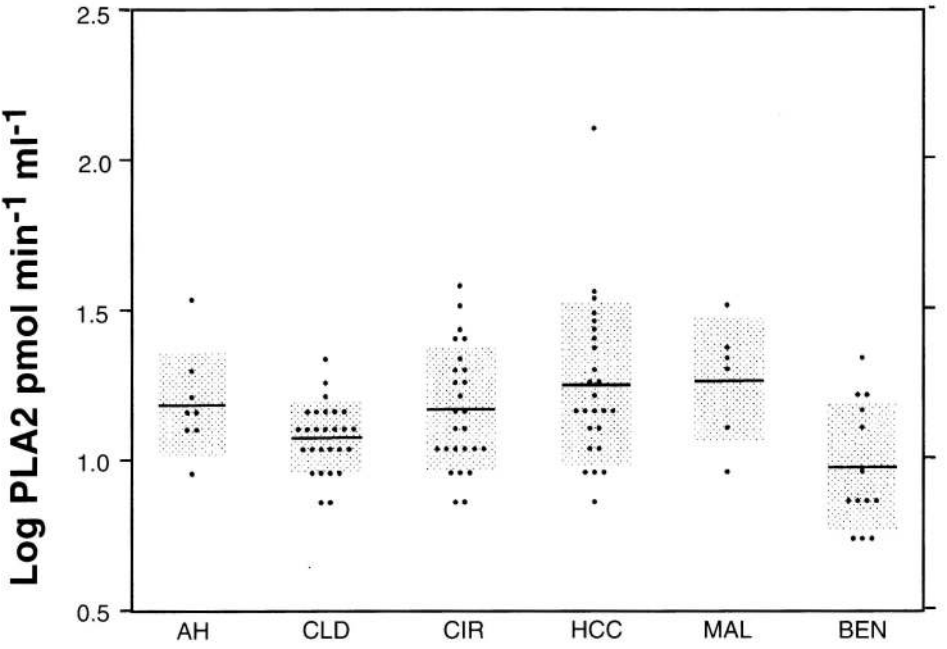

Figure 1. Individual values (logarithmically transformed) of serum PLA2 activity in the studied patients grouped according to diagnosis. Bars and shaded areas represent mean ± standard deviation. One-way analysis of variance: F = 4.53, P<0.001; Bonferroni's test for pairwise comparisons: P<0.01 for HCC Vs BEN, P<0.05 for HCC Vs CLD. *AH*: acute hepatitis; *CLD*: mild chronic liver diseases; *CIR*: cirrhosis; *HCC*: hepatocellular carcinoma; *MAL*: extra-hepatic malignant diseases; *BEN*: extra-hepatic benign diseases; *PLA2*: phospholipase A2.

DISCUSSION

PLA2 represents a heterogenous group of enzymes that can determine hydrolysis of cell membrane phospholipids. Through this process PLA2 produces a number of cytotoxic products such as lysophospholipids and releases free fatty acids like arachidonic acid. These substances in turn act as precursors of biologic mediators such as prostaglandins and tromboxanes (Flower and Blackwell, 1976; Kuehl and Egan, 1980). By immunological methods it is possible to distinguish at least two groups of secretory PLA2 which are functionally and structurally distinct (Kortesuo *et al.*, 1992; Nevalainen *et al.*, 1992). Group I PLA2 is mainly represented by pancreatic phospholipase, while Group II PLA2 derives from several tissues, including liver, spleen, lung and kidney. The pH profiles of Group I and Group II differ (Hoffmann *et al.*, 1992), and PLA2 catalytic activity measured at a pH around 7.5 (as in the method we used) parallels Group II PLA2 concentration measured immunologically. Therefore, we think that in our study we dealt mainly with variations in serum PLA2 activity derived from Group II PLA2.

We found an increase of PLA2 activity in patients with hepatocellular carcinoma as compared to subjects with extra-hepatic benign diseases and mild chronic liver disease.

Table 2. Multiple Regresssion Analysis performed choosing PLA2 activity as dependent variable and α1-fetoprotein (APF), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and C-reactive protein (CRP) as predictor variables. Values of PLA2 activity, AFP and CRP were transformed logarithmically.

Multiple R	0.7056	
Multiple R ^{2}	0.4978	
F	15.36	
P	<0.0001	
variables	Std. Regr. Coefficients	P
AFP	0.24	0.02
ALP	0.15	0.12
BUN	0.28	0.00
CRP	0.40	0.00

Furthermore, the highest frequencies of PLA2 activity pathological values were found in patients with malignancies either hepatic (36.0% of the total of this group) or extra-hepatic (66.7% of the total of this group). These data confirm previously published observations (Funakoshi *et al.*, 1991; Oka *et al.*, 1990): a) PLA2 serum levels increase not exclusively in patients with pancreatic diseases but in a variety of clinical conditions; b) high levels can be detected in particular in a relatively large proportion of patients with cancer. In effect, PLA2 activity correlated particularly well with AFP serum levels both in single and multiple regression analyses. However, the considerable overlap found among groups precludes at present any clinical usefulness of PLA2 as a tumor marker. Liberation of PLA2 is nevertheless an expression of pathophysiological phenomena of great interest. Is there a common mechanism through which liberation of PLA2 occurs, linking together apparently heterogenous conditions? Is it expression of the severity of a disease or of the intensity of the inflammatory process or is it due to the neoplastic burden? We focused our attention mainly on chronic liver diseases. They are characterized in their natural history by a down-hill progression towards deterioration of functions, frequently ending in the development of neoplasia. This often happens through a slow relenting course interspersed with flares of activity. Accordingly, the frequency of pathological values of PLA2 activity increases from low in the milder forms of liver disease to high in the severe ones (cirrhosis, hepatocellular carcinoma). Decline of liver synthesis measured by albumin and cholinesterase levels parallels the increase

in PLA2 activity. Therefore, we might suppose that PLA2 activity increases because of lack of putative inhibitors synthetized by the liver; however, even if there is evidence for the existence of substances with inhibitory activity on PLA2, their physiological role is uncertain at present (Northup *et al.*, 1988). Recently, it has been suggested that albumin could provide a protective buffer against small increases in PLA2 activity (Langton & Dench, 1991). However, most evidence favours a stimulatory rather than inhibitory effect of albumin on PLA2 activity itself (Fauvel *et al.*, 1987). In our patients, cholinesterase had a stronger negative correlation than albumin with PLA2 activity; moreover patients with acute hepatitis had the lowest mean albumin value but did not show a substantial increase in PLA2 activity.

We found no correlation between PLA2 activity and indices of hepatocellular cytonecrosis or serum total bilirubin. Similarly, no significant correlation was observed between PLA2 activity and indices of systemic inflammation such as total white blood cell count and erythrocyte sedimentation rate. Instead, PLA2 activity increased in parallel with proteins (like alpha-1-antitrypsin and CRP) liberated during the acute phase response and produced by the liver after cytokine stimulation (Arai *et al.*, 1990; Heinrich *et al.*, 1990; Snick, 1990). Elevated PLA2 activity in liver disease might therefore have a different basis where the progression of chronic liver disease towards hepatocellular carcinoma might be characterized by activation of cytokine-mediated responses. In fact, it has been observed that endotoxinaemia is frequently detectable in advanced chronic liver disease and this might lead to cytokine liberation by the reticulo-endothelial system (Beutler & Cerami, 1988; Fong *et al.*, 1989; Nolan, 1981). Moreover, there are several observations that PLA2 increases in septic shock both in humans and in animal models, and it has been suggested that endotoxin-induced interleukin-1 and tumor necrosis factor are the proximal stimuli for PLA2 synthesis and active secretion (Crowl *et al.*, 1991; Vadas & Pruzanski, 1990).

We noticed a significant positive correlation in our data between BUN and PLA2, thus suggesting that high PLA2 activity may be accounted for by impaired renal clearance. Since patients with advanced liver disease often have associated renal failure, we wondered whether all variation observed in serum PLA2 activity could be explained by this finding alone. However, analysis of covariance, performed choosing BUN as concomitant variable, confirmed the significant difference among groups found by ANOVA, permitting us to rule out renal dysfunction as the major determinant of PLA2 activity variations observed.

In conclusion, in patients with chronic liver disease, serum PLA2 activity increases in parallel with severity; it accompanies the expression of proteins of the acute phase response while liver synthesis declines.

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