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B S Vishwanath, … , J Reichen, B M Frey

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Maintenance of renal function in liver cirrhosis requires increased synthesis of arachidonic acid derived prostaglandin metabolites. Arachidonate metabolites have been reported to be involved in modulation of liver damage. The purpose of the present study was to establish whether the first enzyme of the prostaglandin cascade synthesis, the phospholipase A2(PLA2) is altered in liver cirrhosis induced by bile duct excision. The mRNA of PLA2(group I and II) and annexin-I a presumptive inhibitor of PLA2 enzyme was measured by PCR using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal standard. The mean mRNA ratio of group II PLA2/GAPDH was increased in liver tissue by 126% (P < 0.001) and in kidney tissue by 263% (P < 0.006) following induction of liver cirrhosis. The increase in group II PLA2 mRNA in cirrhotic animals was reflected by an increase in PLA2 protein and enzyme activity in both liver and kidney tissues. Since the mRNA of group I PLA2 was not detectable and Group IV PLA2 activity measured in liver and kidney tissue samples was very low and not changed following induction of cirrhosis, it is likely that the major PLA2 activity measured in liver and kidney corresponds to group II PLA2 enzyme. The mean mRNA ratio of annexin-I/GAPDH was increased in liver tissue by 115% (P < 0.05) but unchanged in kidney tissue following […]

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Liver Cirrhosis Induces Renal and Liver Phospholipase A\textsubscript{2} Activity in Rats

Bannikuppe S. Vishwanath,* Felix J. Frey,* Geneviève Escher,† Jürg Reichen,‡ and Brigitte M. Frey*

*Division of Nephrology, Department of Medicine, and †Department of Clinical Pharmacology, University of Berne, 3010 Berne, Switzerland

Abstract

Maintenance of renal function in liver cirrhosis requires increased synthesis of arachidonic acid derived prostaglandin metabolites. Arachidonate metabolites have been reported to be involved in modulation of liver damage. The purpose of the present study was to establish whether the first enzyme of the prostaglandin cascade synthesis, the phospholipase A\textsubscript{2}(PLA\textsubscript{2}) is altered in liver cirrhosis induced by bile duct excision. The mRNA of PLA\textsubscript{2} (group I and II) and annexin-I a presumptive inhibitor of PLA\textsubscript{2} enzyme was measured by PCR using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal standard. The mean mRNA ratio of group II PLA\textsubscript{2}/GAPDH was increased in liver tissue by 126% (P < 0.001) and in kidney tissue by 263% (P < 0.006) following induction of liver cirrhosis. The increase in group II PLA\textsubscript{2} mRNA in cirrhotic animals was reflected by an increase in PLA\textsubscript{2} protein and enzyme activity in both liver and kidney tissues. Since the mRNA of group I PLA\textsubscript{2} was not detectable and Group IV PLA\textsubscript{2} activity measured in liver and kidney tissue samples was very low and not changed following induction of cirrhosis, it is likely that the major PLA\textsubscript{2} activity measured in liver and kidney corresponds to group II PLA\textsubscript{2} enzyme. The mean mRNA ratio of annexin-I/GAPDH was increased in liver tissue by 115% (P < 0.05) but unchanged in kidney tissue following induction of cirrhosis. The protein content of annexin-I and -V were not affected by bile duct excision in liver and kidney tissue indicating that upregulation of group II PLA\textsubscript{2} activity was not due to downregulation of annexin-I or -V. Group II PLA\textsubscript{2} activity of glomerular mesangial cells stimulated by interleukin-1\textgamma was enhanced by bile juice and various bile salts. In conclusion, activity of group II PLA\textsubscript{2} is upregulated partly due to enhanced transcription and translation in cirrhosis and is furthermore augmented by elevated levels of bile salts. (J. Clin. Invest. 1996, 98:365–371.) Key words: phospholipase A\textsubscript{2} • annexins • cirrhosis • mesangial cells • bile salts

Introduction

Cirrhotic patients have a cardiovascular disturbance characterized by low arterial pressure, high cardiac output, and increased plasma volume (1–4). Besides arteriolar vasodilation and arteriovenous shunting, these patients exhibit a diminished sensitivity to pressor hormones and impaired hypoxic vasoconstriction (5–7). Eventually liver failure causes extrahepatic organ dysfunction such as hepatorenal or hepatopulmonary syndrome (8–10). These changes resemble those seen in patients or animals with septicemia (11–14).

Changes in arachidonic acid derived prostaglandins have been linked to hemodynamic alterations in cirrhotic disease states (11, 15–19). Renal perfusion and glomerular filtration are only maintained through the vasodilatory effect of prostaglandins. Inhibition of the cyclooxygenase enzyme results in a reduction of the effective renal plasma flow and sodium retention (20, 21) and administration of exogenous prostaglandin E derivatives enhance renal function of patients with cirrhosis (22). One of the rate limiting steps in the synthesis of prostaglandins is the release of arachidonic acid from membrane phospholipids by the enzyme phospholipase A\textsubscript{2} (PLA\textsubscript{2}). In contrast to the abundant literature about prostaglandins in liver cirrhosis, the relevance of PLA\textsubscript{2} has never been addressed. Therefore, in the present investigation we report the effect of biliary cirrhosis induced by bile duct ligation in rats on mRNA, protein levels and enzyme activity of group II PLA\textsubscript{2}. The results revealed an upregulation of group II PLA\textsubscript{2} in cirrhosis.

Materials and Methods

Animals and bile duct excision. Sprague-Dawley male rats (Deutsche Versuchstierfarm Hartmut-Voss, Tuttilingen, Germany) were maintained on standard laboratory chow (Kliba-Futter, Basel, Switzerland) and tap water with a 12-h light–dark cycle at constant temperature and humidity. At the time of surgery, rats weighed 300 to 400 g. Animals were allotted to bile duct excision (n = 7) or sham surgery (n = 5). The experimental protocol had been approved by the Animal Ethics Committee of the state of Berne.

We used bile duct excision to produce cirrhosis (23). A 2-cm upper abdominal midline incision was made under ether anesthesia. In the experimental group, the bile duct was isolated and a 1-cm section excised after double ligature. In the sham group, the bowel and mesentery were manipulated and replaced. 8 wk after surgery the animals were anesthetized using pentobarbital sodium (50 mg/kg i.p.). In situ perfusion with ice-cold saline was performed. Liver and kidneys were removed and immediately frozen in liquid nitrogen, and stored at −80°C until further use.

Bile juice collection. Sprague-Dawley rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.). The body temperature, mea-
sured by a rectal thermometer, was maintained at 37–37.5°C with a heating lamp. Bile juice was collected by cannulating the common bile duct with PE-50 tubing (0.51 × 0.94 mm).

**Extraction of total RNA.** Kidney and liver tissues frozen at −80°C were powdered using pestle and mortar kept in a mixture of dry ice and acetone. Total RNA was extracted from a known amount of tissue according to the method of Chomczynski and Sacchi (24). The RNA concentration was determined by absorbance at 260 nm. The quality of RNA was controlled by running an aliquot on a 1% agarose formaldehyde gel.

**Reverse transcription of mRNAs.** The reverse transcription (RT) reaction mixture of 20 μl contained 50 mM Tris-HCl (pH 8.2), 6 mM MgCl₂, 10 mM dTTP, 100 mM NaCl, 200 μM dNTPs, 11 U RNAse inhibitor, 10 pmol primer (3′ primer of the corresponding cDNA positions 695–719 for group II PLA, cDNA [25], 335–359 for group I PLA; [26], 978–1004 for annexin-I [27], and 980–1004 for GAPDH [28] from Microsynth GmbH, Balgach, Switzerland), 2–5 μg of total RNA and 1 U of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim Biochemicals, Rotkreuz, Switzerland). For competitive RT-PCR 18 fg of modified group II PLA transcript was added to the reverse transcription reaction (29). Initially, the 3′ primer and total RNA with and without modified group II PLA transcript, were incubated together for 5 min at 65°C, and then cooled at room temperature for 15 min. The remaining reaction components, including avian myeloblastosis virus reverse transcriptase, were then added and incubated at 42°C for 60 min.

**PCR.** PCR was performed in a total volume of 30 μl with 10 mM Tris- HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μM dNTPs, 10 pmol of the appropriate 3′ and 5′ cDNA primers (5′ primers of corresponding cDNA positions 58–82 for group II PLA, cDNA [25], 38–62 for group I PLA, 1–29 for annexin-I, and 66–90 for GAPDH), 6.0 μg BSA, 1 μCi [α-³²P]dCTP, 2–10 μl reverse transcribed cDNA, 1 U Thermus aquaticus DNA polymerase (Perkin Elmer Cetus Corp., Norwalk, CT). Before adding the enzyme and cDNA the samples were irradiated under ultraviolet light for 20 min to eliminate any contaminating DNA (30). The mixture was overlaid with mineral oil, and cDNA was amplified on either 1.5 or 0.9% agarose gels containing ethidium bromide.

**Protein extraction.** Small amounts of powdered tissue were extracted with acid according to the method of Märki and Franson (31). The prepared material designated as tissue acid extract was used to determine PLA₂ (group II) protein by Western blot analysis and enzyme activity. To determine specifically group IV PLA₂ protein by Western blot and its enzymatic activity, powdered tissue was extracted with Tris-sucrose (TS) buffer (10.0 mM Tris·HCl, pH 7.0; 25.0 mM sucrose). For the determination of annexin-I and -V by Western blot analysis powdered tissue was extracted with 10 mM Tris·HCl buffer, pH 9.0, containing 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride and 5 mM EDTA using a Dounce homogenizer, stored on ice for 60 min. The extracts were centrifuged at 13,000 rpm at 4°C for 30 min to sediment cell debris. The supernatants from TS buffer and from Triton buffer were designated as TS extract and Triton extract, respectively.

**Determination of protein concentrations.** Protein concentration in tissue acid extract, TS extract and in Triton extract was determined using the bicinchoninic acid protein assay reagent (Pierce Chemical Co., Rockford, IL).

**Monoclonal antibodies.** Rat lung tissues were extracted with acid according to the method of Märki and Franson (31). PLA₂ enzyme from lung acid extract was partially purified on a Sephadex G-50 column (dimensions 0.75 × 60 cm; elution buffer 50 mM sodium acetate buffer, pH 4.5, containing 1.5 M NaCl). The major PLA₂ fraction eluted in the 14-kD protein region were pooled and used to raise monoclonal antibody in mice.

Partially purified rat lung PLA₂ in a concentration of 1 mg/ml of saline was mixed with an equal volume of Ribi adjuvant system (RAS: RIBI Immunochem Research Inc., Hamilton, MT) and injected into the peritoneal cavity of 6-wk-old mice. Mice were boosted twice with 100 μg of PLA₂ in RAS and sacrificed 3 d after the last booster injection. The spleen was excised and somatic–cell fusion was performed by the method initially described by Köhler and Milstein (32). Suspensions of fused cells in selective medium containing hypoxanthine, aminopterine, and thymidine were divided into 10 tissue culture plates (Falcon 3072; 96 wells/plate from Becton Dickinson Labware, Lincoln Park, NJ) at the rate of 200 μl/well. Samples of medium from each well were screened for anti-PLA₂ antibody activity using the dot blot procedure and Western blots. Cells from positive wells were subcloned by limiting dilution in 96-well tissue-culture plates. The selected clone (7G7) was expanded in vitro using Iscoe’s modified Dulbecco’s medium enriched with 10% fetal calf serum. The monoclonal antibody was isolated from the hybridoma supernatants by protein G–Sepharose (Pharmacia Biotech AG, Duebendorf, Switzerland) affinity purification using buffers recommended by the manufacturer.

**Monoclonal antibody (6G6) was prepared similarly using recombinant human annexin-I (33). Monoclonal antibodies against annexin-V and group IV PLA₂ were obtained from Alexis Corporation, (San Diego, CA) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively.**

**Western blot analysis.** For Western blots indicated amounts of proteins from tissue acid extract (to detect group II PLA₂), Triton extract (to detect annexin-I and -V) and 50.0 μg of TS extract (to detect group IV PLA₂) were reduced (100 mM DTT) and denatured (100°C) before being resolved by 12.5 or 8.0% SDS–polyacrylamide gel electrophoresis (34). The proteins were transferred to PVDF Immobilon membrane (Millipore AG, Volketswil, Switzerland) by electroblot using Tris-glycine buffer containing 20% methanol. The transfer was performed at a constant voltage of 65 V for 60 min. Protein transfer was monitored by staining the Immobilon membrane with Ponceau S (Sigma Chemie, Buchs, Switzerland) and the gel with Coomassie blue. Purified recombinant annexin-I (0.94–7.5 ng) was used in each set of Western blots as a standard to measure the annexin-I concentration in the protein samples. The blots were probed with Tris-buffered saline (TBS), pH 7.5, containing 5% BSA for 60 min at room temperature, washed three times with TBS, and incubated with primary antibodies as follows.

To detect group II PLA₂ protein membranes were incubated for 120 min at 37°C with the monoclonal antibody 7G7 in TBS containing 1.0% Tween-20 and 0.05% BSA. To detect group IV PLA₂, annexin-I and -V membranes were incubated for 120 min at 37°C with their corresponding monoclonal antibodies in TBS containing 0.5% BSA. Membranes were washed three times with TBS and blocked again with TBS containing 5% BSA for 60 min at room temperature. Membranes were washed three times with TBS and incubated with peroxidase conjugated goat anti–mouse IgG (H + L) (Bio-Rad, Glattbrugg, Switzerland) for 60 min at room temperature in TBS containing 1% Tween-20 and 0.05% BSA for group II PLA₂ or in TBS containing 0.5% BSA for group IV PLA₂ and annexins. Membranes were washed three times with TBS and the peroxidase activity was determined using the enhanced chemiluminescence Western blotting detection system (Amersham International, Buckinghamshire, UK) according to the manufacturer’s instructions. The bands on the X-ray films were scanned with a transmittance scanning densitometer (Scananalytics CSPI, MA).
Cell culture. Mesangial cells were cultured from isolated rat (Sprague-Dawley) glomeruli (35). Briefly, the cells were grown in RPMI 1640 supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml) transferrin (5 μg/ml), insulin (5 μg/ml), and sodium selenite (5 ng/ml). For the experiments passages 27–30 of mesangial cells were used. Confluent mesangial cells cultured in 15-mm-diam wells (24 wells tissue culture plate; Falcon 3047) were incubated with 500 μl of RPMI 1640 medium containing 10% FCS and 1 mM of interleukin-1β (IL-1β; Pharma Biotechnologie, Hannover, Germany) with and without bile juice (5–50 μl/ml) or bile salts (100 μM) for 24 h at 37°C in a humidified atmosphere of 95% air and 5% CO2. The following bile salts were tested: sodium salts of cholic acid (CA); chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), glycylcholic acid (GLCA), lithocholic acid (LCA), taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCa), taurochenoxycholic acid (TDCA), and tauroursodeoxycholic acid (TUDCA) (Calbiochem AG, Lucerne, Switzerland). After 24 h incubation the medium was removed and centrifuged for 5 min at 4,000 rpm. The supernatant was removed and used for PLA activity measurements.

Assay of PLA activity. [3H]oleic acid (specific activity 10 Ci/mmol; Amersham International, Buckinghamshire, UK) labeled Escherichia coli and [3H]arachidonic acid (specific activity 205 Ci/mmol; Amersham International) labeled E. coli were prepared according to the procedure of Patriarca et al. (36). Group II PLA activity was assayed using [3H]oleate-labeled, autoclaved E. coli as the substrate (37). The reaction mixture of 350 μl contained 100 mM Tris-HCl, pH 8.0, 5 mM CaCl2, 2.85 × 106 cells of autoclaved E. coli (corresponding to 10,000 cpm and 5.5 nmol lipid phosphorus), and tissue acid extracts or supernatants of mesangial cells were incubated at 37°C for 120 min. Activity of group IV PLA was assayed using [3H]arachidonate-labeled, autoclaved E. coli as the substrate for TS-extracted samples (38). The reaction mixture of 350 μl contained 100 mM Tris-HCl, pH 7.0, 1 mM CaCl2, 5.0 mM DTT, and TS-extract. This mixture was incubated for 60 min at 37°C. The reaction was started by adding autoclaved E. coli (2.1 × 106 cells corresponding to 8,000 cpm and 4.0 nmol lipid phosphorus) and the incubation was continued for another 120 min at 37°C. The amount of protein was chosen such that the hydrolysis of the substrate was 6–15%. The reaction was terminated by adding 100 μl of 2 N hydrochloric acid. Fatty acid-free BSA (100 μl, 100 mg/ml) was added, and the tubes were vortexed and centrifuged at 15,000 g for 5 min. An aliquot (140 μl) of the supernatant containing released [3H]oleic acid or [3H]arachidonic acid was mixed with scintillation cocktail (Dyagen, J.T. Baker B.V., Deventer, Holland) and counted in a liquid scintillation counter (model SL 4000; Intertechnique, Nuclotron, Lausanne, Switzerland).

Statistics. All results in the text and in the figures are mean (+ SD). Differences between two groups were analyzed by Student’s t-test.

Results

All animals with bile duct ligation had hepatomegaly; the liver was nodular in appearance and all had portal hypertension. The animal characteristics are given in Table I.

The mean amount of total RNA isolated from liver and kidney (μg/g tissue) was significantly lower in cirrhotic liver (−27%) and kidney (−35%) tissue samples compared to their corresponding sham-operated controls. The mRNA of GAPDH and its activity have been shown to be unchanged during cirrhosis (39). Therefore the mRNA of GAPDH was used as an internal standard. In control and cirrhotic samples the mRNA of group I PLA2, group II PLA2, annexin-I and GAPDH were measured by RT-PCR. The mRNA of group II PLA2 was additionally quantified by RT-PCR using a constant amount of a modified group II PLA2 cDNA transcript. Both specific target mRNA and the modified transcript were coamplified in one reaction using the same primers (29). Visual inspection of the bands on the agarose gels revealed qualitatively that mRNA of group II PLA2 was increased in cirrhotic liver and kidney tissue when compared with their corresponding controls (data not shown). The inclusion and coamplification of the modified group II PLA2 transcript using the same primers (29) excluded the possibility that increased concentrations of group II PLA2 in cirrhotic rats were caused by an artifact during the PCR amplification procedure (40). An increased signal for annexin-I mRNA was observed in cirrhotic liver but not in kidney tissue (data not shown). In accordance with the previous report (39) no change in the mRNA of GAPDH was observed in cirrhotic liver and kidney tissue when compared with the corresponding controls in the present investigation. No signal for mRNA of group I PLA2 was found in liver or in kidney tissue.

Table I. Characteristics of the Animals at the Time of the Experiments

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 5)</th>
<th>Bile duct ligation (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>500±20</td>
<td>404±38*</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>18.8±2.1</td>
<td>32.4±2.5*</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>3.3±0.3</td>
<td>3.3±0.3*</td>
</tr>
<tr>
<td>Portal pressure (cm H2O)</td>
<td>12.4±0.7</td>
<td>20.2±2.1*</td>
</tr>
<tr>
<td>Bilirubin (μmoles/l)</td>
<td>2±0</td>
<td>130±26*</td>
</tr>
<tr>
<td>Serum bile acids (μmoles/l)</td>
<td>3±2</td>
<td>91±51*</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/l)</td>
<td>155±41</td>
<td>605±288*</td>
</tr>
<tr>
<td>Alamine aminotransferase (IU/l)</td>
<td>47±4</td>
<td>100±37*</td>
</tr>
</tbody>
</table>

Mean±1 standard deviation are given. *Denotes a statistically significant difference (P < 0.05).
group II PLA2 protein was increased in liver by 230% \((P < 0.001)\) and in kidney tissue by 690% \((P < 0.001, \text{Fig. 1})\). In these tissue samples group IV PLA2 protein was also measured using a specific monoclonal antibody. Kidney tissue exhibited a threefold higher amount of group IV PLA2 protein than liver tissue \((P < 0.04)\), however, cirrhosis did not influence the mean amount of group IV PLA2 in these tissues (data not shown).

For the assessment of group II PLA2 activity in liver and kidney tissue the PLA2 enzyme from these tissues was solubilized by acid extraction. The extracted PLA2 enzyme showed an optimum pH of 7.5–9.0 and required 5 mM Ca\(^{2+}\) for optimal activity (data not shown). The results from PLA2 activity measurements are given in Fig. 1. As previously shown, liver exhibited a higher PLA2 activity \((487\%, \ P < 0.001)\) than kidney tissue in control animals \((29)\). PLA2 activity was increased by 138% in liver \((P < 0.002)\) and by 135% in kidney tissue \((P < 0.02)\) when samples from cirrhotic animals were compared with those from control animals (Fig. 1). In the TS extract of these tissues group IV PLA2 activity was measured using [\(^{3}H\)]arachidonate–labeled E. coli in the presence of DTT \((38)\). Control liver and kidney exhibited a 47- and 20-fold lower activity when compared with group II PLA2 activity \((0.0133\pm 0.006 \text{ vs} 0.63\pm 0.27 \text{ nmol fatty acid/mg protein/min in liver and} 0.0148\pm 0.007 \text{ vs} 0.29\pm 0.07 \text{ nmol fatty acid/mg protein/min in kidney tissues})\). Cirrhosis did not influence the group IV PLA2 activity in these tissue samples (control vs cirrhosis: 0.0133\pm 0.006 \text{ vs} 0.0162\pm 0.009 \text{ nmol fatty acid/mg protein/min in liver and} 0.0148\pm 0.007 \text{ vs} 0.0146\pm 0.005 \text{ nmol fatty acid/mg protein/min in kidney tissue}).

When the ratio of group II PLA2 mRNA/GAPDH mRNA was plotted versus the corresponding group II PLA2 protein or activity for each animal separately the following correlation coefficients were found: mRNA vs protein 0.66 \((P < 0.02)\) for liver and 0.65 \((P < 0.05)\) for kidney; mRNA vs activity 0.59 \((P < 0.05)\) for liver and 0.72 \((P < 0.01)\) for kidney. Since there was no change in the activity of group IV PLA2 and its protein content during cirrhosis the increased PLA2 activity was most likely due to an increase in group II PLA2 enzyme activity. The
Discussion

PLA$_2$ enzymes characterized so far are classified into group I, II, III, and IV based on their primary and secondary structure (41,42). PLA$_2$ enzymes purified from human platelets and from human synovial fluid are classified under group II and are considered to account for inflammatory reactions, whereas the group I from pancreas appears not to be involved in inflammation (29, 43, 44). Group III enzyme has not been detected in mammals and the function of cytosolic PLA$_2$ (group IV) is not very well defined yet (42). Inflammatory human synovial fluid PLA$_2$ (group II) is resistant to low pH, optimally active in the presence of mM concentrations of calcium and at basic pH (38, 45). In the present study PLA$_2$ activity measured in the acid extracts of kidney and liver exhibited similar biochemical/biophysical properties as those known for PLA$_2$ derived from inflammatory synovial fluid PLA$_2$. Group IV PLA$_2$ selectively hydrolyzes sn-2-arachidonoyl-containing phospholipids and is resistant to reducing agents like DTT (38). Group IV PLA$_2$ activity measured in kidney and liver was 20–47-fold lower compared to the activity of group II PLA$_2$. Since no mRNA for group I PLA$_2$ was detected its contribution for total PLA$_2$ activity is very unlikely. Therefore the PLA$_2$ activity measured in these tissues corresponds mainly to group II PLA$_2$ enzyme.

The present investigation revealed an upregulation of group II PLA$_2$ enzyme in obstructive jaundice but not of group I or of group IV PLA$_2$. For such an enhanced PLA$_2$ activity three mechanisms have to be considered. First, an enhanced transcription and translation of the group II PLA$_2$ enzyme, second an increased concentration of PLA$_2$ enzyme activating agents and third, a decline of an endogenous PLA$_2$ inhibitor. The quantitative RT-PCR study revealed that group II PLA$_2$ mRNA levels were elevated after bile duct ligation in renal and liver tissue. The upregulation of PLA$_2$ was not a nonspecific phenomenon, since mRNA of GAPDH was unaffected and was used as internal standard. The increase of group II PLA$_2$ mRNA was reflected by an increase of group II PLA$_2$ protein and group II PLA$_2$ enzyme activity. Thus, an enhanced transcription and translation of group II PLA$_2$ enzyme accounted at least partly for the augmented enzyme activity in cirrhotic rats.

With respect to the second mechanism involving the PLA$_2$ activating agents during cirrhosis we analyzed the impact of bile juice and different bile salts known to be increased during cirrhotic conditions on the secretion and on the activation of group II PLA$_2$ enzyme activity. Bile juice and bile salts did not activate PLA$_2$ enzyme activity by inducing the transcription and translation of PLA$_2$ protein (data not shown). However, bile juice by itself and several bile salts like GCA, TCA, TCDCA, TDCA, and TUDCA individually activated IL-1β released group II PLA$_2$ enzyme activity indicating the synergetic action of these salts. The present finding, that some bile acids enhance PLA$_2$ activity is novel, and in line with the description of other endobiotics such as PLA$_2$ activating protein enhancing PLA$_2$ activity (46).

For the third mechanism, a decline of an endogenous inhibitor of PLA$_2$ enzyme during cirrhosis, the role of the presumptive PLA$_2$ inhibitor annexin-I has been studied together with annexin-V. The annexin family presently consists of some 13 members capable of binding phospholipids in the presence of calcium (27, 47–49). It had been suggested that annexin-I (sive lipocortin-I or calpain-II) is a mediator of glucocorticoid action and acts as a specific inhibitor of PLA$_2$ (50, 51), a concept which remains controversial, however (33, 52). In the present investigation annexin-I and -V have been quantitatively assessed. Bile duct ligation did not affect the concentrations of these proteins. Thus, enhanced transcription of PLA$_2$ and subsequent activation by bile salts is the most likely factor accounting for the enhanced PLA$_2$ activity following bile duct ligatation.

The mechanism accounting for the enhanced release of PLA$_2$ in obstructive cirrhosis is unknown; however, the following factors have to be considered. First, PLA$_2$ enzyme is activated by endotoxins and cytokines like IL-1β and tumor necrosis factor-α (53–55). Chronic endotoxia due to increased intestinal absorption and/or decreased hepatic clearance of lipopolysacharide has been documented in patients with cirrhosis (56, 57) and may thus account for the activation of PLA$_2$. Second, glucocorticoids which are known to regulate the activity of group II PLA$_2$ enzyme could be involved in the observed activation of PLA$_2$. Glucocorticoid deficiency enhances and pharmacological doses reduce PLA$_2$ activity by means of inhibiting cAMP-dependent, but not cAMP-independent expression of group II PLA$_2$ mRNA (43, 58). The increased PLA$_2$ activity under glucocorticoid deficiency was positively correlated to the decreased expression of the presumptive inhibitor annexin-I (29). Since there was no change in the level of annexin-I during cirrhosis, the enhanced PLA$_2$ activity in biliary obstruction is probably not related to deranged glucocorticoid effects. Third, activation of PLA$_2$ by catecholamines and angiotensin II has to be considered (59). Numerous studies have demonstrated an increased release of both pressor agents in humans and various animal models with cirrhosis (60, 61), suggesting a role of these endobiotics for PLA$_2$ activation in liver disease.

Arachidonate metabolites have been reported to be involved in liver damage. For instance there is evidence for 16,16-dimethyl PGE$_2$ to attenuate liver cell damage induced by carbon tetrachloride and aflatoxin (62-64). Similarly, a PG$_I_2$ analogue protects against carbon tetrachloride or acetaminophen-induced liver injury (65, 66) and 5-lipooxygenase inhibitors or LT receptor antagonists reduce liver injury from D-galactosamine (67–71). On the other hand, there is evidence that
leukotrienes and thromboxane could play a major role in the pathogenesis of cirrhosis and portal hypertension. Thus, the demonstration of increased leukotriene excretion by patients with liver cirrhosis is compatible with PLA2 activation (72). This contention is further supported by the presence of elevated prostacyclin levels in patients with cirrhosis which correlate with portal hypertension (73).

While the role of prostanooids in the liver is ambiguous, at least exogenous prostaglandins being protective but leukotrienes, prostacyclin, and thromboxane being pathogenetically involved in cirrhogenesis and maintenance of portal hypertension and hyperdynamic circulation, the situation appears much clearer in the kidney. Through their vasodilator effect, prostaglandins modify intrarenal vascular resistance and thereby maintain glomerular filtration rate (74). Furthermore, they promote natriuresis and free water clearance (20, 22). This explains the deleterious effect of cyclooxygenase inhibitors on renal function in cirrhosis (75).

In conclusion, our studies demonstrate an upregulation of group II PLA2 at the enzyme and message level in biliary cirrhosis in rat kidney and liver. While this could account for the renal protective effects of prostaglandins in the kidney, its role in cirrhogenesis in the liver remains to be established.

Acknowledgments

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Interleukin-1 generation. The effect of glucocorticoids.


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