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Expression and antioxidant function of liver fatty acid binding protein in normal and bile-duct ligated rats

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Abstract

Liver fatty acid binding protein has recently been shown to possess antioxidant properties but its role in liver disease, such as cholestasis, is not known. Since oxidative stress has been recognized as an important contributing factor in liver disease, we investigated the expression and antioxidative function of this protein using the bile-duct ligated model of cholestasis. Rats were divided into 3 groups: sham, bile-duct ligated and bile-duct ligated plus clofibrate. Animals were sacrificed at various time points after bile-duct ligation. RT-PCR and Western blot were used to analyze liver fatty acid binding protein expression. Cellular lipid peroxidation products were assessed by measuring thiobarbituric acid-reactive substances. Liver function was evaluated by measuring serum total bilirubin, alanine aminotransferase and ammonia. Liver fatty acid binding protein mRNA and protein levels were reduced to 51% and 20% of sham, respectively at 2 weeks following bile-duct ligation ($p<0.05$). The decreased liver fatty acid binding protein was associated with a statistical increase in hepatic lipid peroxidation products (224%) and decrease in hepatic function. Clofibrate treatment restored protein level and improved hepatic function. Clofibrate treatment also reduced hepatic lipid peroxidation products by 68% as compared with the bile-duct ligated group ($p<0.05$). Liver fatty acid binding protein likely has important antioxidant function during hepatocellular oxidative stress.

Keywords: Fatty acid binding protein; Cholestasis; Bile-duct ligation; Free radicals; Liver; Oxidative stress

1. Introduction

Fatty acid binding proteins (FABP) are lipid-binding proteins that play an important role in the trafficking of intracellular ligands, metabolism, cell proliferation and signal transduction (Glatz and van der Vusse, 1996; Storch and Thumser, 2000; Wang et al., 2004; Zimmerman and Veerkamp, 2002). Some tissue-specific isoforms of fatty acid binding protein include heart (H-FABP), liver (L-FABP), intestine (I-FABP), brain (B-FABP). Although they are similar in protein structure and function, they are encoded by different genes located on different chromosomes (Zimmerman and Veerkamp, 2002).

Liver fatty acid binding protein is a 14 kDa protein that accounts for 3–5% of the total cytosolic protein pool (Burnett et al., 1979). It contains seven methionine and one cysteine group in its amino acid sequence (Thompson et al., 1999). It has been postulated that this protein functions as an intracellular buffer of long chain fatty acids and their CoA and carnitine esters thus maintaining a low concentration of their unbound form. Liver fatty acid binding protein also has been suggested to trap or scavenge cytotoxins and superoxide species, thus protecting cells from reactive oxygen species (Ek-Von Mentzer et al., 2001; Kaikaus et al., 1993; Khan and Sorof, 1990; Luebker et al., 2002). Because liver fatty acid binding protein forms a large portion of the intracellular protein pool and contains a large number of methionines and cysteine, it may

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have an important function as a cytoprotectant (Levine et al., 1999; Thomas et al., 1995). We previously reported that Chang liver cells were devoid of liver fatty acid binding protein. Transfecting those cells with liver fatty acid binding protein cDNA produced a new stably transfected cell line. Inducing oxidative stress in the liver fatty acid binding protein cDNA transfected and vector transfected cells, we reported that the liver fatty acid binding protein cDNA transfected cells were associated with lower reactive oxygen species levels than the same cells transfected with the vector (Wang et al., 2005), suggesting that the protein indeed has important intracellular antioxidative properties.

In this report we examined the role of liver fatty acid binding protein in a cholestatic liver disease model. The mechanism of cholestatic liver disease is not well understood and several hypotheses have been proposed including the involvement of oxidative stress (Aboutwerat et al., 2003; Ljubuncic et al., 2000). According to the oxidative stress hypothesis, endogenous antioxidant systems could prevent liver damage during cholestatic liver disease progression. The experimental model widely used to study cholestatic liver disease is the bile-duct ligation model (Kountouras et al., 1984) which is associated with decreased antioxidant activities of hepatic catalase, superoxide dismutase and glutathione peroxidase (Orellana et al., 2000). Moreover, liver mitochondria antioxidative capacity and glutathione are decreased in bile-duct ligated rats (Krahenbuhl et al., 1995). While exogenous antioxidants, vitamin E (lipophilic) and Trolox (hydrophilic) improved lipid peroxidation and oxidation of glutathione in bile-duct ligated rats, it had no effect on liver injury (Baron and Muriel, 1999). Whether other endogenous antioxidant systems are available within the liver to improve liver function is not clear. Interestingly, liver fatty acid binding protein levels are known to be reduced in steatosis (Hung et al., 2005). Since liver fatty acid binding protein has been thought to function as an effective antioxidant, it may play an important role in the prevention of cholestatic liver disease. In this report we demonstrated the expression and antioxidative function of liver fatty acid binding protein in an animal model of cholestatic liver disease induced by bile-duct ligation.

2. Materials and methods

2.1. Materials

Trizol LS Reagent was purchased from GIBCO/BRL (Burlington, ON). All other chemicals were purchased from Sigma-Aldrich Canada LTD (Oakville, ON). Rat L-FABP antibody was raised in our lab (Wang et al., 2004). Rabbit anti-rat IgG and the enhanced chemiluminescence Western blot kit were purchased from Amersham-Pharmacia Biotech Inc. (Baie d'Urfe, Quebec). Advantage RT-for-PCR Kit, Advantage cDNA PCR Kit and Polymerase Mix were purchased from Clontech Laboratories Inc. (Palo Alto, CA). Male Sprague–Dawley rats (250–350 g) were obtained from the Animal Breeding Facility of the University of Manitoba. All rats were maintained under 12-hour light/dark cycles with food and water *ad libitum*. In conducting the research described in this report, all animals received humane care in compliance with institutional guidelines, which are in accordance with criteria set by the Canadian Council on Animal Care.

2.2. Bile-duct ligation animal model time study

Adult male Sprague–Dawley rats were divided into two groups ($n=4$ for each time point in each group); group 1 included sham operated animals and group 2 included common bile-duct ligated animals as described by (Shen et al., 2005). Blood by cardiac puncture at different time points was obtained just prior to animals being sacrificed while the animals were anesthetized with ether. Livers were removed and immediately frozen in liquid nitrogen. Liver and serum samples were stored at −70 °C until required.

2.3. Bile-duct ligation animal model with clofibrate treatment

Adult male Sprague–Dawley rats were divided into three groups ($n=5$ for each group; total time for each group was 12 days); group 1 — sham operated; group 2 — common bile-duct ligation as described by Shen et al. (2005); group 3 — common bile-duct ligation and clofibrate treatment (50 mg/100 g body weight/day) for 5 days after the bile-duct had been ligated for 7 days (total time was 12 days). Clofibrate was suspended in glycerol and administrated (1.0 ml) by gastric gavage. Control animals were administered the same volume of glycerol (without drug). Blood was sampled by cardiac puncture at different time points just prior to animals being sacrificed while the animals were anesthetized with ether. Livers were removed and immediately frozen in liquid nitrogen. Liver and serum samples were stored at −70 °C until required.

2.4. Histopathologic examination

Sections of liver tissue from sham and bile-duct ligated animals were fixed in 10% buffered formalin. The fixed tissues were embedded in paraffin and then sectioned and stained with Hematoxylin and Eosin for histopathological examination. Slides were reviewed for degree of bile-duct proliferation and liver inflammation.

2.5. Reverse transcriptase polymerase-chain reaction (RT-PCR)

Total RNA was isolated by Trizol LS reagent as described in the manufacturer's instruction and the first cDNA strand synthesis and PCR procedure were followed. The specific primers for rat liver fatty acid binding protein were designed from the rat liver fatty acid binding protein sequence (GenBank M35991) by Oligo 5.1 program on a Macintosh computer. The sense primer was 5′-GGA AAC CTC ATT GCC ACC A-3′ (position at 22) and the antisense primer was 5′-GCC TTG TCT AAA TTC TCT TGC TGA-3′ (position at 407). The expected product size was 409 base pairs. Specific rat glyceraldehyde 3-phosphate dehydrogenates amplimers were obtained from Clontech (Cat. No. 5507-3) with an expected size of 986 base pairs. PCR amplification was carried out by applying 30 cycles

comprising of denaturation at 94 °C for 1 min, annealing at 56 °C (liver fatty acid binding protein) and 60 °C (glyceraldehyde 3-phosphate dehydrogenates) for 30 s, elongation at 72 °C for 45 s, followed by a final elongation at 72 °C for 8 min using Eppendoff MasterCycler (Eppendoff, Westbury, NY). PCR products were analyzed by electrophoresis in a 1.2% agarose gel.

2.6. Western blot analysis

Liver protein was isolated by homogenizing liver tissue in Phosphate Buffered Saline (containing 1% of Protease Inhibitor Cocktail, Sigma) using a Tissue Tearor homogenizer. Protein concentration was determined by the Lowry protein assay (Lowry et al., 1951) and 20 μg of protein was subjected to Western blot analysis. Specific liver fatty acid binding protein was determined by incubating membranes with the antibody against rat liver fatty acid binding protein and bands were visualized using the enhanced chemiluminescence kit as outlined by the manufacturer's instruction.

2.7. Lipid peroxidation (thiobarbituric acid-reactive substances) assay

Supernatants from homogenized livers were subjected to a modified lipid peroxidation assay (Chirico et al., 1993). Liver protein fraction (2 mg) was added to 1.5 ml microcentrifuge tube containing 25 μl ethanolic antioxidant butylated hydroxytoluene (2 g/l). 750 μl 0.44 M H_3PO_4 was added to the tube. Volume of the mixture was adjusted to 1000 μl with doubled-distilled water. The mixture was set aside for 10 min at room temperature and then 250 μl thiobarbituric acid (6 g/l ethanol solution) added. The mixture was vortexed and heated to 100 °C for 30 min. The mixture was subsequently cooled on ice for 5 min and then centrifuged at 1000 *g* for 10 min. To eliminate the hemolyzed and icteric interference or other possible chromogens from thiobarbituric acid malondialdehyde adducts at wavelength 532 nm, the cooled sample was subjected to high-performance liquid chromatography. The thiobarbituric acid reaction mixture (20 μl) was injected onto an reversed-phase C-18 column (Xterra MS, 4.6 × 250 mm) in a Waters 2690 alliance HPLC system (Waters Corporation, USA) and eluted with 50% 50 mM KH_2PO_4–KOH at pH 7.0 and 50% methanol at a flow rate of 0.75 ml/min. Absorbance was read at 532 nm with the retention time (R_T) at 5.8 min. Malondialdehyde standard (1,1,3,3-tetramethoxypropane; Sigma) was used to construct the standard curve. The degree of lipid peroxidation was expressed as concentration of thiobarbituric acid-reactive substances in terms of malondialdehyde equivalents per gram of liver protein.

2.8. Serum total bilirubin, alanine aminotransferase and ammonia assay

Hepatic function was assessed by determining serum bilirubin, alanine aminotransferase and ammonia. Total bilirubin was determined by Sigma Diagnostic kit (550-A). Serum alanine aminotransferase and ammonia were assayed by a commercially available kit (Diagnostic Chemicals Limited). All assays were performed in a Cary Win UV spectrometer at 25 °C.

2.9. Statistical analysis

Results were expressed as mean ± S.E.M. Statistical difference was determined by Analysis of Variance using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). Level of statistical significance was taken as $P < 0.05$.

3. Results

3.1. Liver injury following bile-duct ligation

As shown in Fig. 1, bile-duct proliferation and mononuclear cell infiltration were detected in the portal area of the bile-duct ligated rat liver sections. Macrovascular cytoplasmic alterations of hepatocytes and many lysed cell areas (arrows in Fig. 1) were observed in this group. Bile-duct ligation also was associated with significant proliferation of bile-duct epithelial cells, inflammation and altered liver structure (circle arrow in Fig. 1).

3.2. Liver fatty acid binding protein expression in bile-duct ligated rats

Liver fatty acid binding protein mRNA and protein level determined at different time points following bile-duct ligation in rat liver tissue. Liver fatty acid binding protein mRNA expression started to decline significantly ($P < 0.05$) after 2 days

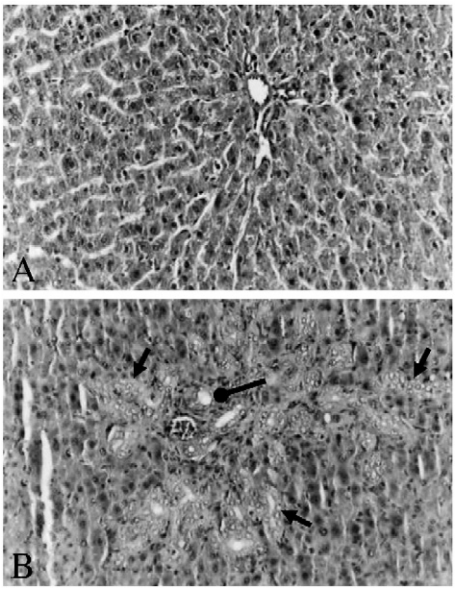

Fig. 1. Liver sections from sham and bile-duct ligated rats. (A) Normal hepatocytes in portal area from the rat of sham group. (B) Mild bile-duct proliferation and mononuclear cell infiltration in portal area from two weeks post-bile-duct ligation group. Macrovascular cytoplasmic alterations of hepatocytes and many lysed cell areas (arrows) were observed in this group. Bile-duct ligated rats were associated with significant proliferation of bile-duct epithelial cells, inflammation and altered liver structure (circle arrow).

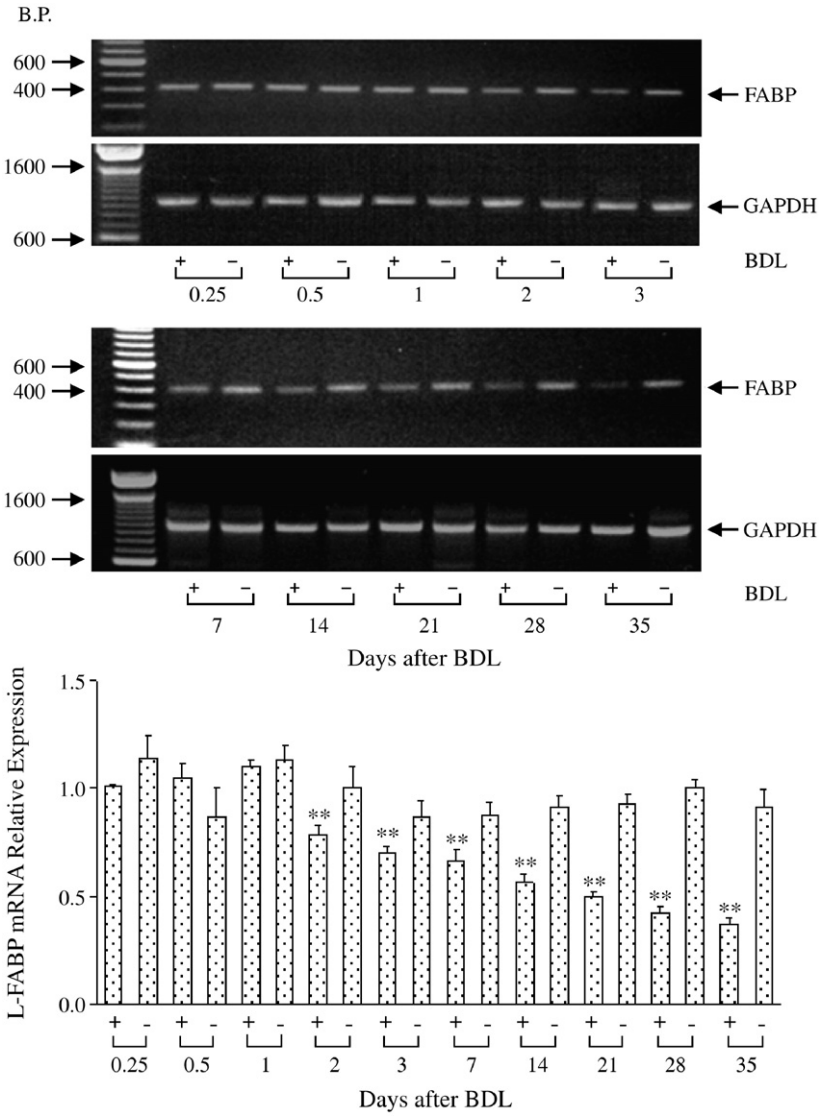

Fig. 2. RT-PCR analysis of L-FABP mRNA at different time intervals following bile-duct ligation (+) and sham (−). GAPDH was used as loading control. The optical density of each band was obtained using the NIH Image program. The relative expression of L-FABP mRNA *versus* GAPDH was displayed against time following bile-duct ligation. The significant decrease of L-FABP mRNA level occurred after 2 days of bile-duct ligation (**$P<0.01$). The data represent mean±S.E.M., $n=4$.

of bile-duct ligation. A dramatic decrease in the liver fatty acid binding protein mRNA expression was observed after one week of bile-duct ligation (Fig. 2). Reduction in liver fatty acid binding protein mRNA abundance caused a decrease in protein level. After two weeks of bile-duct ligation, liver fatty acid binding protein level was almost undetectable by Western blot (Fig. 3).

3.3. Thiobarbituric acid-reactive substances, serum bilirubin, alanine aminotransferase and ammonia levels of rats

Serum bilirubin, alanine aminotransferase and ammonia levels were examined in sham and bile-duct ligated rats (Table 1). Bile-duct ligation was associated with a significant increase in serum bilirubin, alanine aminotransferase and ammonia (increased by 3760%, 245% and 266%, respectively) reflecting liver dysfunction. Following clofibrate treatment, serum bilirubin, alanine aminotransferase and ammonia levels were statistically reduced. We used the thiobarbituric acid-reactive substances assay to assess lipid peroxidation levels and tissue oxidative stress. As shown in Fig. 4, the thiobarbituric acid-reactive substances increased following bile-duct ligation in a time dependent manner. A significant ($P<0.01$) increase occurred after 14 days of bile-duct ligation, indicating animals were experiencing hepatic oxidative stress and liver dysfunction. The increase in thiobarbituric acid-reactive substances production was closely associated with the decrease in liver fatty acid binding protein content.

To further elucidate the association between thiobarbituric acid-reactive substances production and liver fatty acid binding protein, we examined whether the reduction in liver fatty acid binding protein may be responsible for the for some of the released liver lipid peroxides. To this end, rats were administered clofibrate to up-regulate liver fatty acid binding protein expression. Liver fatty acid binding protein expression is known to be regulated by PPAR agonists such as clofibrate (Burczynski et al., 1999). As shown in Fig. 5, after bile-duct ligation liver

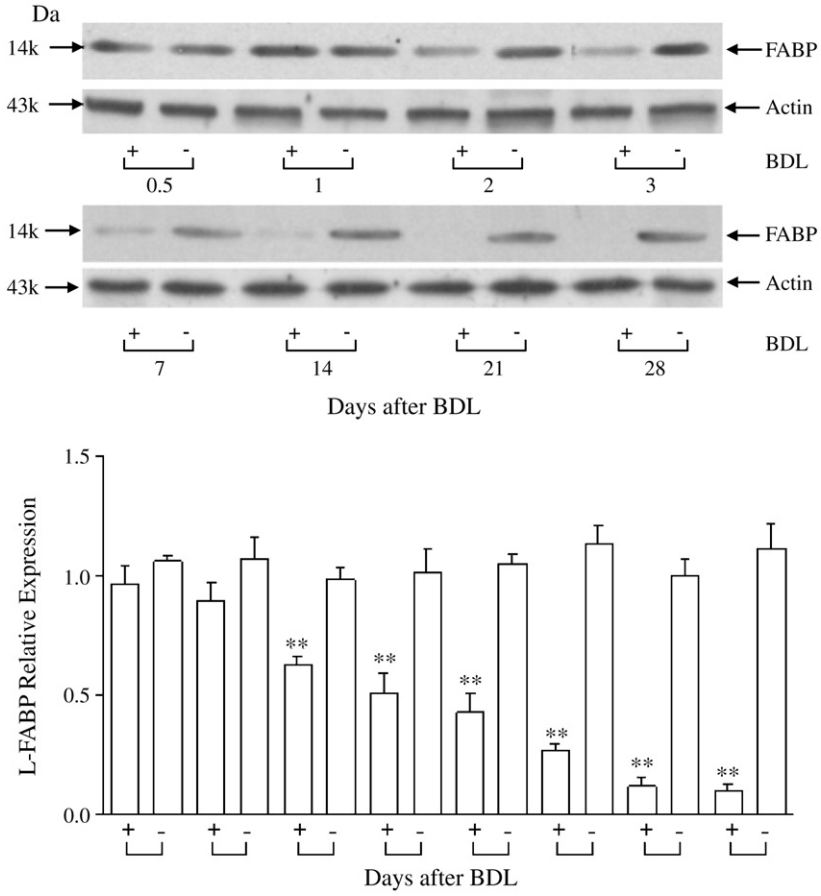

Fig. 3. Western blot analysis of L-FABP levels in bile-duct ligated (+) and sham (−) rat livers at different time intervals following bile-duct ligation. Actin expression was the loading control. The L-FABP relative expression (optical density units) of each band was obtained using the NIH Image program and expressed as L-FABP *versus* actin. Data represent mean±S.E.M., $n=4$. Statistical changes in L-FABP with time started after 2 days of bile-duct ligation, $^{**}P<0.01$.

fatty acid binding protein mRNA level was reduced to 58% as compared to the sham group while the protein level was reduced by 70% compared to the sham group ($P<0.01$, $n=5$). Five day treatment with clofibrate restored both the liver fatty acid binding protein mRNA and protein level back to 74% and 79% of the sham group, respectively. Compared to the bile-duct ligated group, the bile-duct ligated clofibrate treated animals had significantly elevated the mRNA and protein levels ($P<0.01$, $n=5$). The restoration of liver fatty acid binding protein level was associated with reduced lipid peroxidation products in both serum and liver (Table 1). Moreover, the up-regulation of liver fatty acid binding protein was associated with a significant decrease in serum bilirubin, alanine aminotransferase and ammonia levels by 193%, 139% and 133%, respectively, as compared to bile-duct ligated only animals (Table 1). These data indicate that liver fatty acid binding protein may likely be involved in the reduction of hepatic oxidative stress and improvement of hepatic function in bile-duct ligated rats.

4. Discussion

Bile-duct ligation is a typical model of biliary disease in animals, which features proliferation of bile-duct epithelial cells, hepatocellular necrosis and apoptosis, stellate cell activation and eventually the formation of liver fibrosis and cirrhosis (Kountouras et al., 1984; Scobie and Summerskill, 1965). Bile-duct ligation has been associated with hepatic mitochondrial dysfunction that includes oxidative damage to mitochondrial proteins and lipids and cytotoxicity of bile components such as the lipophilic bile acids (Hino et al., 2001;

Table 1
Serum levels of liver thiobarbituric acid-reactive substance levels, bilirubin, alanine aminotransferase and ammonia in sham, bile-duct ligation and bile-duct ligation plus clofibrate (clofibrate treated) rats

	Sham	Bile-duct ligation	Bile-duct ligation+ clofibrate
Thiobarbituric acid-reactive substances (nmol/mg protein)	0.18±0.04	0.39±0.03[b]	0.27±0.05[a]
Thiobarbituric acid-reactive substances (nmol/ml serum)	0.10±0.02	0.31±0.04[b]	0.21±0.03[a]
Total Bilirubin (mg/dl)	0.25±0.07	9.40±0.51[b]	4.87±0.90[b]
Alanine aminotransferase (U/l)	17.43±0.99	42.67±3.35[b]	30.70±3.26[a]
Ammonia (μmol/l)	102.6±9.4	273.1±29.6[b]	205.7±16.6[a]

Clofibrate was administered to rats for 5 days (50 mg/day/100 g body weight) by gavage after 7 days bile-duct ligation (total bile-duct ligation time was 12 days). All groups of animals were sacrificed on the 12th day. Data are presented as mean±S.E.M., $n=5$, [a]$P<0.05$, [b]$P<0.01$.

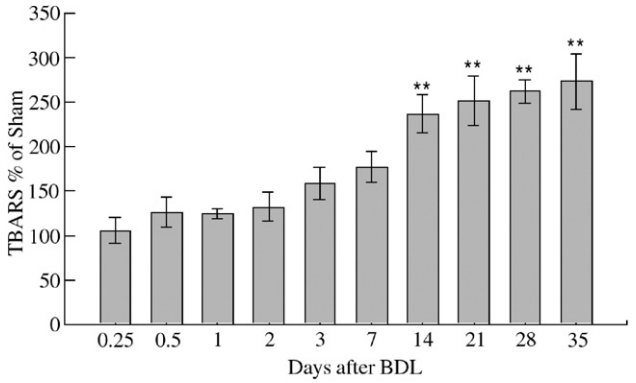

Fig. 4. Increased thiobarbituric acid-reactive substances production in liver tissues in bile-duct ligated rats. Thiobarbituric acid-reactive substances production in liver was determined by measurement of absorbance at 532 nm using HPLC. The degree of lipid peroxidation was expressed as concentration of thiobarbituric acid-reactive substances in terms of malondialdehyde equivalents per gram of liver proteins. The plot shows a significant thiobarbituric acid-reactive substances increase in bile-duct ligated rats compared to sham following 14 days of bile-duct ligation. Data represent mean ± S.E.M., $n=4$, $^{**}P<0.01$.

Krähenbühl et al., 1994a,b). The detergent action and cytotoxicity of bile salts is partly responsible for the plasma membrane damage seen in bile-duct ligated models which leads to further oxidative stress (Sokol et al., 1995). Extensive oxidative damage results from the release of reactive oxygen species (free radicals) that most likely result from a lack of adequate reactive oxygen species scavengers. For example, hepatic glutathione in bile-duct ligated animals shows a continuous decrease in reduced glutathione (GSH) and an increase in oxidized glutathione (GSSG) levels (Baron and Muriel, 1999; Neuschwander-Tetri et al., 1996; Purucker et al., 1998). Hepatic ubiquinones (lipophilic membrane associated antioxidants) also were reported to be decreased in bile-duct ligated animals (Krahenbuhl et al., 1995), as were the enzyme activities of cytosolic water soluble antioxidants such as superoxide dismutase, catalase and glutathione peroxidase (Orellana et al., 2000). While the bile-duct ligated model is known to be associated with reduced absorption of vitamin E, exogenous antioxidants such as vitamin E or Trolox, vitamin C and silymarin do not seem to prevent the damage induced by bile-duct ligation (Baron and Muriel, 1999; Muriel and Moreno, 2004). Bile-duct ligation also is associated with decreased uptake of long chain fatty acids (De Vriese et al., 2001) and decreased microsomal and peroxisomal fatty acid metabolism which may partly be due to reduced amounts of peroxisomes (Orellana et al., 1997a,b). Since processing of intracellular long-chain fatty acids is disrupted, it is likely that liver fatty acid binding protein is involved in this disease state. Our results are consistent with this notion. Liver fatty acid binding protein levels were observed to be statistically lower by day 2 of bile-duct ligation and declined with time following bile-duct ligation. The decrease in protein level may, however, be due to the reduced number of hepatocytes rather than a decline in liver fatty acid binding protein level within the existing hepatocytes. It is known that liver weight increases with the proliferation of biliary epithelial cells and other cell and tissue types, but the volume proportion of hepatocytes is reduced (Gall and Bhathal, 1990). The reduced liver fatty acid binding protein levels (65% to 90%) in bile-duct ligated rats compared with control rats one to four weeks after surgery greatly exceeded the

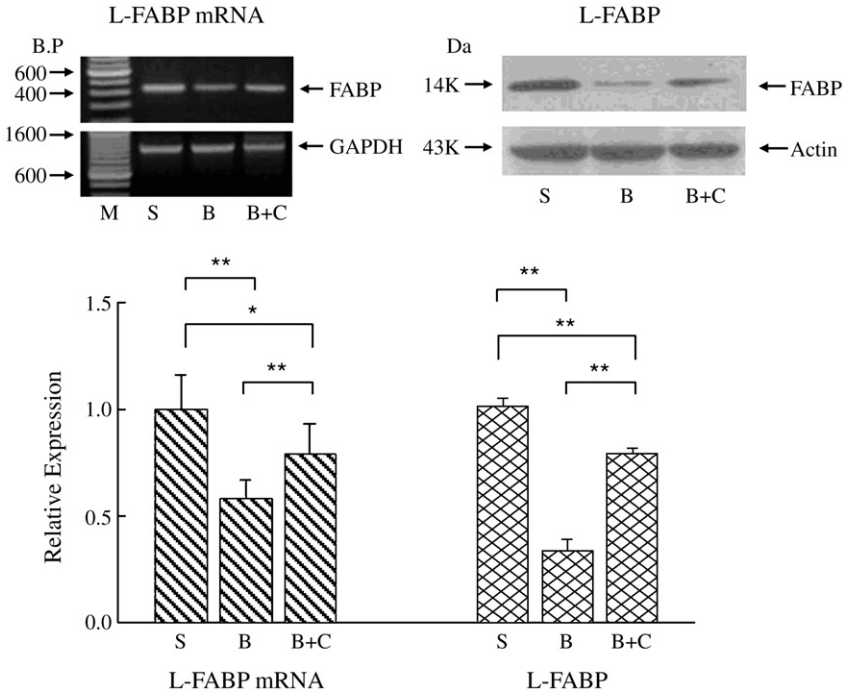

Fig. 5. Clofibrate reverses reduction of L-FABP mRNA and L-FABP level in bile-duct ligated rats. Clofibrate was administered to rats for 5 days (50 mg/day/100 g body weight) by gavage after 7 days bile-duct ligation (total bile-duct ligation time was 12 days). S represents sham rats (total time was 12 days); B represents bile-duct ligated rats (total bile-duct ligation time was 12 days); B+C represent bile-duct ligated rats with clofibrate treatment. Data represent mean ± S.E.M., $n=5$. Statistical significance was documented by analysis of variance with $^{*}P<0.05$ and $^{**}P<0.01$.

10% to 30% loss of hepatocyte fraction in bile-duct ligated rats at the same period after surgery (Gall and Bhathal, 1990; Krahenbuhl et al., 1996; Yamauchi et al., 1976). Thus, we conclude that the dramatic reduction in liver fatty acid binding protein level in bile-duct ligated animals was related to reduced protein level within the existing hepatocytes. This reduction is due to reduced liver fatty acid binding protein transcription.

Since liver fatty acid binding protein forms a large portion of the intracellular protein pool, together with its methionine and cysteine residues and great binding capacity, liver fatty acid binding protein is likely an important intracellular antioxidant. Work within our group has demonstrated that the protein likely protected hepatocytes against oxidative stress induced by H_2O_2 and hypoxia-reoxygenation in a liver fatty acid binding protein stably transfected Chang liver cell line (Wang et al., 2005). In that report we observed that cells expressing a higher level of liver fatty acid binding protein were associated with significantly reduced levels of reactive oxygen species within the cells, suggesting that the reduced reactive oxygen species levels was likely due to the increased protein level. The current study also indicated that liver fatty acid binding protein may act as an antioxidant in liver injury induced by bile-duct ligation. The significant decease in protein level occurred 2 days following bile-duct ligation, which preceded the decrease in GSH that is known to occur 5 days following bile-duct ligation (Purucker et al., 1998). Although thiobarbituric acid-reactive substances activity started to increase at day 1 following bile-duct ligation, the increase was more pronounced on day 3 and showed statistical significance by day 14 following bile-duct ligation. It is likely that the antioxidant capacity of hepatocytes is able to deal with the increased levels of reactive oxygen species within the first few days of bile-duct ligation. However, long term the antioxidant complement is unable to maintain a low reactive oxygen species level. Interestingly, restoration of liver fatty acid binding protein expression in the liver by clofibrate (a peroxisome proliferator) reduced the reactive oxygen species levels which was accompanied with reduced serum total bilirubin, alanine aminotransferase and ammonia. Although ammonia could increase in plasma from intestinal bacterial proliferation following bile-duct ligation, the difference between bile-duct ligation and bile-duct ligation plus clofibrate data suggests that the ammonia likely reflects liver cell damage rather than intestinal origin. Ciprofibrate treatment has been reported to significantly reduce the activities of hepatic antioxidant enzymes NAD(P)H quinine oxidoreductase, glutathione *S*-transferase, glutathione peroxidase and superoxide dismutase, but increase total cellular catalase activity in rats (Dhaunsi et al., 1994; Mesia-Vela et al., 2004). Several observations indicate that clofibrate treatment contributes to a heightened defense against oxidative stress in liver (Mesia-Vela et al., 2004; Nicholls-Grzemski et al., 2000; Rajaraman et al., 2006). Nicholls-Grzemski et al. suggested that a putative antioxidant protein may contribute to this protection against liver toxicity (Nicholls-Grzemski et al., 2000). The antioxidant factor was not glutathione, but was inactivated by proteases and heat treatment. Thus, collectively with our previous report (Wang et al., 2005), our current results lead us to suggest that liver fatty acid binding protein P likely acts as an antioxidant protein. Its antioxidation function may be due to the binding of peroxidized fatty acids, bile salts and/or scavenging of reactive oxygen species through its cysteine and methionine groups. Liver fatty acid binding protein compensates for the decreased activity of the nascent antioxidant systems. Although clofibrate treatment improved liver function by upregulating liver fatty acid binding protein expression, the resulting morphological changes of liver associated with bile-duct ligation were not found to be improved over the treatment period in this study.

The direct antioxidant function of liver fatty acid binding protein is likely due to its amino acid composition. The protein possesses one cysteine residue at position 69 and seven methionine residues in its 127 amino acid sequence. Cysteine may be involved in the binding of other hydrophobic ligands or serve as an antioxidant participating in *S*-thiolation/dethiolation (Sato et al., 1996), while methionine residues have nucleophilic sulfur atoms and are regarded as a cellular scavenger of activated xenobiotics such as carcinogens (Bassuk et al., 1987). Cellular oxidative stress may be suppressed by oxidation of methionine in liver fatty acid binding protein to sulfoxides, that are then reduced back by the protein methionine sulfoxide reductase (Levine et al., 1999). This cyclic oxidation–reduction of protein methionine residues may serve an important antioxidant function (Levine et al., 1996). The net effect of these catalytic reactions is the conversion of reactive oxygen species to innocuous products, driving NADPH oxidation reactions (Stadtman, 2004). Liver also has a high expression level of methionine sulfoxide reductase (Moskovitz et al., 1996) providing for the catalytic methionine redox homeostasis. Moreover, liver fatty acid binding protein binds metabolic oxidative products such as oxidized fatty acids (Ek-Von Mentzer et al., 2001; Raza et al., 1989) and inactivates these reactive molecules. For these reasons we suggest that liver fatty acid binding protein serves as an endogenous cellular protectant, participating as a scavenger of highly reactive products resulting from metabolic reactions and/or binding of products that induce cellular oxidative damage on the surface of membranes or in the cytosol. Further studies are required to delineate the mechanism of its antioxidant function. In conclusion, our data lead us to suggest that decreased expression of liver fatty acid binding protein contributes to hepatic oxidative stress and that the protein plays an important role in the pathogenesis of biliary disease.

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