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BASIC RESEARCH

Enhanced expressions and activations of leukotriene C4 synthesis enzymes in D-galactosamine/lipopolysaccharide-induced rat fulminant hepatic failure model

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Abstract

AIM: To investigate the expression and activity of leukotriene C4 (LTC4) synthesis enzymes and their underlying relationship with cysteinyl leukotriene (cys-LT) generation in a rat fulminant hepatic failure (FHF) model induced by D-galactosamine/lipopolysaccharide (D-GalN/LPS).

METHODS: Rats were treated with D-GalN (300 mg/kg) plus LPS (0.1 mg/kg) for 1, 3, 6, and 12 h. Enzyme immunoassay was used to determine the hepatic cys-LT content. Reverse transcription-polymerase chain reaction (RT-PCR), Western blot or immunohistochemical assay were employed to assess the expression or location of LTC4 synthesis enzymes, which belong to membrane associated proteins in eicosanoid and glutathione (MAPEG) metabolism superfamily. Activity of LTC4 synthesis enzymes was evaluated by determination of the products of LTA4 after incubation with liver microsomes using high performance liquid chromatography (HPLC).

RESULTS: Livers were injured after treatment with D-GalN/LPS, accompanied by cys-LT accumulation at the prophase of liver injury. Both LTC4 synthase (LTC4S) and microsomal glutathione-S-transferase (mGST) 2 were expressed in the rat liver, while the latter was specifically located in hepatocytes. Their mRNA and protein expressions were up-regulated at an earlier phase after treatment with D-GalN/LPS. Meantime, a higher activity of LTC4 synthesis enzymes was detected, although the activity of LTC4S played the main role in this case.

CONCLUSION: The expression and activity of both LTC4S and mGST2 are up regulated in a rat FHF model, which are, at least, partly responsible for cys-LT hepatic accumulation.

Key words: Cysteinyl leukotriene; Microsomal glutathione S-transferase 2; Leukotriene C4 synthase; D-galactosamine/lipopolysaccharide; Fulminant hepatic failure

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INTRODUCTION

Leukotriene (LT) is a potent lipid mediator with biological activities related to inflammation and allergy[1]. It was reported LT is involved in a variety of diseases, such as allergic airway disease, dermatological disease, cardiovascular disease and liver injury[2-5]. Fulminant hepatic failure (FHF) is a severe liver injury accompanying hepatic encephalopathy[6] and causes multi-organ failure with an extremely high mortality rate, even if intensive care is provided. Treatment is directed at an early recognition of the complications and general supportive measures. The only proven therapy for those who are unlikely to recover is liver transplantation[7,8]. Although it has been considered to be caused by several hepatitis viruses, various drugs, toxins, and metabolic disorders, its pathogenesis is extremely complicated, with indeterminate mechanisms[9,10]. There is evidence that inflammatory response and microcirculatory disturbances contribute to FHF[11-14]. Suppression of the inflammatory process in the liver is an effective therapy for fulminant or severe acute hepatic failure[15]. An experimental model of FHF induced

by D-galactosamine (D-GalN)/lipopolysaccharide (LPS) well simulates the situation in clinical FHF[11]. D-GalN can deplete liver UTP, disrupt protein synthesis and sensitize hepatocytes to challenging agents such as LPS[10,16]. It was reported that arachidonate metabolism is changed and LT formation is stimulated in D-GalN/LPS-induced acute liver injury[17,18]. Inhibition of LT biosynthesis or blocking binding of LT at the receptor level could also prevent liver injury induced by D-GalN/LPS[19,20]. Therefore, LT as an inflammatory factor is probably involved in the pathogenesis of FHF.

LT is a metabolite of arachidonic acid synthesized *via* the 5-lipoxygenase pathway[21]. LTC4 synthase (LTC4S) catalyzes LTA4 and reduced glutathione (GSH) generating LTC4 is the first committed step in the synthesis of cysteinyl leukotrienes (cys-LT), including LTC4, LTD4, and LTE4[22]. Like LTC4S, members of membrane associated proteins in eicosanoid and glutathione metabolism (MAPEG) superfamily, namely microsomal glutathione-S-transferase 2 (mGST2) and mGST3 also catalyze LTC4 synthesis[23-25]. However, it was reported that rat mGST3 is unable to synthesize LTC4 even in the presence of the conserved Arg/Try catalytic pair[26]. LTC4S is a pivotal enzyme for cys-LT biosynthesis in human lung membranes and platelet homogenates where it is prevalently expressed[27,28]. A recent study in LTC4S knockout mice suggested that LTC4S is a predominant *in vivo* source for cys-LT synthesis[29]. Its crystal structure that was recently interpreted provides structural insights into the mechanism of LTC4 formation[30,31]. It is up-regulated in LPS- induced systemic inflammatory reactions[32]. Our previous study showed that hepatic ischemia/reperfusion (I/R) injury up-regulates the mRNA and protein expression of LTC4S and its activity[33]. However, other studies reported that mGST may account for LTC4S-like activity in non-inflammatory cells[24]. mGST2, 44% identical to LTC4S in primary structure, is the principal enzyme responsible for LTC4 production in human liver microsomes and endothelial cells[34,35].

However, no reports are available on the alterations in both mRNA and protein of LTC4 synthesis enzymes including LTC4S and mGST2, their activities, and the cell types responsible for their expression during the FHF course. The mechanism underlying cys-LT generation at the early phase of FHF has not been extensively elucidated. Therefore, the present study aimed to investigate the expression and activity of LTC4 synthesis enzymes and the possible mechanism underlying cys-LT generation in a rat FHF model induced by D-GalN/LPS.

MATERIALS AND METHODS

Animals and experimental protocol

Male Sprague-Dawley rats, weighing 200 ± 20 g, were obtained from Experimental Animal Center, Zhejiang University. The animal study protocol, in compliance with the Guidelines of China for Animal Care, was approved by the Ethics Committee of Zhejiang University and conformed to the internationally accepted principles in the care and use of experimental animals.

Rats were randomly divided into five groups. Vehicle rats (control) were injected with saline, and decapitated immediately after the second injection. Other animals were intraperitoneally injected with D-GalN (300 mg/kg), followed by LPS (0.1 mg/kg) 1 h later. Rats were decapitated at 1, 3, 6 and 12 h, respectively, after LPS administration. Sera and liver tissue were collected for further evaluation.

Aminotransferase determination and histological evaluation

Serum ALT and AST were measured with an automatic analyzer (Hitachi 7600, Tokyo, Japan). The right lobe of liver was fixed in 4% paraformaldehyde for hematoxylin and eosin or immunohistochemistry staining. The left lobe of liver was snap-frozen in liquid nitrogen and stored at -80℃ until further preparation.

Paraffin-embedded liver tissue was cut into 5-μm thick sections, deparaffinized in xylene, and rehydrated through a series of decreasing concentrations of ethanol. The sections were stained with hematoxylin and eosin and analyzed under a light microscope.

Enzyme immunoassay

An aliquot of liver tissue (0.4 g) was individually homogenized in Dulbecco's phosphate-buffered saline and de-proteined with two volumes of ice-cold acetonitrile. Supernatant was collected before extraction with Sep-pak C18 cartridges (Water Corporation, USA). Methanol fraction was collected and evaporated to dryness under nitrogen for cys-LT measurement.

Cys-LT content in liver tissue was quantified with an enzyme immunoassay (EIA) kit (Cayman Chemical Company, USA) which recognizes LTC4, LTD4 and LTE4 for a combined quantitative value.

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from rat liver tissues using the TRIzol reagent (Sanggon) according to its manufacturer's instructions and quantified by measuring the ultraviolet absorption at 260 nm. For the synthesis of cDNA, 3 μg of total RNA from each sample was reverse-transcribed. Primers (Shanghai Sanggon, China) and application parameters for PCR analysis of LTC4S, mGST2 and β-actin are listed in Table 1. PCR products were analyzed by electrophoresis, visualized by ultraviolet transillumination of ethidium bromide-stained gels.

Western blotting analysis

Total protein was harvested from liver tissues. An aliquot of protein (80 μg) was electrophoresed on 12.5% SDS-polyacrylamide gels. After separation, proteins were transferred onto a pure nitrocellulose blotting membrane (Pall, Dreieich, Germany). Membranes were incubated with their corresponding antibody (1:200 for LTC4 synthase antibody from Santa Cruz). Blots were then exposed to the corresponding secondary antibody (horseradish peroxidase-conjugated IgG, Beijing Zhongshan Biotechnology Company, 1:4000). Immunocomplexes

Table 1 Primers and application parameters for PCR analysis of the respective genes

Gene	Primer	Designed size (bp)	Annealing temperature (℃)	Mg^{2+} (mmol/L)	Cycles
LTC4S	Sense 5′-GAAGAACTTTCCACGTGTCG-3′ Antisense 5′-GTGCAGCCATTGCCACTAGC-3′	282	55	2.0	35
mGST2	Sense 5′-TTCAATCAAGTTTTTGCAACC-3′ Antisense 5′-TCTTGGCAACATGAAAGTCC-3′	204	57	1.5	35
β-actin	Sense 5′-TGACGGGGTCACCCACACTGTGCCCATCTA-3′ Antisense 5′-CTAGAAGCATTTGCGGTGGACGATGGAGGG-3′	660	58	1.5	28

were visualized autoradiographically with an enhanced chemiluminescent substrate (ECL, Pierce, USA), and scanned using a bio-imaging analyzer (Bio-Rad, USA).

Immunohistochemistry

Immunohistochemistry was performed to determine the localization and protein expression of LTC4S or mGST2. Liver tissue was cut into 5-μm thick sections with a microtome and the sections were then mounted on slides. Streptavidin-biotin-complex (SABC, Boster Co, China) immunohistochemistry was used to explore the expression of mGST2. Briefly, endogenous peroxidase was inactivated. After treated with 5% BSA for 30 min, specimens were incubated at 4℃ overnight with primary antibody (mGST2 antibody from Abnova Corporation, Taiwan, final dilution 1:100; LTC4S antibody from Santa Cruz, USA, final dilution 1:200). Diaminobenzidine (DAB) was used for peroxidase reaction and the slides were counterstained with hematoxylin.

Reverse-phase HPLC analysis of LTC4 synthesis enzyme activity

Activity of LTC4 synthesis enzymes was assayed by measuring the amount of LTC4 and its isomer produced after incubation with liver microsomal fraction and LTA4 -free acid. LTA4 was saponified in acetone with 50 mmol/L NaOH (20% v/v) for 40 min at room temperature (RT) from LTA4 methyl ester (Cayman Chemical Co., USA). Microsomal fraction was harvested from liver tissue homogenates. An aliquot (100 μg) of microsomal fraction was incubated with 60 μmol/L LTA4- free acid, 10 mmol/L GSH and 0.2% BSA for 5 min at 37℃. The reaction was terminated by adding 100 μL stop solution (acetonitrile/methanol/acetic acid, 150:50:0.3, v/v) containing a defined amount of internal standard prostaglandin B2 (PGB2). Metabolites of LTA4 were resolved by isocratic RP-HPLC on an Agilent Zorbax SB-C18 column (4.6 mm × 250 mm, 5 μm). The mobile phase was at 1.0 mL/min comprised of acetonitrile: water: acetic acid (38:62:0.3, v/v, pH adjusted to 5.6 with $NH_3{\cdot}H_2O$). LTC4 and LTC4 isomer peaks were identified by comparing the retention time of LTC4 standard and on-line analysis of UV absorbance spectra. The amount of products formed was calculated by comparing the internal standard.

LC/MS assay

LTC4 generation was further identified by LC/MS. Assay was performed on a HP 1100 HPLC system with an Esquire-LC 00075 quadrupole mass spectrometer detection system. Agilent Zorbax SB-C18 column (4.6 mm × 250 mm, 5 μm) was used. The mass spectrometer was equipped with an electrospray ionization source. Typical source conditions were as follows: positive ionization mode, capillary 3.5 kV, skimmer 38.3 V, dry inert gas N_2 (10 L/min), temperature of the capillary 350℃, scan beginning at 200 m/z, scan ending at 700 m/z.

Statistical analysis

All data were expressed as mean ± SD. The results in different groups were compared by one-way analysis of variance (ANOVA) with SPSS 10.0 for WINDOWS. $P < 0.05$ was considered statistically significant.

RESULTS

Serum ALT and AST examination and histological evaluation

Rat liver injury was evaluated histologically and serologically after D-GalN/LPS treatment. As shown in Figure 1B, serum ALT and AST levels were continuously elevated and peaked at 12 h (392.2 ± 104.6 IU/L and 888.2 ± 190.8 IU/L respectively in the 12 h group, and 116.8 ± 39.4 IU/L and 156.2 ± 15.5 IU/L in the control group). ALT at 6, 12 h and AST at 3, 6, 12 h were significantly elevated in the D-GalN/LPS treatment group than those in the control group ($P < 0.05$).

Liver sections from the control group showed cord-like arrangement of hepatocytes with clear nucleus and intact endothelium in central veins without vascular congestion, observed with HE staining. Liver inflammation, found at 1 and 3 h after treatment with D-GalN/LPS, was characterized by congested central vein, infiltrated inflammatory cells and swollen hepatocytes. Dramatic injuries occurred in liver 6 and 12 h after treatment with D-GalN/LPS, and were characterized by disarranged hepatocytes, appearance of massive necrosis and broken cytolemma (Figure 1).

Hepatic cys-LT accumulation after D-GalN/ LPS treatment

Cys-LT in liver, determined with a cys-LT EIA kit, was significantly higher at 1 h in the D-GalN/LPS treatment group than in the control group (1167.9 ± 340.5 pg/g *vs* 236.3 ± 88.5 pg/g, $P < 0.05$), increased to 4.9 folds at 3 h, and gradually returned to its basal values at 12 h (Figure 2), suggesting that D-GalN/LPS injection at an early stage could lead to accumulation of cys-LT, which may play an important role in the onset of FHF.

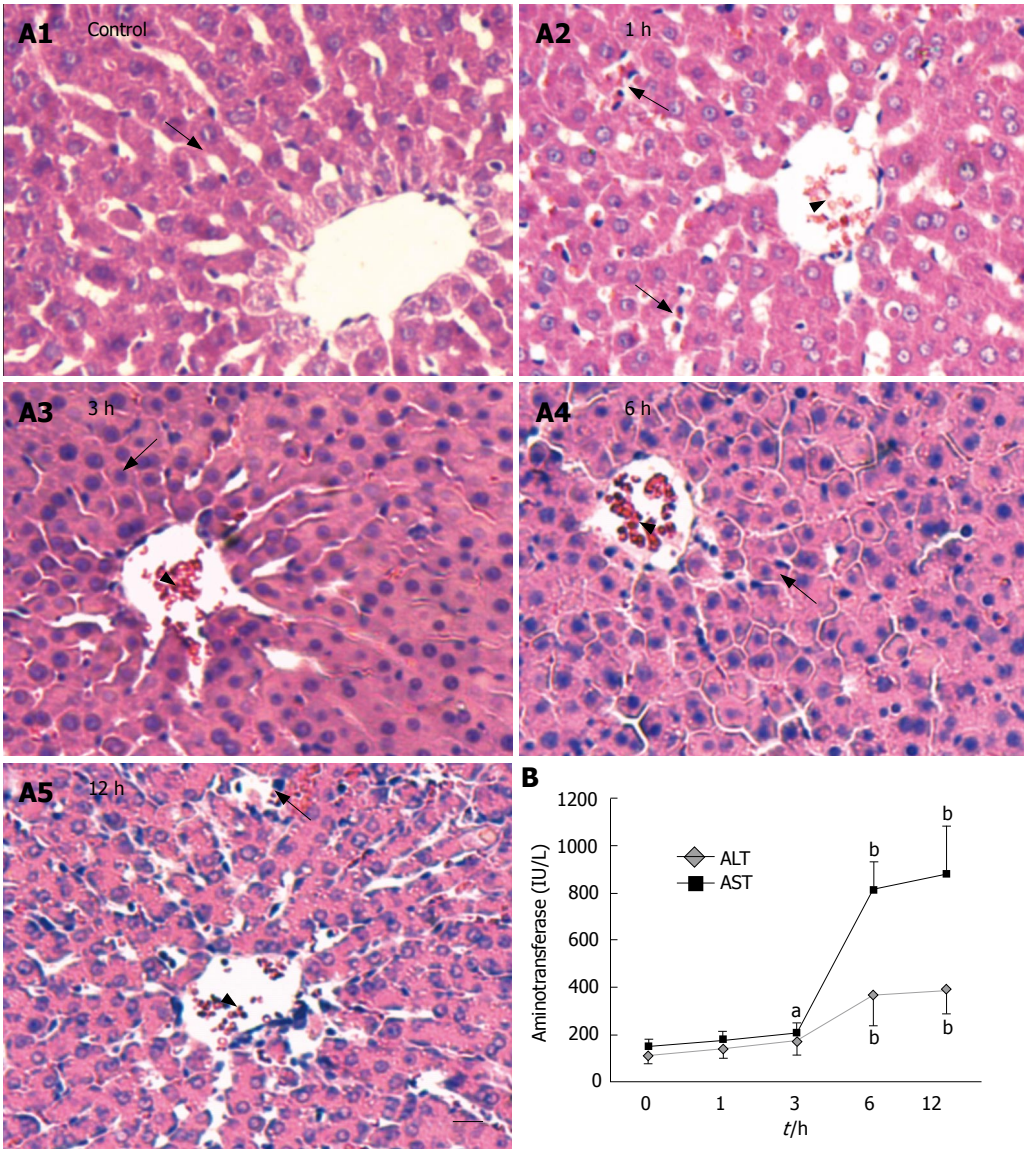

Figure 1 Histology and enzymology evaluation of rat livers treated with D-galactosamine/lipopolysaccharide (D-GalN/LPS). **A**: Histological examination of the liver at indicated times after GalN/LPS treatment (hematoxylin-eosin staining, bar = 100 μm). Arrow heads indicate congested central veins. The arrow in the control group **A1** indicates cord-like arrangement of hepatocytes with clear nucleoli. The arrows in the 1 h group **A2** indicate infiltrated inflammatory cells. The arrow in the 3 h group **A3** indicates swollen hepatocytes. The arrow in the 6 h group **A4** indicates disarranged hepatocytes. The arrow in the 12 h group **A5** indicates massive necrosis and broken cytolemma; **B**: Time course of serum ALT and AST in rats received intraperitoneal injection of D-GalN/LPS. Data are represented as mean ± SD, $n = 6$, $^aP < 0.05$ and $^bP < 0.01$ *vs* control group.

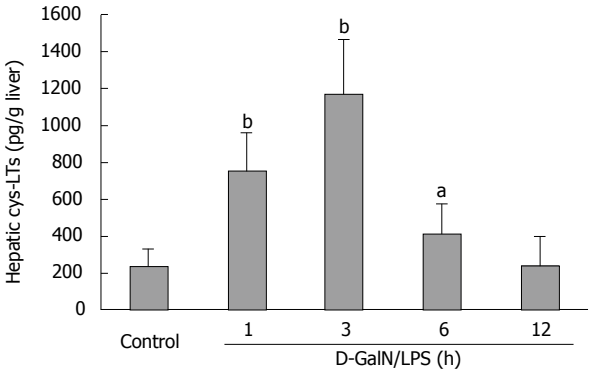

Figure 2 Time course of hepatic cys-LT content in D-GalN/LPS-injured rats. Rats were treated with D-GalN/LPS as described. Cys-LT was quantified with an enzyme immunoassay kit which recognizes LTC4, LTD4 and LTE4 for a combined quantitative value. Each value represents the mean obtained from six rats in each group. $^aP < 0.05$, $^bP < 0.01$ *vs* control group.

Expressions of LTC4S and mGST2 in liver tissues

mRNA and protein expressions of LTC4S and mGST2 were detected in D-GalN/LPS-injured liver tissues. Figure 3A shows a representative of hepatic mRNA expression of LTC4S and mGST2 in rat liver during FHF. Densitometric analysis of their PCR products revealed that mRNA expressions of LTC4S and mGST2 were significantly increased at 1 h after treatment with D-GalN/LPS ($P < 0.05$).

Considering the above changes in hepatic cys-LT content and the gene expressions of LTC4 synthesis enzymes, we examined their protein levels in rat liver tissues by Western blotting analysis. The protein level of mGST2 was not detectable by Western blotting analysis because the proper antibody was not commercially available. As shown in Figure 3B, the protein expression of LTC4S was obviously increased after D-GalN/LPS treatment.

Immunohistochemical localization of LTC4 synthesis enzymes in liver tissue

To clarify what cell types increased the expression of LTC4 synthesis enzymes in the rat FHF model, we performed immunohistochemical staining for paraffin-embedded liver sections from control and D-GalN/LPS treated rats.

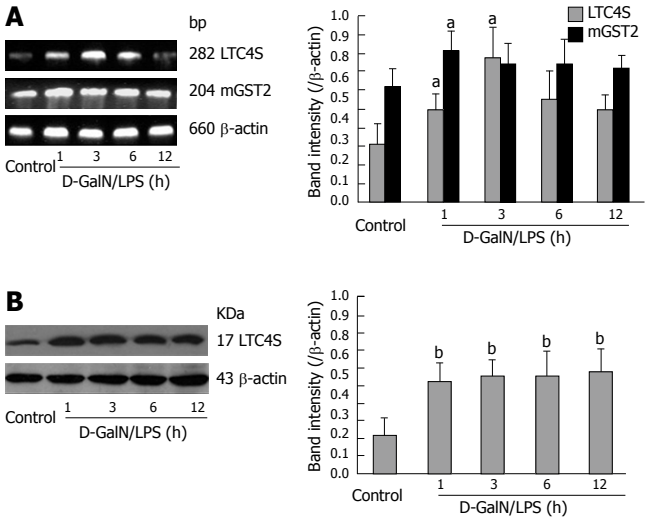

Figure 3 mRNA and protein expressions of LTC4S and mGST2 in D-GalN/LPS-treated liver tissues. **A**: mRNA expression of LTC4S and mGST2 detected by RT-PCR. cDNA synthesized from total RNA (3 μg) was used for PCR amplification with 35 cycles for *LTC4S* and *mGST2*. PCR products were electrophoresed on a 1.5% agarose gel. The intensity of each band was quantified by computer-assisted densitometry, and the data were compared to those of the corresponding band of β-actin (Right panel); **B**: Protein expression of LTC4S detected by Western blotting. An aliquot of total protein (80 μg) was subjected to immunoblot analysis as described in Methods. Left panel: Immunoreactive bands corresponding to LTC4S and β-actin in D-GalN/LPS-injured liver; right panel: Densities of the products quantified by computer-assisted densitometry, and the data were normalized to β-actin expression. Data are represented as mean ± SD, $^aP < 0.05$, $^bP < 0.01$ *vs* control group.

In normal liver, liver cells exhibited a low immunological staining for LTC4S (Figure 4A). In D-GalN/LPS treated rats, LTC4S was detected in endothelial cells and most hepatocytes which were strongly stained, exhibiting a heterogeneous intralobular distribution. However, no positive infiltrated inflammatory cells or Kupffer cells were found. As shown in Figure 4B, mGST2 was mainly located in hepatocytes and its expression was increased in D-GalN/LPS-injured rat livers.

Activity of LTC4 synthesis enzymes in D-GalN/ LPS-treated rats

The activity of LTC4 synthesis enzymes in liver microsomal fraction were determined by RP-HPLC. A peak at the retention time of 6.06 min in the indicated mobile phase appeared using an LTC4 standard (Figure 5A), which was also detected at the same retention time in lung microsomes, as a positive control, when the microsomal fraction was incubated with LTA4 and GSH (Figure 5B). MS analysis of the peak displayed a protonated molecular ion at m/z 625.8, corresponding to the LTC4 ion form (Figure 5C). We assessed the activity of mGST2 by identifying the isomer of LTC4, since mGST2 may produce not only LTC4, but also an isomer of LTC4 when incubated with LTA4 which is different from LTC4S[23,34]. A peak assessed as the LTC4 isomer was observed when the rat liver microsomal fraction was incubated with exogenous LTA4. The time course of LTC4 production in incubated samples is illustrated in Figure 5D. LTC4 production was increased after treatment with D-GalN/ LPS, suggesting that the activity of LTC4 synthesis was strengthened ($P < 0.05$). The activity of isomer of LTC4 was also increased, indicating that the activity of mGST2 was also initiated, demonstrating that the activity of both mGST2 and LTC4S in rat liver microsomes was increased after D-GalN/ LPS treatment.

DISCUSSION

Cys-LT has a wide variety of physiological and immunological effects on liver function and disease[36-39]. Cys-LT, as an inflammatory factor, is involved in the pathogenesis of FHF[17,18], which has attracted the interest of clinicians[40]. However, the mechanism of cys-LT alteration during FHF has not been well elucidated yet. Thus, we employed a D-GalN/LPS-induced rat model of FHF to explore the possible reason of LTC4 alteration in the pathogenesis of FHF.

In the present study, we evaluated the inflammatory cell infiltration, hepatocytes damage and structure disorder after D-GalN/LPS treatment. Serological data further confirmed the impairment of liver function at a later phase of treatment. However, the hepatic cys-LT content was transiently increased before the occurrence of liver injuries. It was reported that inhibiting cys-LT biosynthesis or blocking binding of cys-LT at the receptor level can prevent liver injury[19,20]. D-GalN not only acts directly on hepatocytes by depleting liver UTP and disrupting protein synthesis, but also sensitizes hepatocytes to challenging agents such as LPS[10,16]. In addition, liver injury induced by D-GalN/LPS well simulates the situation in clinical FHF[11]. Our results suggested that cys-LT accumulation plays a role in D-GalN/LPS-induced FHF.

To elucidate whether cys-LT accumulation is related to LTC4 synthesis enzymes in the rat FHF model, we examined their expression and activity owing to lack of enough information in this model. The committed step in cys-LT biosynthesis in rats is catalyzed by LTC4 synthesis enzymes, but not by mGST3[22-24]. It was reported that mRNA expression of LTC4S in liver is so low that Northern blotting analysis is not sufficiently sensitive to detect it[29,41]. In the present study, the mRNA expression of mGST2 was significantly higher than that of LTC4S by RT-PCR. Protein expression of LTC4S was detected in rat liver instead of purified hepatocytes by Western blotting assay. Immunohistochemical staining was used to clarify which cell types expressed LTC4 synthesis enzymes and whether the expression was changed in the rat FHF model. The results showed that LTC4S was expressed in hepatocytes and sinusoidal endothelial cells, while mGST2 was only located in hepatocytes. Unlike the report of Schröder[32] that mGST2 remained unchanged after a single dose of LPS-induced systemic inflammatory reaction, our results showed that both LTC4S and mGST2 were increased in liver after D-GalN/LPS treatment. The result suggested that mGST2 may play a special role in the pathogenesis of FHF. The discrepancy may be due to the pretreatment with D-GalN and the different treatment time or dose of LPS[42]. D-GalN, as a specific hepatotoxic agent, renders hepatocytes sensitive to LPS.

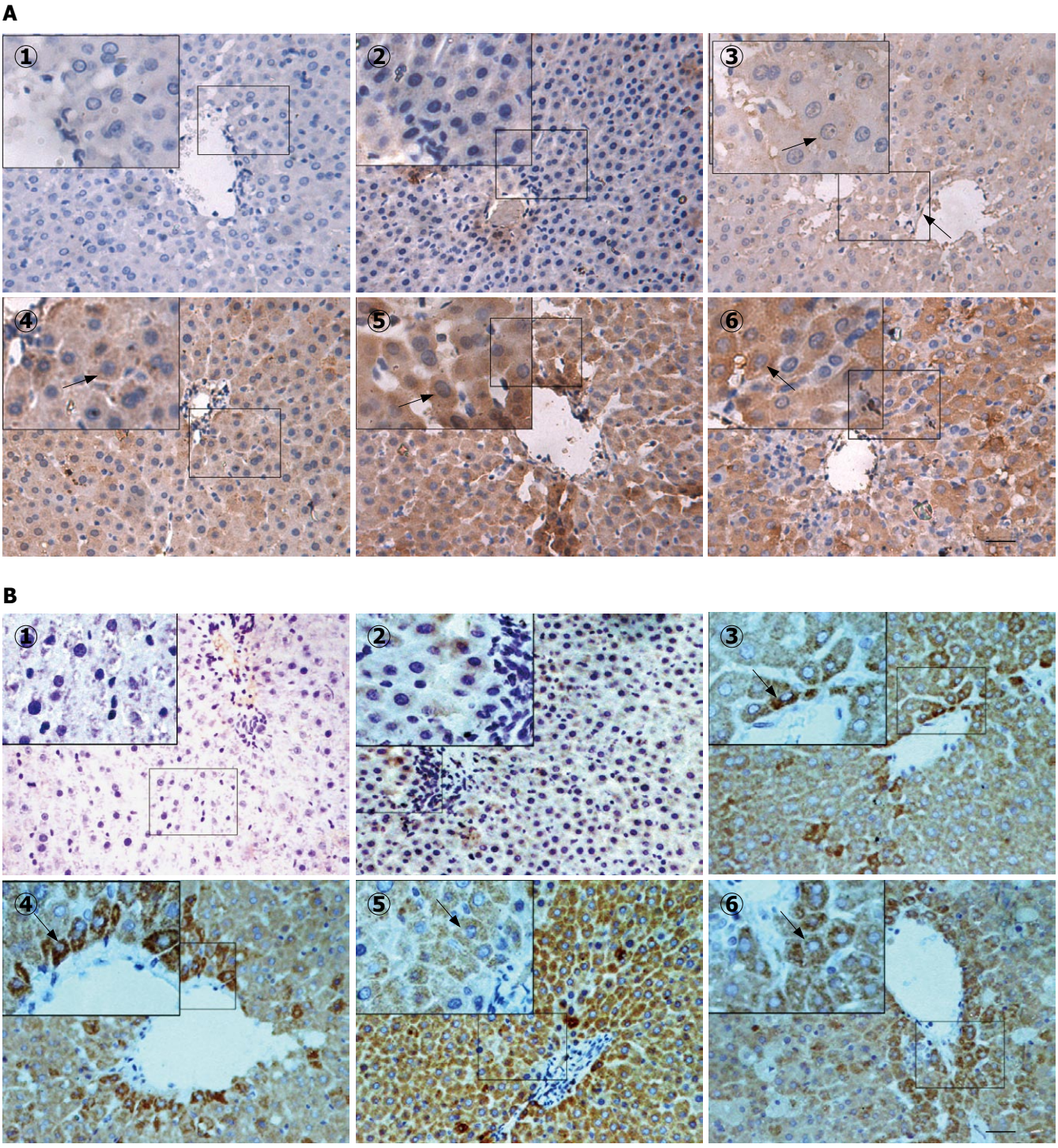

Figure 4 Immunohistochemical assay of the expression and localization of LTC4S (**A**) and mGST2 (**B**) in control and D-GalN/LPS-treated liver tissues. Immunohistochemical staining for paraffin-embedded liver sections from the control and D-GalN/LPS-treated rats was examined as described in Materials and Methods. Arrows indicate the representative positive cells expressing the brown granules. 1: Absence of staining on omission of primary antibody; 2: Control; 3-6: Groups treated with D-GalN/LPS at 1, 3, 6 and 12 h, respectively. Bar = 100 μm.

Pretreatment with D-GalN creates a different pathogenesis of liver injury. Different treatment time or dose of LPS could also produce different effects. For instance, LPS is usually thought to be a potent stimulus for LT production *in vivo*[43,44]. However, it was reported that LPS down-regulates cys-LT release and LTC4S gene expression in mononuclear phagocytes[45]. In the present study, LTC4S and mGST2 expressions were up-regulated after treatment with D-GalN (300 mg/kg) and LPS (0.1 mg/kg). Furthermore, the liver not only plays a major role in metabolism and elimination of LT, but also produces cys-LT[46]. It was reported that hepatocytes generate cys-LT when co-cultured with Kupffer cells[47,48]. Therefore, increased expression of LTC4 synthesis enzymes in liver may partly contribute to cys-LT accumulation.

The activity of LTC4 synthesis enzymes was subsequently detected in D-GalN/LPS-induced rat FHF. The appearance of isomer of LTC4 in HPLC traces

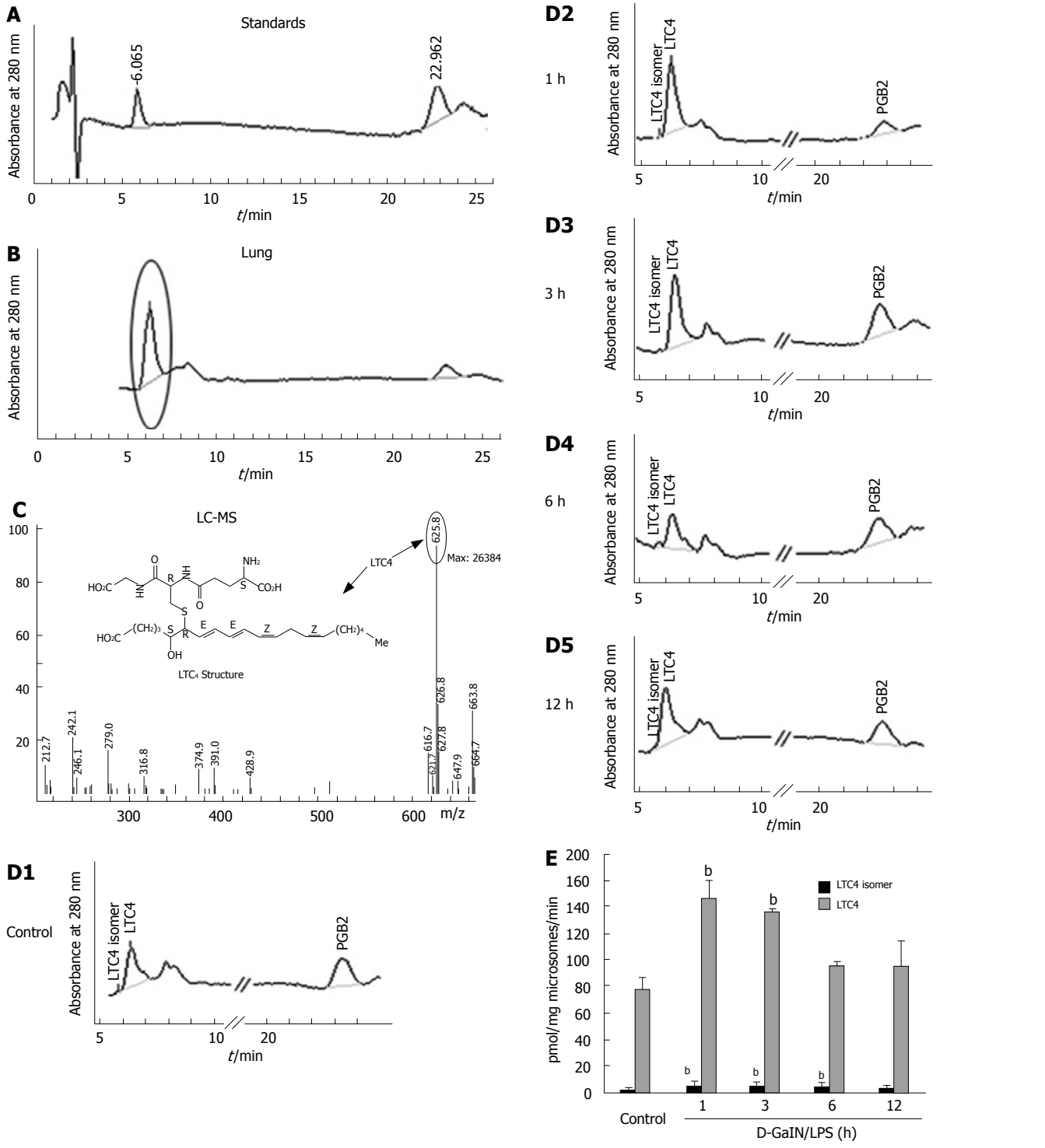

Figure 5 LTC4 synthesis enzyme activity in rat liver microsomal fraction. **A**: HPLC traces showing standard LTC4 and internal standard PGB2; **B**: HPLC traces showing the main products when 100 000 × g pellets from lung incubated with LTA4 (60 μmol/L) and glutathione (10 mmol/L); **C**: MS assay of the product showing its peak at the retention time of 6.06 min in HPLC; **D**: HPLC traces showing generated LTC4 and isomer of LTC4 by a microsomal fraction from D-GalN/LPS treated rat livers with indicated times; **E**: The amount of generated LTC4 and isomer of LTC4 was calculated by an area peak compared with the internal standard PGB2 and then plotted. Each value represents the mean obtained from duplicate experiments with liver tissue from six rats in each group. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control group.

suggested the activity of mGST2 in rat liver, since the isomer was used to distinguish between mGST2 and LTC4S activities involved in the biosynthesis of LTC4[23,34]. According to Scoggan *et al*[34], a ratio of LTC4/LTC4 isomer < 50 is typical for a system containing mostly mGST2 whereas a ratio > 50 is seen in tissues containing mostly LTC4S. The minimal ratio in our study was 32, suggesting that mGST2 and LTC4S have activities in liver microsomes, but LTC4S takes the main responsibility in this case. The mGST2 activity was simultaneously increased, as demonstrated by the increased LTC4 isomer. mGST2 in liver is a functional enzyme and plays a role in LTC4 biosynthesis. Since mGST2 was not changed after a signal dose LPS treatment, the synergistic effect of D-GalN and

LPS may be the reason why the expression and activity of mGST2 were increased, which may partly contribute to the pathogenesis of FHF.

In conclusion, the expressions and activities of both LTC4S and mGST2 are up-regulated in a rat FHF model, which are, at least, partly responsible for cys-LT hepatic accumulation.

ACKNOWLEDGMENTS

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COMMENTS

Background

Cysteinyl leukotriene (cys-LT), as an inflammatory factor, is probably involved in fulminant hepatic failure (FHF). LTC4 synthesis enzymes catalyze LTA4 to generate LTC4. However, no report on the alterations in mRNA and protein of LTC4S and mGST2 and their activities during FHF is available. The mechanism underlying cys-LT generation at an early phase of FHF has not been elucidated. Therefore, we focused on the expression and activity of LTC4 synthesis enzymes and their relationship with cys-LT generation in a rat FHF model induced by D-galactosamine/ lipopolysaccharide (D-GalN/LPS).

Research frontiers

LTC4S is a pivotal enzyme for cys-LT biosynthesis in human lung membranes and platelet homogenates where it is prevalently expressed. Studies in the LTC4S knockout mice suggest that LTC4S is a predominant *in vivo* source for cys-LT synthesis. Its crystal structure was recently interpreted, which provides structural insights into the mechanism of LTC4 formation. It is up-regulated in LPS-induced systemic inflammatory reactions. Our previous study showed that hepatic infusion/ reperfusion (I/R) injury can up-regulate mRNA and protein expression of LTC4S and enhance its activity. However, other studies reported the mGST may account for LTC4S-like activity in non-inflammatory cells. mGST2, 44% identical to LTC4S in primary structure, is the principal enzyme responsible for LTC4 production in human liver microsomes and endothelial cells.

Innovations and breakthroughs

Increased expression of LTC4 synthesis enzymes may partly contribute to cys-LT accumulation and liver injury in a rat FHF model. mGST2 and LTC4S contribute to the pathological process of this model.

Applications

The results provide a rationale for the pathphysiological process of FHF and potential drug molecular targets.

Terminology

Cysteinyl leukotriene: A potent lipid mediator with biological activities related to inflammation and allergy. LTC4 synthesis enzymes include LTC4S, mGST2 and mGST3.

Peer review

The authors showed that cys-LT accumulation in FHF was due to enhanced expression and activity of LTC4S and mGST2. The present study is interesting and instructive. The data are well described in this paper. The paper provides useful information on the daily management of patients with viral hepatitis.

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