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• ORIGINAL RESEARCH •

Expression of lipopolysaccharide binding protein and its receptor CD14 in experimental alcoholic liver disease

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Abstract

AIM: **To evaluate the relationship between the expression of lipopolysaccharides (LPS) binding protein (LBP) and CD14 mRNA and the severity of liver injury in alcohol-fed rats.**

METHODS: **Twenty Wistar rats were divided into two groups: ethanol-fed group (group E) and control group (group C). Group E was fed with ethanol (5-12 g·kg^{-1}·d^{-1}) and group C received d extrose instead of ethanol. Rats of the two groups were sacrificed at 4 weeks and 8 weeks. Levels of endotoxin and alanine transaminase (ALT) in blood were measured, and liver pathology was observed under light and electronic microscopy. Expressions of LBP and CD14 mRNA in liver tissues were determined by RT-PCR analysis.**

RESULTS: **Plasma endotoxin levels were increased more significantly in group E (129±21) ng·L^{-1} and (187±35) ng·L^{-1} at 4 and 8 wk than in control rats (48±9) ng·L^{-1} and (53±11) ng·L^{-1}, respectively (P<0.05). Mean values of plasma ALT levels were (1867±250) nkat·L^{-1} and (2450±367) nkat·L^{-1} in Group E. The values were increased more dramatically in ethanol-fed rats than in Group C after 4 and 8 weeks. In liver section from ethanol-fed rats, there were marked pathological changes (steatosis, cell infiltration and necrosis). In ethanol-fed rats, ethanol administration led to a significant increase in LBP and CD14 mRNA levels compared with the control group (P<0.05).**

CONCLUSION: **Ethanol administration led to a significant increase in endotoxin levels in serum and LBP and CD14 mRNA expressions in liver tissues. The increase of LBP and CD14 mRNA expression might wake the liver more sensitive to endotoxin and liver injury.**

Subject heading lipopolysaccharides/analysis, antigens, cd14/analysis, liver diseases, alcoholic/pathology, liver/pathology, liver/ultrastr ucture, rat, animal

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INTRODUCTION

There is an accumulating evidence suggesting a role for endotoxin or lipopoly-sac charide(LPS) in the cause of alcohol-induced liver disease (ALD)[1-5]. Circulating LPS consists of several compounds including its specific carrier, the LPS binding protein (LBP)[6-8]. The LPS-LBP complex has high affinity to the LPS receptor CD14 located on monocytes/macrophages. CD14 is a 55-kD myeloid membrane glycoprotein, expressed mainly by monocytes and macrophages[7,9-11]. Attachment of LPS-LBP complex to the CD14 initiates a process lead in g to the release of cytokines and liver injury[13-17]. Although indirect evidence cited previously suggested an interaction among LPS, LBP and CD14 during ALD[18-20], a direct link is lacking. To evaluate the role of LBP and CD14 in ALD, the intragastric ethanol porat model for ALD was used to study the relationship between the expression of LBP and CD14 genes and the severity of liver injury in ALD rats.

MATERIALS AND METHODS

Animals and treatments

Twenty adult female Wistar rats weighing 180 and 220 g were fed ad libitum a liquid diet. They were divided into two groups (ten rats/group): et hanol liquid diet group (group E) and control liquid diet group (group C). Group E were fed ethanol, and group C received the same diet but with isocaloric amounts of dextrose instead of ethanol. In the ethanol-fed rats, an initial dose of 5 g·kg^{-1}·d^{-1}. The ethanol concentration within the diet was gradually increased up to 12 g·kg^{-1}·d^{-1} in 8 wk. All diets were kept fresh daily. They were anesthetized with sodium pentobarbital (30 mg kg^{-1} intraperitoneally) and sacrificed at different time points (4 wk and 8 wk). Blood was withdrawn from the tail vein and liver samples were frozen in liquid nitrogen and stored at -70℃ before use.

Blood endotoxin and ALT

For determination of endotoxin, blood was collected into pyrogen-free tubes containing heparin. Plasma was immediately separated at 4℃ by centrifugation at 200 g for 8 minutes and stored in pyrogen-free tubes at -70℃. Plasma en dotoxin levels were measured within a week using the Limulus Amebocyte Lysate assay. Levels of endotoxin in plasma from normal rats were below the limits of detection. Serum alanine transaminase (ALT) was measured by standard enzymatic procedures.

Liver pathology

Liver samples from different liver lobes were fixed with 100 ml·L^{-1} buffered formalin or 25 g·L^{-1} glutaraldehyde immediately. For optical microscopy, the tissue blocks were embedded in paraffin, and stained with hematoxylin and eosin (HE). For electronic microscopy, the tissue blocks were embedded in Epon 618 resin and ultrathin sections

were stained with urany acetate and lead citrate. A H-2000 transmission electron microscope was used.

RNA isolation and complementary DNA synthesis

Total RNA was isolated from rat liver tissue using the TRIZOL Reagent (Life Technologies, USA). The quality of RNA was controlled by the intactness of ribosomal RNA bands. A total of 0.5 mg of each intact total RNA samples was reverse-transcribed to complementary DNA (cDNA) using the reverse transcription polymerase chain reaction (RT-PCR) kit (Roche, USA). cDNA was stored at -70¡æ until polymerase chain reaction (PCR) analysis.

Determination of LBP and CD14 mRNA by RT-PCR

The PCR primers used were LBP: sense (5'-GAGGCCTGAGTCTCTCCATCT-3'), antisense (5'-TCTGAGATGGCAAAGTAGACC -3'); CD14: sense (5'-CTCAACCTAGAGCCGTTTCT-3'), anti-sense (5'-CAGGATTGTCAGACAGGTCT-3'); β-actin: sense (5'-ACCACAGCTGAGAGGGA-A ATCG-3'), antisense (5'-AGAGGTCTTTACGGATGTC-AACG-3'). The sizes of the amplified PCR products were 552 bp for LBP, 267 bp for CD14, and 281 bp for β-actin. The conditions for amplification were as follows: denaturation at 94℃ for 1 min, annealing at 58℃ for 2 min, and extension at 71℃ for 2 min for 28 cycles. The PCR products were electrophoresed in 20 $g \cdot L^{-1}$ agarose gels, and the gels were ethidium bromide stained and videophotographed on an ultraviolet transilluminator.

Statistical analysis

All results were expressed as $X \pm S_x$. Statistical difference between means were determined using two-way ANOVA or Student's *t* test. A *P* value of $\leqslant 0.05$ was considered significant.

RESULTS

Blood endotoxin and ALT levels

Plasma endotoxin levels in control rats were (48±9) $ng \cdot L^{-1}$ and (53±11) $ng \cdot L^{-1}$ at 4 wk and 8 wk, respectively. Plasma endotoxin levels in ethanol -fed rats were increased significantly by ethanol to values of (129±21) $ng \cdot L^{-1}$ at 4 wk and (187±35) $ng \cdot L^{-1}$ at 8 wk. The Levels of endotoxin were about 2-and 3-fold higher than the values from control rats (Figure 1). Me an values for ALT in the control animals were (517±200) $nkat \cdot L^{-1}$ and (550±150) $nkat \cdot L^{-1}$ at 4 wk and 8 wk, respectively. Plasma ALT levels were increased dramatically to (1867±250) $nkat \cdot L^{-1}$ and (2450±367) $nkat \cdot L^{-1}$ in ethanol-fed rats after 4 wk and 8 wk, respectively (Figure 2).

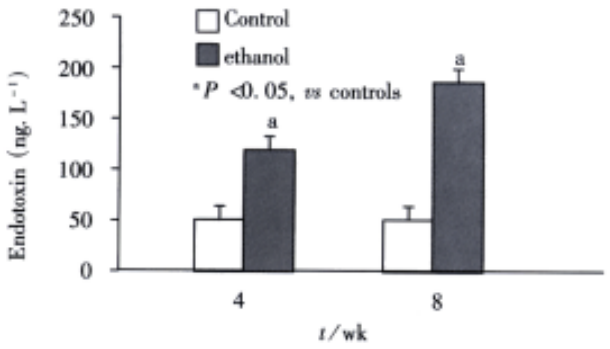

Figure 1 Chenges of endotoxin levels in two groups at different time points.

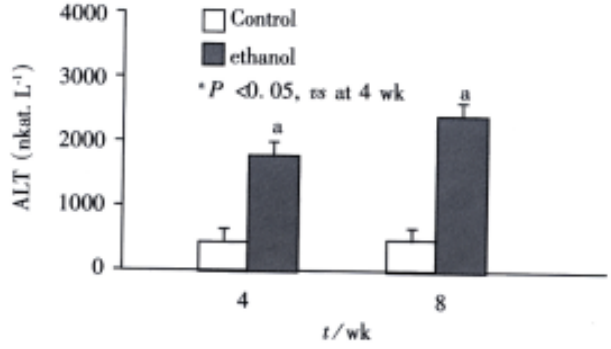

Figure 2 Changes of serum transaminase levels in two groups at different time points.

Pathological changes

Rats in the two groups, increased their body weight. Although the average weight gain was lower in ethanol-fed rats than the control rats, the differences between the two groups were not significant. Histopathological changes of the liver tissues were depicted in rep resentative photomicrographs in Figure 3A and 3B. None of the rats in the control group deve loped pathological changes in the liver at 4 wk or 8 wk. But, in liver section from rats after 4 wk on ethanol liquid diet, steatosis which was both microvesicular and macrovesi cular and few in flammation but accumulation of blood cells in the sinusoidal lining can be seen. In liver section from rats after 8 wk on ethanol diet, there was marked pathological changes (steatosis, cell infiltration and necrosis). Under electron microscopy, focal cytoplasmic degeneration and necrosis could be seen in hepatocytes of ethanol-fed rats(Figure 4A and B).

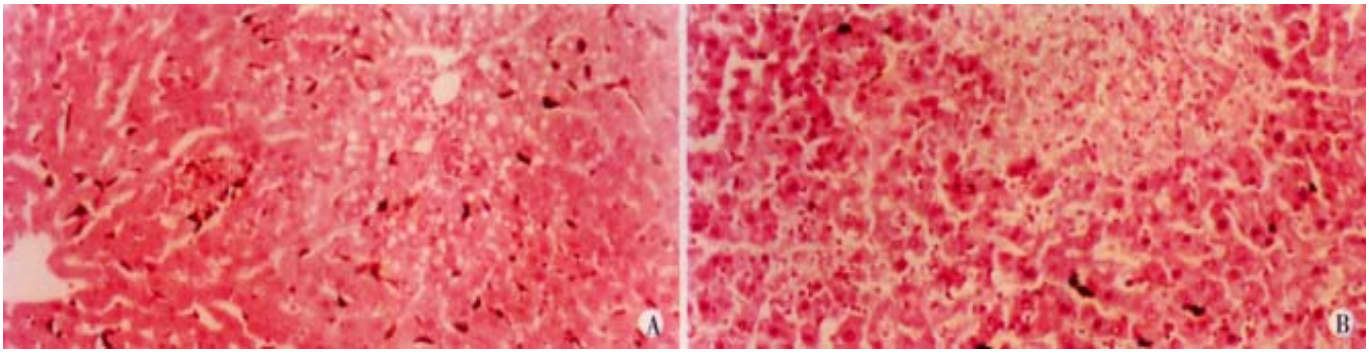

Figure 3 Liver section from rats (HE×200) A: 4 wk after ethanol liquid diet, steatosis and accumulation of blood cells in the sinusoidal lining; B: 8 wk after ethanol diet, steatosis, cell infiltration and necrosis.

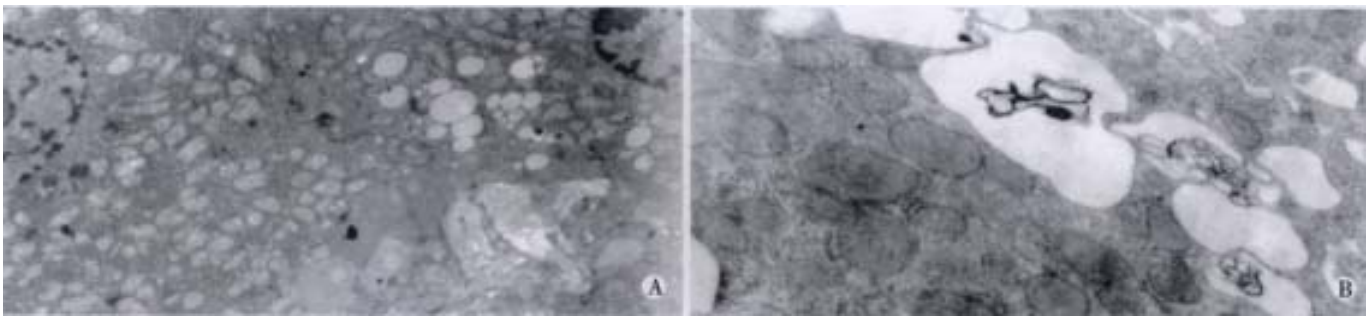

Figure 4 Ethanol-fed rats. A: Steatosis degeneration and necrosis in hepatocytes TEM×5000 B: Focal cytoplasmic degeneration and many myelin figures (TEM×20 000).

Expression of LBP and CD14 mRNA in the liver

The livers of rats in the individual group were examined for LBP and CD14 mRNA expression by RT-PCR (Figure 5). In the control rats, there was no significant difference in the levels of LBP and C D14 at 4 wk and 8 wk. Ethanol administration led to a significant increase in LBP and CD14 mRNA levels compared with the control group ($P<$ 0.05). The levels of LBP and CD14 mRNA in ethanol-fed rats were significantly higher in 8 wk than in 4 wk ($P<0.05$). The highest levels of CD14 mRNA were seen in ethanol-fed rats after 8 wk (Figure 6).

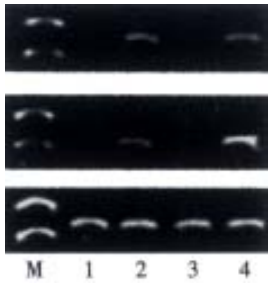

Figure 5 Expression of LBP and CD14 mRNA by RT-PCR analysis. M: Marker; Lane 1, 3: Group C in 4, 8 wk respectively. Lane 2, 4: Group E in 4, 8 wk.

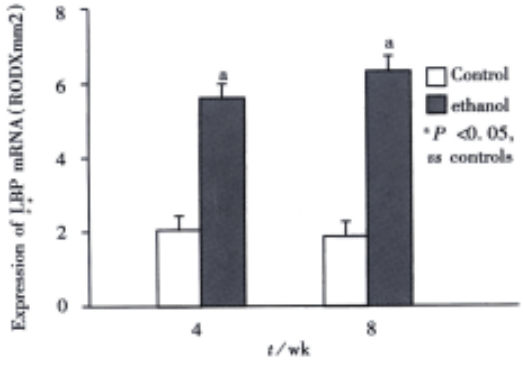

Figure 6 Expression of LBP mRNA.

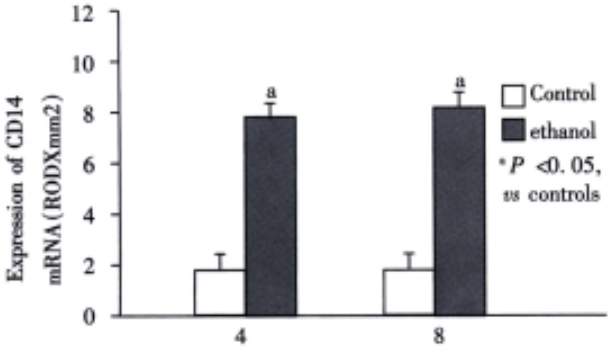

Figure 7 Expression of CD14 mRNA.

DISCUSSION

It is well documented that liver disease can result from the dose- and time-dependent consumption of alcohol[23-25] , female rats exhibit greater susceptibity to early alcohol-induced injury than males[26-28], Glycine prevents alcohol-induced liver injury by decreasing alcohol in the stomach[29]. However, mechanisms re main unclear. There appears to be increasing evidences that ethanol toxicity is associated with increased level of endotoxin in plasma[1,2,31]. Endotoxin or LPS is believed to exert many of its effects on the liver injury via interaction with LBP and CD14[11-18]. LBP and CD14 are clearly implicated in the mo lecular and cellular basis of the interaction between endotoxin and monocytes/ macrophages. LBP in serum can recognize and bind LPS to form LPS-LBP complexes and activate cells through the CD14 receptor on membrane of these cells, initiate a process leading to the release of cytokines (e.g. tumor necrosis factor a and interleukines), prostanoids, and other soluble mediators[24,31-34].The release of these mediators is considered to be an early key step in the pathogenesis of liver disease because they trigger inflammatory events in the liver and alter the parenchymal homeostasis, ultimately initiating liver injury[35-38].

A major goal of this study was to observe the expression of LBP and CD14 mRNA in ethanol-fed rats an d evaluate the in role in ALD. It was found that endotoxin levels in the plasma of rats treated with ethanol were increased significantly when compared with control animals and fatty liver, necrosis, and inflammation were developed in the ethanol treated rat. Control rats showed no liver pathology. In the present study, we found the severity of pathological changes in ethanol-fed rats were accompanied by an increase in intrahepatic LBP and CD14 mRNA levels and serum ALT levels. The increase in LBP and CD14 mRNA levels in the ethanol-fed rats is correlated with the degree of inflammation and necrosis in the livers of these animals. A similar pattern of changes was observed by Yin, *et al*[39]. They found that blood end otoxin and hepatic levels of CD14 messenger RNA and protein were increased by ethanol. Therefore, the sensitivity of rat liver to alcohol-induced injury is directly related to CD14 expression in the liver that lead to increasing the production of TNF-á, free radicals, interleukins and other cytokines[40-44]. The marked increase in CD14 expression suggests a new mechanism by which alcohol increases the LPS-mediated cytokine signaling by the liver macrophages, thus promoting the interaction between alcohol and endoto xins in the development of liver damage[45-50].

It has been well established that the role of LBP is to augment the response of monocytes/macrophages to low levels of endotoxin via interaction with CD14 protein and play an important role in alcoholic liver injury[36,]. The increase in intrahepatic CD14 m RNA expression may represent either an increase in the expression of CD14 within cells that reside in the liver or may represent recruitment of inflammatory cells(e.g., infiltrating mononuclear cells or macrophages) that have high expression of CD14 gene and CD14 protein[39,51-53]. In either case, an increase of C D14 may result in greater sensitivity to

endotoxin and NF-kB activation and production of pro-inflammatory cytokines which mediate liver injury[54-57].

In summary, our results show that ethanol administ ration led to a significant increase in LBP and CD14 mRNA levels in ethanol-fed rats when compared with the control rats. Increase of LBP and CD14 mRNA expression may result in greater sensitivity to endotoxin and liver injury. However, the mechanism of LBP increase and CD14 mRNA express ion is thus as yet unclear and needs further studies.

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