Liver sinusoidal endothelial cell injury by neutrophils in rats with acute obstructive cholangitis

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AIM: The objective of this study is to elucidate the potential role of polymorphonuclear neutrophils (PMN) in the development of such a sinusoidal endothelial cell (SEC) injury during early acute obstructive cholangitis (AOC) in rats.

METHODS: Twenty one Wistar rats were divided into three groups: the AOC group, the bile duct ligated group (BDL group), and the sham operation group (SO group). The common bile duct (CBD) of rats in AOC group was dually ligated and 0.2ml of the E. coli O111: B4 (5×10^10cfu/ml) suspension was injected into the upper segment, in BDL group, only the CBD was ligated and in SO group, neither injection of E. coli suspension nor CBD ligation was done, but the same operative procedure. Such group consisted of seven rats, all animals were killed 6h after the operation. Morphological changes of the liver were observed under light and electron microscope. Expression of intercellular adhesion molecule-1 (ICAM-1) mRNA in hepatic tissue was determined with reverse transcription polymerase chain reaction (RT-PCR). The serum levels of alanine aminotransferase (ALT) which was performed with autoanalyger. Hepatic injury was assessed by measuring the serum alanine aminotransferase (ALT) were performed with autoanalyger.

RESULTS: Neutrophils was accumulated in the hepatic sinusoids and sinusoidal endothelial cell injury existed in AOC group. In contrast, in rats of BDL group, all the features of SEC damage were greatly reduced. Expression of ICAM-1 mRNA in hepatic tissue in three groups were 7.54±0.82, 2.87±0.34, and 1.01±0.12, respectively. There were also significant differences among the three groups (P<0.05). Activity of the serum ALT was 917±167nkat·L⁻¹, 901±171nkat·L⁻¹, and 908±164nkat·L⁻¹, respectively, (P>0.05).

CONCLUSION: Hepatic SEC injury occurs earlier than hepatic parenchymal cells during AOC. Recruitment of circulating neutrophils in the hepatic sinusoidal space might mediate the SEC injury, and ICAM-1 in the liver may modulate the PMN of accumulation.

INTRODUCTION

Biliary tract infection, especially acute obstructive cholangitis (AOC) is common[5-9]. Despite a multitude of advances in treatment of surgical infection, this remains a significant cause of morbidity and mortality[5-9], where sepsis and endotoxemia from AOC are important causes of multiple organ failure (MOF) and death[10,11]. Ohtsuka et al[10] reported that polymorphonuclear neutrophils (PMN) accumulated in the hepatic sinusoidal space increased obviously in rats with obstructive jaundice and there were interaction between PMN and sinusoidal endothelial cells (SEC) causing injury during AOC. This study was study the potential role of PMN in the development of SEC injury and the mechanism of accumulation of PMN during early period of AOC.

MATERIALS AND METHODS

Animal Experiment

Male Wistar rats weighing 200-230g were purchased from Laboratory Animal Center of Chongqing University of Medical Science. These animals were divided into three groups: the AOC group, bile duct ligated group of (BDL group), and sham operation group (SO group). All the animals were killed 6h after operation, the surgical procedures were carried out under light diethyl ether anaesthesia. The animal models were performed as follows. In AOC group, a 1.5cm medium incision was made over the upper abdomen, the common bile duct (CBD) was mobilized and dually ligated, and 0.2ml of the E. coli O111: B4 (5×10^10cfu·L⁻¹) (Sigma, USA) suspension was injected into the upper segment. In BDL group, the CBD was doubly ligated but without injection of E. coli O111: B4 suspension. In SO group, neither E. coli injection of suspension nor CBD ligation was done, but only routine operative procedure was performed. Seven rats constituted each group.

Serum ALT & CINC

Hepatic injury was assessed by measuring the serum alanine aminotransferase (ALT) which was performed with autoanalyger. The serum cytokine-induced neutrophil chemoattractant (CINC) concentration was measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s direction with a lower limit of 10ng·L⁻¹. For CINC, microtitre plates were coated with anti CINC mAb overnight at room temperature on a plate shaker, after the blockage, samples were then added. The detected antibody was biotinylated anti-CINC. Standard curves were made with a natural CINC calibrated against the WHO interim International Standard.

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Abstract

AIM: The objective of this study is to elucidate the potential role of polymorphonuclear neutrophils (PMN) in the development of such a sinusoidal endothelial cell (SEC) injury during early acute obstructive cholangitis (AOC) in rats.
Expression of ICAM-1 mRNA in Hepatic Tissue

Total RNA was isolated from rat liver tissue by using the Trizol Reagent (Life Technologies, USA). The quality of RNA was controlled by the intactness of ribosomal RNA bands. A total of 0.5mg of each intact total RNA sample was reverse-transcribed to complementary DNA (cDNA) by using reverse transcription polymerase chain reaction (RT-PCR) kit (Roche, USA). cDNA was stored at -70°C until PCR analysis. The PCR primers used were ICAM-1: sense (5'-CTTCTCTGTCTGCAACCC-3'), antisense (5'-GGGAGACATTCAGGTC-3'); β-actin: sense (5'-ACCACAGCTGAGAGGGAAATCG-3'), antisense(5'-AGAGGTCTTTACGGATGTCAACG-3'). The sizes of the amplified PCR products were 326 bp for ICAM-1 and 281 bp for β-actin. The procedure was as follows: denaturation at 95°C for 30sec, annealing at 55°C for 1min, and extension at 71°C for 1min for 28 cycles. The PCR products were electrophoresed in 20g·L⁻¹ agarose gels, and the gels were ethidium bromide stained and video photographed on an ultraviolet transilluminator. The bands representing reactive product were scanned by densitometer of a Bio-Image Analysis System (Doc Gel 2000). The relative optical density (ROD) values were expressed as the level of ICAM-1 mRNA in hepatic tissue.

Morphologic Observations of Hepatic Tissue

Liver samples from different liver lobes were fixed with 100mL·L⁻¹ buffered formalin or 25g·L⁻¹ glutaraldehyde immediately. For light microscopy, the tissue blocks were embedded in paraffin, and the sections were stained with hematoxylin and eosin (H&E). For transmission electron microscopy (TEM), the tissue blocks were embedded in Epon 618 resin and ultrathin sections were stained with uranyl acetate and lead citrate. A H-2000 transmission electron microscope was used.

Statistical Analysis

Results were presented as x±s. Statistical difference was analysed by means of the analysis of Variance (ANOVA). P<0.05 is considered significant.

RESULTS

Accumulation of PMN in hepatic sinusoidal space

Accumulation of PMN in the hepatic sinusoidal space was found, under light microscopy, PMN counts in hepatic sinusoidal space increased significantly after 6h in AOC group in comparison with BDL group or SO group. Under electron microscopy, PMN were seen easily in hepatic sinusoidal space in AOC group (Figure 1A).

Sinusoidal endothelial cell injury

Under light microscopy, no distinct change in SEC could be shown in any of the above groups. Electron microscopically, however, focal detachment, decreased electron density of cytoplasm, and swollen or even vacuolated mitochondria in SEC could often be observed in the AOC group (Figure 1B). In this group, the Kupffer cells were enlarged, but normal surface structures were retained and no degenerative changes of the nucleus or cytoplasm were shown (Figure 1-C). In contrast such changes could be occasionally seen in the SEC of BDL group. No evident morphological changes of SEC could be observed in SO group.

Expression of ICAM-1 mRNA in hepatic tissue

Expression of ICAM-1 mRNA in hepatic tissues was distinctly enhanced after 6h in AOC group when compared to other two groups (P<0.05). There was less expression of ICAM-1 gene in BDL group and no expression of ICAM-1 gene in SO group (Figure 2).
The serum ALT level and CINC concentration

The serum ALT level and CINC concentration were shown in Table 1. The serum ALT activity in the three groups was evidently unchanged in the same period (P<0.05). but, the serum CINC concentration in the AOC group was significantly higher than that in the BDL group or the SO group (P<0.05).

| Table 1 | The changes of serum ALT level and CINC concentration in the three groups (Tas, n=7) |
|---------|-----------------------------------------------|
| Serum parameters | AOC | BDL | SO |
| ALT (nkat-L⁻¹) | 917±167 | 901±171 | 908±164 |
| CINC (ng·L⁻¹) | 188±24 | 94±11 | 57±8 |

P<0.05, vs other two groups. P<0.05, vs SO group.

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