Reduction of the amount of intestinal secretory IgA in fulminant hepatic failure

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Dong-yan Liu1, Weiguo Jiang2 and Pei Liu3

1Research Center, China Medical University Affiliated Shengjing Hospital and Key Laboratory of Congenital Malformation Research, Ministry of Health, Shenyang, China
2Pathobiology Department, China Medical University Affiliated Shengjing Hospital, Shenyang, China
3China Medical University Affiliated Shengjing Hospital, Shenyang, China

Abstract

Intestinal barrier dysfunction plays an important role in spontaneous bacterial peritonitis. In the present study, changes in the intestinal barrier with regard to levels of secretory immunoglobulin A (SIgA) and its components were studied in fulminant hepatic failure (FHF). Immunohistochemistry and double immunofluorescent staining were used to detect intestinal IgA, the secretory component (SC) and SIgA in patients with FHF (20 patients) and in an animal model with FHF (120 mice). Real-time PCR was used to detect intestinal SC mRNA in the animal model with FHF. Intestinal SIgA, IgA, and SC staining in patients with FHF was significantly weaker than in the normal control group (30 patients). Intestinal IgA and SC staining was significantly weaker in the animal model with FHF than in the control groups (normal saline: 30 mice; lipopolysaccharide: 50 mice; D-galactosamine: 50 mice; FHF: 120 mice). SC mRNA of the animal model with FHF at 2, 6, and 9 h after injection was 0.4 ± 0.02, 0.3 ± 0.01, 0.09 ± 0.01, respectively. SC mRNA of the animal model with FHF was significantly decreased compared to the normal saline group (1.0 ± 0.02) and lipopolysaccharide group (0.89 ± 0.01). The decrease in intestinal SIgA and SC induced failure of the intestinal immunologic barrier and the attenuation of gut immunity in the presence of FHF.

Key words: Fulminant hepatic failure; Secretory IgA; IgA; Secretory component; Intestine

Introduction

Fulminant hepatic failure (FHF) is a rare but potentially fatal complication of acute hepatitis. Spontaneous bacterial peritonitis (SBP) has been defined as culture positivity with a neutrophilic response (≥250 cells/mm3) in ascitic fluid (1,2). SBP, which occurs in 15-25% of patients with cirrhosis (3,4), has been reported to occur in FHF (5-8). It has been shown that mortality is 2.5-fold higher and morbidity 2-fold higher in patients with SBP than in those without SBP (8).

Secretory IgA (SIgA), which is composed of IgA, secretory component (SC) and the J chain, is an important component of the intestinal immunologic barrier. IgA is a predominant Ig in mucosal secretions and serves as the first line of humoral defense on intestinal mucosal surfaces. The majority of these IgA proteins are monomers, while the rest are predominantly polymeric to facilitate efficient transport to the mucosal secretions mediated via the polymeric Ig receptor (PIgR). SC is the extracellular component of the PIgR responsible for the transcytosis of newly synthesized IgA (polymeric IgA). SIgA and its components, SC and IgA, are important components of the intestinal immunologic barrier. Up to now, investigations of the intestinal barrier in patients with FHF have focused mainly on the mechanical barrier and barrier to the microorganism. In the present study, SIgA, SC and IgA were measured in patients with FHF and in an animal model of FHF in order to elucidate the state of the intestinal immunologic barrier.

Material and Methods

Tissue samples

Paraffin-embedded sections of intestinal tissue from 20 patients with FHF who had died were obtained from Youan Hospital in Beijing. For controls, paraffin-embedded sections of normal intestine tissue from 30 patients who had died in a traffic accident were obtained from China Medical University, affiliated with Shengjing Hospital.
experimental procedures were approved by the Ethics Committee of China Medical University before the beginning of the study.

Animal model of FHF

Male BALB/c mice weighing 18-22 g were randomly divided into four groups. Group 1 (30 mice): normal saline (NS); group 2 (50 mice): lipopolysaccharide (LPS); group 3 (50 mice): D-galactosamine (D-GalN); group 4 (120 mice): FHF (LPS and GalN; Sigma, USA). Mice were injected intraperitoneally with LPS (10 µg/kg) and/or GalN (800 mg/kg). Mice of group 4 were killed by decapitation 2, 6, 9, 12, and 24 h after injection. The NS, LPS and GalN mice were killed by decapitation 9 h after the injection of NS, LPS or GalN (9). The study was approved by the animal Ethics Committee of China Medical University.

Immunohistochemistry of tissue samples

Intestinal IgA and SC of patients and mice with FHF were determined by immunostaining. Paraffin-embedded intestinal sections of patients and mice with FHF were deparaffinized, rehydrated, and stained with goat anti-human IgA or anti-mouse IgA (Sigma) and mouse anti-human SC (SigA; Sigma) or anti-mouse SC mAb (Bethyl, USA) by sequential incubation. The slides were rinsed three times with 0.02 M PBS, pH 7.4, after each incubation, and sections were counterstained with hematoxylin. The sections from the same patient or the mouse were processed without the primary antibody and then examined by the procedure detailed above as a control for nonspecific binding of the secondary antibody. The absorbance values of IgA and SC were determined with the Image analysis software (China) after scanning.

Double immunofluorescent staining of intestinal SigA

Paraffin-embedded intestinal sections of patients with FHF were deparaffinized, rehydrated, and double-stained by immunofluorescence with rhodamine-conjugated goat anti-human IgA (Southern Biotech, USA) and fluorescein-conjugated mouse anti-human SC (SigA; ICN, USA) or anti-mouse SC mAb (Bethyl, USA) by sequential incubation. The slides were rinsed three times with 0.02 M PBS, pH 7.4, after each incubation, and sections were counterstained with hematoxylin. Sections from the same patient or the mouse were processed without the primary antibody and then examined using the procedure detailed above as a control for nonspecific binding of the secondary antibody.

Quantitative real-time polymerase chain reaction of intestinal SC mRNA of mice with FHF

Total RNA was measured as described earlier in Refs. 10,11. Briefly, total RNA was extracted from mouse gut tissue with FHF using an RNA Mini Kit from Takara (Takara Biotechnology Co., Ltd., China). The quality of extracted RNA was determined by the number and size of the bands obtained with agarose gel electrophoresis. cDNA was synthesized using 100 ng RNA. The levels of individual RNA transcripts were quantified by the quantitative real-time polymerase chain reaction (PCR). The primers of SC were as follows: PIgR-F: 5’-CAGACATTAGCATGGCAGACTTCAA-3’; PIgR-R: 5’-TGCCGAGTAGGCCATGTCAAG-3’; GAPDH-F: 5’-AATGGTGGAAGGTGGTGAAT-3’; GAPDH-R: 5’-TGAAGGGGTCGTTGATG-3’.

The primers and fluorescent probes for SC and standard were purchased from Takara. The PCR conditions were a preliminary cycle at 95°C for 10 s, followed by 45 cycles at 95°C for 5 s and at 60°C for 20 s, followed by 1 min at 60°C and 5 s at 95°C. We also confirmed that the efficiency of amplification for each target gene (GAPDH) was 100% in the exponential phase of PCR. The levels of SC mRNA and GAPDH mRNA were determined according to the standard (Takara). The mRNA levels were normalized to GAPDH mRNA by dividing SC gene copies of the samples by the SC gene copies of GAPDH. The intestinal RNA level of the NS group was assumed to be 1, with which the intestinal RNA levels of other groups were compared.

Statistical analysis

The statistical differences between treatment groups were determined by the t-test using the SPSS 10.0 software.

Results

Intestinal IgA and SC staining in patients

In the normal control group, SC staining of the cytoplasm and membranes of intestinal epithelial cells was brown (Figure 1A). SC staining of the cytoplasm and of the membranes of intestinal epithelial cells in patients with FHF was pale and considerably weaker than in the normal control group (Figure 1B). IgA staining of the cytoplasm of plasma cells within the lamina propria and of intestinal epithelial cells was brown in the normal control group (Figure 1C). However, in patients with FHF, IgA staining of the cytoplasm of plasma cells within the lamina propria was pale brown and IgA staining of intestinal epithelial cells was pale. IgA staining in patient samples was notably weaker than that of the normal control group (Figure 1D). The mean absorbance values of SC and IgA in patients with FHF were significantly lower than those of the normal control group (P < 0.01; Figure 1E).

Double immunofluorescence staining

The results of intestinal double immunofluorescence showed that the cytoplasm of normal intestinal epithelial cells stained orange (SC, SigA) and the membranes stained yellow (SC, IgA, SigA), and that the plasma cells within the lamina propria stained orange (SigA) and yellow (IgA, SigA; Figure 2A). However, in patients with FHF, the staining of
the intestinal epithelial cell cytoplasm and membranes as well as plasma cells within the lamina propria was green (Figure 2B). The staining results proved that intestinal SC, IgA and SlgA were decreased compared to the normal control group.

**Intestinal IgA staining in the mouse model of FHF**

IgA staining of the cytoplasm and/or membranes of intestinal epithelial cells, as well as of plasma cells within the lamina propria, was weaker in the mouse FHF group (Figure 3D) compared to the NS (Figure 3A), LPS (Figure...
3B) and GalN groups (Figure 3C). The mean absorbance values of IgA in the mouse FHF group were significantly decreased from 2 to 12 h compared to the NS control group (P < 0.01; Figure 3E).

**Intestinal SC staining in mice with FHF**

SC staining of the cytoplasm and/or the membranes of intestinal epithelial cells was significantly weaker in the mouse FHF group (Figure 4D) compared to the NS group (Figure 4A), LPS group (Figure 4B), and GalN group (Figure 4C). The mean absorbance values of SC in the mouse FHF group were significantly decreased from 2 to 12 h compared to the NS group (P < 0.01; Figure 4E).

**SC mRNA of intestinal tissues in the animal model of FHF**

The relative content of intestinal SC mRNA in mice with FHF at 2, 6, and 9 h after injection was 0.4 ± 0.02, 0.3 ± 0.01, 0.09 ± 0.01, respectively. In the mice with FHF, intestinal SC mRNA was significantly lower at 2 to 9 h compared to the NS group (1.0 ± 0.02) and LPS group (0.89 ± 0.01; P < 0.01). In the GalN group, intestinal SC mRNA (0.2 ± 0.00) was also lower compared to the NS group and LPS group (P < 0.01; Figure 5).

**Discussion**

SBP is a common illness in patients with cirrhosis and ascites, which occurs without any apparent focus of infection. Bacterial translocation, gut motility alterations, and intestinal barrier dysfunction, along with bacterial overgrowth and changes in intestinal permeability play important roles in SBP, and it is evident from a number of studies that the gut is a major source of these bacteria (12). Intestinal SlgA levels are inversely correlated with bacterial overgrowth, bacterial translocation and changes in intestinal permeability (13). SlgA may weaken bacterial translation and restrict bacteria through intestinal epithelia (14). Secretory IgA is the first line of defense against microorganisms through immune exclusion that prevents interaction of neutralized antigens with the intestinal epithelium. SlgA plays an important role in the immune balance of the intestinal epithelial barrier (15-17). SC plays a protective role in preventing the proteolytic degradation of polymeric IgA, thus enhancing the mucosal immunity provided by IgA at these sites. SC is a key defense against bacteria (18-21) and
parasites (22) as well as playing a critical role in the immunologically mediated neutralization of cholera toxin (23). In vitro, free SC has been shown to fix the complement component C3b, suggesting a role for SC in enhancing local immune responses (24).

In liver diseases, notably alcoholic cirrhosis of the liver, intestinal IgA is decreased (25). In the present study, intestinal IgA, SC and SIgA were markedly decreased in patients with FHF, this possibly being the reason why gut immunity is weakened in patients with FHF. Changes in intestinal IgA, SC and SIgA in the animal model of FHF were consistent with those of the patients with FHF. SC mRNA was simultaneously detected by real-time PCR. A decrease in intestinal SC mRNA was observed in the animal model of FHF. These results confirm that at the mRNA and protein levels, SC is decreased in the animal model of FHF. It has been reported that in mildly inflamed ulcerative colitis lesions, SC levels showed no change but tended to decrease with increasing degree of inflammation. Decreased SC expression thus seemed to be related to intensified inflammatory activity (26). Therefore, we think that in patients with FHF, damaged intestinal mucus results in lower levels of intestinal SC and IgA. Malnourished animals exhibit low levels of hepatic and intestinal IgA. The concentration of biliary SC was also low in malnourished animals (27). In patients with FHF, the disease is usually complicated by malnutrition. Therefore, malnutrition may cause low concentrations of intestinal SC in FHF.

In conclusion, intestinal IgA and SC (SIgA) were lower in patients with FHF, with consequent failure of the intestinal immunologic barrier and attenuation of gut immunity.

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References