Effects of *Salmonella* infection on hepatic damage following acute liver injury in rats

Yong-Tao Li, Cheng-Bo Yu, Dong Yan, Jian-Rong Huang and Lan-Juan Li

Hangzhou, China

BACKGROUND: Acute liver injury is a common clinical disorder associated with intestinal barrier injury and disturbance of intestinal microbiota. Probiotic supplementation has been reported to reduce liver injury; however, it is unclear whether enteropathogen infection exacerbates liver injury. The purpose of this study was to address this unanswered question using a rat model.

METHODS: Oral supplementation with *Salmonella enterica serovar enteritidis* (*S. enteritidis*) was given to rats for 7 days. Different degrees of acute liver injury were then induced by intraperitoneal injection of D-galactosamine. The presence and extent of liver injury was assayed by measuring the concentrations of serum alanine aminotransferase, aspartate aminotransferase, and total bilirubin. Histology was used to observe liver tissue damage. Additionally, we measured the changes in plasma endotoxin, serum cytokines and bacterial translocation to clarify the mechanisms underlying intestinal microbiota associated liver injury.

RESULTS: The levels of liver damage and endotoxin were significantly increased in the *Salmonella* infected rats with severe liver injury compared with the no infection rats with severe liver injury ($P<0.01$); The peyer's patch $CD3^+$ T cell counts were increased significantly when the *Salmonella* infection with severe injury group was compared with the normal group ($P<0.05$). *S. enteritidis* pretreatment enhanced intestinal barrier impairment and bacterial translocation.

CONCLUSIONS: Oral *S. enteritidis* administration exacerbates acute liver injury, especially when injury was severe. Major factors of the exacerbation include inflammatory and oxidative stress injuries induced by the translocated bacteria and associated endotoxins, as well as over-activation of the immune system in the intestine and liver.

(*Hepatobiliary Pancreat Dis Int 2016;15:399-405*)

KEY WORDS: acute liver injury; *Salmonella enteritidis*; endotoxin; cytokine; bacterial translocation

Introduction

The gut and the liver are the key organs in nutrient absorption, metabolism and immune defense. The functions of the two organs affect each other through gut-liver axis.[1] Acute liver injury is commonly found in clinical practices. The liver injury often accompanies with intestinal barrier impairment and imbalance of intestinal microbiota. The imbalanced microbiota are often shown as excessive growth of aggressive bacteria and decrease of protective species, which contribute to the risk of spontaneous bacterial peritonitis and sepsis.[2]

The gut microbiota has been recognized as a significant factor influencing our health and well-being.[3] The main beneficial effects were achieved by stimulating the proliferation of epithelial cells, providing the host colonization resistance to invading pathogens and regulating intestinal immune system.[4, 5] Repeated alterations of the intestinal bacterial equilibrium can impair homeostasis, resulting in immune imbalance and the emergence of infectious inflammatory or allergic diseases.[6, 7] The ecological changes in intestinal system may also affect the liver function significantly. For example, liver and biliary abnormalities are common in patients with inflammatory bowel disease, celiac disease and in patients with jejunoileal bypass or short bowel syndrome.[1] A breakdown in intestinal barrier function and increased endotoxins result in the activation of macrophages in liver. The production of nitric oxide and cytokines also impairs liver

Author Affiliations: State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, and Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China (Li YT, Yu CB, Yan D, Huang JR and Li LJ)

Corresponding Author: Lan-Juan Li, MD, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, and Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China (Email: ljli@zju.edu.cn)

doi: 10.1016/S1499-3872(16)60113-3
Published online July 4, 2016.

function.[8] Several studies have demonstrated that probiotics, such as *Lactobacillus* and *Bifidobacterium*, can palliate liver injury through restoring the balance of intestinal microbiota and increase colonization resistance to enteropathogens.[9, 10] On the basis of these findings, we hypothesized that infection with enteropathogens would aggravate liver injury through interrupting the intestinal barrier, perturbing the balance of intestinal microbiota and enhancing bacteria translocation.

In this study, *Salmonella*, one of the most common food-borne bacteria,[11] was used to investigate whether oral infection exacerbates acute liver injury with different degrees of damages induced by D-galactosamine (GalN) and to what extent it affects.

Methods

Animals and *Salmonella*

Specific pathogen-free (SPF) male Sprague-Dawley rats weighing 180 to 220 g were purchased from Zhejiang Academy of Medical Sciences, Hangzhou, China. The experimental rats were individually caged at 21 ℃ and exposed to a 12 hours light/dark cycle. The rats were fed with sterilized water and standard rat chow. All animal experiments were conducted according to the *Guide for the Care and Use of Laboratory Animals* and approved by the Research, Animal Sources and Animal Care Committee of The First Affiliated Hospital, Zhejiang University School of Medicine. *Salmonella enterica serovar enteritidis* (*S. enteritidis*) was isolated from a patient and maintained in culture as described.[12]

Experimental design and animal treatment

Forty-eight rats were randomized into six groups (n=8/group): (i) *Salmonella* infection with no liver injury group (the S+no injury group); (ii) *Salmonella* infection with moderate liver injury group (the S+moderate injury group); (iii) *Salmonella* infection with severe liver injury group (the S+severe injury group); (iv) no infection and no liver injury group (the normal group); (v) no infection but with moderate liver injury group (the moderate injury group) and (vi) no infection but with severe liver injury group (the severe injury group). *Salmonella* infection was performed by gastric gavage with 2 mL/day (2.0×10^{10} CFU/mL suspended in physiologic saline) of live *S. enteritidis* to the *Salmonella* infection groups. Animals in the no infection groups received 2 mL physiologic saline only. On day 7, the moderate liver injury was induced by intraperitoneal injection of 0.7 g/kg body weight of GalN (Sigma Chemical Co., St. Louis, MO, USA) and the severe liver injury with 1.1 g/kg GalN.[13] Twenty-four hours later, animals were sacrificed by intramuscular injection of ketamine hydrochloride (50 mg/kg body weight) (Shanghai First Biochemical & Pharmaceutical Co., China) and ether inhalation.

Assessment of liver injury and endotoxin measurement

Blood samples from the inferior vena cava were centrifuged at 3000 g/min for 15 minutes at room temperature. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBiL) were measured using a Hitachi 7600 automatic analyzer (Hitachi, Tokyo, Japan).

Blood from the inferior vena cava (0.5 mL) was placed in an endotoxin-free tube with heparin and centrifuged at 3000 g/min for 15 minutes at 4 ℃. Plasma endotoxin concentrations were determined using quantitative, chromogenic Limulus Amebocyte Lysate (LAL; Eihua Medical Co., Shanghai, China) according to the manufacturer's instructions. Endotoxin levels were expressed as ng/mL.

Bacterial translocation

Samples from the liver and mesenteric lymph node (MLN) tissues were weighed and placed in a sterile glass homogenizer containing a nine-fold amount of anaerobic buffer (PBS with 0.5 g cysteine HCl, 0.5 mL tween 80 and 0.5 g agar/L). They were homogenized and 50 μL homogenate was planted on a blood agar base (bioMerieux, Inc., Durham, NC, USA) and an anaerobic blood agar base (bioMerieux) within 30 minutes of sample collection. The plates were incubated for 48 hours at 37 ℃ in anaerobic or aerobic environments, respectively. Sterile swabs were passed over the parietal peritoneal surface and then cultured on the same medium as an index of aseptic technique. The number of CFU on each plate was counted and the number of bacteria in each sample was determined based on the original weight of the sample at the time of collection. The results were expressed as the mean $\log_{10}$ CFU/g. Bacterial translocation (BT) was thought to occur if bacteria grew in the culture medium with the tissues homogenate.[14]

Liver histology

Liver tissue was fixed in 10% buffered formalin and embedded in paraffin. Five μm thick serial sections were stained with hematoxylin and eosin (HE) for histological analysis. The degree of liver injury and inflammation was semiquantitatively graded on a scale of 0 (absent), 1 (mild), 2 (moderate) and 3 (extensive).[15] At least three slides were studied in a blinded fashion from each specimen. Images were taken with a Philips light microscope (Philips Research, Eindhoven, The Netherlands) using a 20× objective.