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Role of Kupffer cells in ischemic injury in alcoholic fatty liver

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ABSTRACT

Background: This study was designed to evaluate the role of Kupffer cells (KCs) in hepatic drug metabolizing dysfunction after hepatic ischemia–reperfusion (IR) in alcoholic fatty liver.

Materials and methods: Rats were fed the Lieber-DeCarli diet for 5 wk to develop alcoholic fatty liver, then were subjected to 90 min of hepatic ischemia and 5 h of reperfusion. For ablation of KCs, rats were pretreated with gadolinium chloride ($GdCl_3$) 48 and 24 h before the IR procedure.

Results: After the IR procedure, ethanol diet (ED)-fed rats had higher serum aminotransferase activity compared with the control diet-fed rats. These changes were attenuated by $GdCl_3$. The ED-fed rats exhibited increased hepatic microsomal total cytochrome P450 (CYP) content and nicotinamide adenine dinucleotide phosphate-CYP reductase and CYP1A1, 1A2, 2B1, and 2E1 isozyme activity. After hepatic IR, these increases were reduced to lower levels than observed in the sham group, except CYP2E1 activity. Increases in CYP2E1 activity and its expression were augmented after hepatic IR in ED-fed animals, but were attenuated by $GdCl_3$. Finally, toll-like receptor 4 and myeloid differentiation primary response gene 88 protein expression, nuclear translocation of nuclear factor-κB and activator protein 1, and levels of proinflammatory mediators were further increased in ED-fed animals compared with control diet-fed animals after IR. These increases were attenuated by $GdCl_3$.

Conclusions: We suggest that KCs contribute to hepatic drug metabolizing dysfunction during hepatic IR in alcoholic fatty liver via the toll-like receptors 4-mediated inflammatory response.

1. Introduction

Fatty liver, or steatosis, is an early stage liver pathology primarily induced by drugs, ethanol, and abnormal metabolic states including disturbed triglyceride cycle and insulin resistance. More than 12,000 deaths per year are attributed to alcoholic liver disease in the United States [1]. Chronic liver diseases have also been identified as a potential source of inter-individual variation in drug metabolism, which can cause dose-dependent drug toxicity resulting from impaired *in vivo* drug clearance. Although

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the expression and activity patterns of the phase I and II drug metabolizing enzymes were observed recently in nonalcoholic fatty liver [2], the hepatic drug metabolizing function in alcoholic fatty liver has not yet been characterized.

Liver transplantation is an effective treatment option for a variety of end-stage liver diseases. Although the number of patients in need of a liver transplantation has increased, the number of donor organs available has not grown. Fatty liver occurs in up to 20% of potential liver donors, and is known to accentuate the severity of ischemia–reperfusion (IR) injury. The mechanisms of primary graft failure are not fully understood; injury or death of liver sinusoidal endothelial cells, excessive reactive oxygen species production or inflammatory response involving hepatocytes, and Kupffer cells (KCs) are recognized to have key roles in primary graft failure or accelerated rejection [3].

Infectious or inflammatory stimuli cause changes in the catalytic activity and expression of hepatic cytochrome P450 (CYP) enzymes in experimental animals and in humans [4]. In most cases, CYP enzyme content and activities are suppressed, but some enzymes are unaffected or induced under the same conditions [5]. We previously demonstrated that individual CYP isozymes appear to be affected differently by inflammatory response during polymicrobial sepsis or hepatic IR [6,7]. Moreover, other studies have reported that CYP action can act as an oxidative stressor on hepatocytes [8]. However, there is little information indicating exactly how the activities of CYP isozymes are altered in alcoholic fatty liver or how they are affected by secondary stress, such as IR.

Toll-like receptors (TLRs) and associated downstream signaling molecules play a critical role in innate immune response; especially TLR4, which senses lipopolysaccharide (LPS), has significant involvement in the pathogenesis of both alcoholic liver disease and ischemic condition [9]. Moreover, the alteration of metabolic processes in the liver during LPS-mediated inflammation is dependent on TLR4, suggesting cross-talk between hepatic drug metabolizing enzymes and TLR4 signaling [10]. Activation of KCs, resident hepatic macrophages, leads to the release of proinflammatory cytokines through multiple pathways, including that of TLR4, during acute liver failure, which can be induced by various hepatotoxins, such as acetaminophen [11]. Activated by a gut-derived endotoxin during chronic ethanol consumption, KCs are considered to be one of the major components of alcoholic liver disease [12]. KCs also trigger the development nonalcoholic steatohepatitis in a diet-induced mouse model through tumor necrosis factor (TNF)-α production [13]. Our previous study indicated that the depletion of KCs protects against liver injury by the sequential stresses of trauma and sepsis [14].

Thus, the aims of this study were to determine the role of KCs in changes in hepatic microsomal CYP isozymes during hepatic IR in alcoholic fatty liver by evaluating inflammatory response.

2. Materials and methods

2.1. Reagents and animals

The Lieber-DeCarli liquid diet was purchased from Dyets (Bethlehem, PA). Unless otherwise specified, all other reagents were purchased from Sigma–Aldrich (St. Louis, MO). All animals received care in compliance with the Principles of Laboratory Animal Care formulated by the National Institutes of Health (NIH publication No.86-23, revised 1985) and the guidelines of the Sungkyunkwan University Animal Care Committee. Male Sprague–Dawley rats weighing 150–170 g were obtained from Hyundai-Bio (Anseong, Korea) and were acclimatized to laboratory conditions at Sungkyunkwan University for at least 1 wk. Rats were maintained in a room with controlled temperature and humidity (25 ± 1°C and 55 ± 5%, respectively) with a 12-h light–dark cycle.

2.2. Induction of alcoholic fatty liver

In this study, we used the Lieber-DeCarli chronic ethanol consumption model to mimic human alcoholic fatty liver disease, as this model may closely resemble its clinical status [15]. As animals given alcohol show a tendency to reduce their solid food consumption, animals were provided with a liquid diet. The rats were randomly assigned to the groups specified and given free access to the Lieber-DeCarli liquid diet; 36% of the total calories were supplied from ethanol (ethanol diet group [ED]) or maltodextrin (control diet group [CD]) [16]. Ethanol was introduced progressively, beginning at 30 g/L of liquid diet for 2 d, 40 g/L for the two following days, and 50 g/L thereafter. Animal body weight was monitored twice a week and liquid diet intake was determined daily. Animals were assigned randomly to receive a CD for 5 wk or an ethanol isocaloric liquid diet for the same period. Ethanol was incorporated into the liquid feed directly before feeding. Our previous work presented the increases in liver weight and serum and hepatic triglyceride levels in the ED-fed rats, indicating the induction of alcoholic fatty liver [7].

2.3. Hepatic IR procedure

Rats were anesthetized with an intraperitoneal injection of ketamine (55 mg/kg) and xylazine (8 mg/kg). The abdomen of the anesthetized rats was incised to expose the abdominal contents. The left branches of the portal vein and hepatic artery were clamped to induce complete ischemia of the median and left lobes of the liver. The right lobes remained perfused to prevent venous congestion of the intestine. After 90 min of ischemia, the clamp was removed to allow 5 h of reperfusion. Sham-operated rats were prepared in a similar manner; however, vasculature leading to the median and left lobes was not clamped. The rats were sacrificed, and blood from the abdominal aorta and liver tissue were collected. Liver tissue was analyzed immediately by histologic staining of sections from the left lobe, and the remaining portions of the median and left lobes were frozen in liquid nitrogen and kept at −80°C until biochemical analyses were performed.

2.4. Depletion of KCs

Gadolinium chloride ($GdCl_3$), a selective KCs inhibitor, was administered to examine the effect of KC depletion during hepatic IR in alcoholic fatty liver. $GdCl_3$ (7.5 mg/kg) was injected intravenously 24 h before ischemia or sham operation. In the vehicle-treated rats, saline was injected in the