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The role of heme oxygenase-1 in drug metabolizing dysfunction in the alcoholic fatty liver exposed to ischemic injury

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ABSTRACT

This study was designed to investigate the role of heme oxygenase-1 (HO-1) in hepatic drug metabolizing dysfunction after ischemia/reperfusion (IR) in alcoholic fatty liver (AFL). Rats were fed a Lieber–DeCarli diet for five weeks to allow for development of AFL and were then subjected to 90 min of hepatic ischemia and 5 h of reperfusion. Rats were pretreated with hemin (HO-1 inducer) or ZnPP (HO-1 inhibitor) for 16 h and 3 h before hepatic ischemia. After hepatic IR, ethanol diet (ED)-fed rats had higher serum aminotransferase activities and more severe hepatic necrosis compared to the control diet (CD)-fed rats. These changes were attenuated by hemin and exacerbated by ZnPP. The activity and gene expression of HO-1 and its transcription factor (Nrf2) level increased significantly after 5 h of reperfusion in CD-fed rats but not in ED-fed rats. After reperfusion, cytochrome P450 (CYP) 1A1, 1A2, and 2B1 activities were reduced to levels lower than those observed in sham group, whereas CYP2E1 activity increased. The decrease in CYP2B1 activity and the increase in CYP2E1 activity were augmented after hepatic IR in ED-fed animals. These changes were significantly attenuated by hemin but aggravated by ZnPP. Finally, CHOP expression and PERK phosphorylation, microsomal lipid peroxidation, and levels of proinflammatory mediators increased in ED-fed rats compared to CD-fed rats after reperfusion. These increases were attenuated by hemin. Our results suggest that AFL exacerbates hepatic drug metabolizing dysfunction during hepatic IR via endoplasmic reticulum stress and lipid peroxidation and this is associated with impaired HO-1 induction.

1. Introduction

Alcohol abuse is a major public health problem worldwide because of its extensive detriments to the alimentary, circulatory, immune, and nervous systems. Alcoholic fatty liver, or alcoholic steatosis, is an early-stage liver pathology primarily induced by ethanol, which can lead to cirrhosis and hepatocellular carcinoma. Hepatic ischemia and reperfusion (IR) injury remains an important complication of liver surgery or transplantation. Despite being the last hope for selective patients with end-stage liver disease, liver transplantation is facing the dilemma of a huge donor deficit. Fatty liver disease that affects at least 30% of hepatocytes might render donor organs more susceptible to IR injury, causing primary graft failure or accelerating rejection in transplanted patients (Nativ et al., 2012). The susceptibility of fatty livers to IR injury has been attributed to fat-laden hepatocytes, ATP depletion, oxidative stress, and a different profile of inflammatory mediators (Farrell et al., 2008).

Drug elimination is frequently altered in patients with liver diseases. One major reason for this is thought to be the impaired activity of hepatic microsomal drug metabolizing enzymes, which can cause drug toxicity. Chronic liver diseases such as fatty liver disease 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. Indeed, altered expression and activity patterns of phase I and II drug metabolizing enzymes were recently reported in non-alcoholic fatty liver (Merrell and Cherrington, 2011). Cytochrome P450 (CYPs) are the main enzymes involved in drug metabolism. CYPs can be induced or suppressed in pathological conditions such as steatosis and IR. Recently, our group suggested that the resident liver macrophages, Kupffer cells, contribute to CYPs dysregulation during hepatic IR in alcoholic fatty liver via the toll-like receptor 4-mediated inflammatory response (Park et al., 2015).

Microsomal CYPs are highly inducible enzymes whose endoplasmic reticulum (ER) accumulation results in extensive proliferation of smooth ER membranes (Pahl, 1999); however, the mechanism and signals involved in this process are not known. It is conceivable that the ER

Abbreviations: AFL, alcoholic fatty liver; ALT, alanine aminotransferase; ARE, antioxidant response element; AST, aspartate aminotransferase; CD, control diet; CHOP, C/EBP homologous protein; CO, carbon monoxide; CYP, cytochrome P450; ED, ethanol diet; ER, endoplasmic reticulum; GSH, glutathione; HO, heme oxygenase; iNOS, inducible nitric oxide synthase; IR, ischemia and reperfusion; MDA, malondialdehyde; Nrf2, NF-E2-related factor 2; PERK, protein kinase R-like ER kinase; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; ZnPP, zinc protoporphyrin.

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accumulation of CYPs activates a general ER stress response. Hepatocytes are rich in ER, and ER stress response accompanies acute and chronic liver diseases, thus playing an important role in the pathogenesis of the liver disorders (Dara et al., 2011). Accumulating evidence indicates that reactive oxygen species (ROS) trigger ER stress in vivo and in vitro. Sun et al. (2014) recently reported that *N*-acetylcysteine attenuates ROS-mediated ER stress during liver IR.

Heme oxygenase (HO), also known as inducible heat shock protein 32, has attracted particular interest because of its extensive physiological modulating function. Upregulation of HO-1 is among the most critical cytoprotective mechanisms activated during cellular stress, such as inflammation, ischemia, hypoxia or hyperthermia, and it is thought to play a key role in antioxidant/oxidant homeostasis during times of cellular injury (Choi and Alam, 1996). The rate of heme synthesis is balanced by the rate of its degradation through HO. The liver is one of the body compartments with the highest rates of heme synthesis. More than 50% of the heme synthesized in the liver is committed to the synthesis of CYPs (Guengerich, 2006). As the prosthetic moiety of all CYPs, heme is responsible for the catalytic activity of these enzymes. Our group previously showed that HO-1 induction alleviates hepatic injury in alcoholic steatotic liver exposed to cold IR (Kim et al., 2012). Furthermore, bilirubin, a catabolite of heme, has been shown to increase insulin sensitivity in obese mice through suppression of ER stress (Dong et al., 2014). Transcription of HO-1 gene is modulated by the antioxidant response element (ARE) located in its promoter. The transcription factor NF-E2-related factor 2 (Nrf2) directly binds to ARE and activates HO-1 transcription (Cheng et al., 2015).

Thus, we hypothesized that HO-1 induction via Nrf2 improves the drug metabolizing function of alcoholic fatty liver exposed to IR injury. In this study, we fed rats a Lieber–DeCarli liquid diet to induce alcoholic fatty liver and determined the role of HO-1 in alterations in CYP isozymes during hepatic IR in alcoholic fatty liver using HO-1 inducer and inhibitor.

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 one week. 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 employed the Lieber–DeCarli chronic ethanol consumption model to mimic human alcoholic fatty liver disease. As animals given alcohol show a tendency for reduced solid food consumption, animals were provided a liquid diet. The rats were randomly assigned to the groups specified and given free access to the Lieber–DeCarli liquid diet in which 36% of the total calories were supplied from ethanol (ethanol diet group, ED) or maltodextrin (control diet group, CD) (Lieber and DeCarli, 1989). Ethanol was introduced progressively, beginning at 30 g/l of liquid diet for two days, 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 randomly assigned to receive a control diet for five weeks or an ethanol isocaloric liquid diet for the same period. Ethanol was incorporated into the liquid feed directly before feeding. Our previous work reported increases in liver weight and serum and hepatic triglyceride levels in the ED-fed rats, indicating the induction of alcoholic fatty liver (Cho and Lee, 2007).

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 each 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, the 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 using histological 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. Drug treatment

Hemin (HO-1 inducer, 30 mg/kg, ip) and zinc protoporphyrin (HO-1 inhibitor, ZnPP; 10 mg/kg, ip) were prepared as described previously (Kaizu et al., 2003; Yun et al., 2010). Rats were pretreated twice with hemin or ZnPP, at 16 h and 3 h prior to ischemia, and an equal volume of saline was administered to control animals. The doses and injection times of each reagent were determined based on a previous report (Yun et al., 2014) and on our own preliminary studies.

2.5. Serum aminotransferase activities

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined through standard spectrophotometric procedures using ChemiLab ALT and AST assay kits (IVDLab, Uiwang, Korea), respectively.

2.6. Histological analysis

Liver tissues were fixed overnight in 10% formalin buffered phosphate solution. After automated dehydration through a graded alcohol series, transverse liver slices were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). To evaluate macro- and micro-vesicular steatosis, hepatic necrosis, and portal inflammation, H&E stained sections were digitally photographed under an Olympus CKX41 microscope (Olympus Optical, Tokyo, Japan) (Karaa et al., 2005).

2.7. Isolation of the hepatic microsomal fraction and analytical procedures

The excised liver was minced and then homogenized in four volumes of ice-cold 1.15% KCl per 1 g of liver and centrifuged at 9000 ×*g* for 60 min. The supernatant was collected and centrifuged at 105,000 ×*g* for 60 min. The precipitate (microsomal fraction) was resuspended with 10 volumes of 1.15% (w/v) KCl solution containing 10 mM HEPES and 1 mM EDTA, pH 7.6. Aliquots of these hepatic microsomal fractions were stored at −80 °C until assayed. CYP content was calculated using the molar extinction coefficient for the absorbance difference between 450 and 480 nm, as measured with a differential spectrophotometer (Omura and Sato, 1964). The catalytic activities of CYP1A1, 1A2, and 2B1 in liver microsomal fractions were measured as the activities of 7-ethoxyresorufin *O*-deethylase, methoxyresorufin *O*-demethylase, and pentoxyresorufin *O*-dealkylase, respectively, using the method previously described by Burke et al. (Burke et al., 1985).