Food and Chemical Toxicology 108 (2017) 339–350

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Induction of mitochondrial biogenesis protects against acetaminophen hepatotoxicity

Kuo Du [a, 1, 2], Anup Ramachandran [a, 1], Mitchell R. McGill [a, 3], Abdellah Mansouri [b, c], Tarik Asselah [b, c], Anwar Farhood [d], Benjamin L. Woolbright [a], Wen-Xing Ding [a], Hartmut Jaeschke [a, *]

[a] Department of Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS, USA
[b] Hepatology Department, Beaujon Hospital, AP-HP, Clichy, France
[c] INSERM U1149, Centre de Recherche sur l'Inflammation, Université Denis Diderot, PRES Paris Sorbonne Cité, Paris, France
[d] Department of Pathology, St. David's North Austin Medical Center, Austin, TX, USA

ARTICLE INFO

Article history:
Received 21 July 2017
Received in revised form
15 August 2017
Accepted 16 August 2017
Available online 18 August 2017

Keywords:
Acetaminophen
Hepatotoxicity
Mitochondria biogenesis
Regeneration
PPARγ co-activator 1-α
SRT1720

ABSTRACT

Mitochondrial biogenesis (MB) is an adaptive response to maintain metabolic homeostasis after mitochondrial dysfunction. Induction of MB during APAP hepatotoxicity has not been studied. To investigate this, mice were treated with toxic doses of APAP and euthanized between 0 and 96 h. At early time points, APAP caused both mitochondrial dysfunction and reduction of mitochondrial mass, indicated by reduced activity of electron transport chain (ETC) complexes I and IV and depletion of mitochondrial DNA (mtDNA), respectively. Both ETC activity and mtDNA gradually recovered after 12 h, suggesting that MB occurs at late time points after APAP overdose. Immunofluorescent staining of mitochondria with mitochondrial outer membrane protein Tom20 further demonstrated that MB occurs selectively in hepatocytes surrounding necrotic areas. MB signaling mediators including PPARγ co-activator 1-α (Pgc-1α), nuclear respiratory factor-1 (Nrf-1) and mitochondrial fission protein dynamin-related protein-1 (Drp-1) were induced. Pgc-1α was selectively increased in hepatocytes surrounding necrotic areas. In addition, the time course of MB induction coincides with increased liver regeneration. Post-treatment with the known MB inducer SRT1720 increased Pgc-1α expression and liver regeneration, resulting in protection against late liver injury after APAP overdose. Thus, induction of MB is an important feature during APAP hepatotoxicity and liver regeneration.

1. Introduction

Acetaminophen (APAP)-induced liver injury is the leading cause of acute liver failure in the United States and many other Western countries (Lee, 2008). Numerous studies have established the critical role of mitochondria in the initiation and progression of APAP hepatotoxicity in both mice and humans (Placke et al., 1987; Meyers et al., 1988; Jaeschke, 1990; Kon et al., 2004; LoGuidice and

Abbreviations: ALT, alanine aminotransferase; APAP, acetaminophen; Drp-1, dynamin-related protein-1; ETC, electron transport chain; GSH, reduced glutathione; GSSG, glutathione disulfide; JNK, c-jun N-terminal kinase; MB, mitochondrial biogenesis; MPT, mitochondrial permeability transition; mtDNA, mitochondrial DNA; NAPQI, *N*-acetyl-*p*-benzoquinone imine; Nrf-1, nuclear respiratory factor-1; PCNA, proliferating nuclear antigen; Pgc-1α, PPARγ co-activator 1-α; RIP3, receptor-interacting protein kinase-3; Tom20, the central component of the TOM (translocase of outer membrane) receptor complex; ROS, reactive oxygen species; TUNEL, terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling assay.

* Corresponding author. Dept. of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, 3901 Rainbow Blvd, MS 1018, Kansas City, KS 66160 USA.
E-mail addresses: kuo.du@duke.edu (K. Du), aramachandran@kumc.edu (A. Ramachandran), mmcgill@wustl.edu (M.R. McGill), abdel.mansouri@inserm.fr (A. Mansouri), tarik.asselah@aphp.fr (T. Asselah), farhood.a.i@gmail.com (A. Farhood), bwoolbright@kumc.edu (B.L. Woolbright), wxding@kumc.edu (W.-X. Ding), hjaeschke@kumc.edu (H. Jaeschke).
[1] These authors contributed equally to the study.
[2] Department of Medicine, Division of Gastroenterology, Duke University, Durham, NC, USA.
[3] Department of Pathology, Washington University, St. Louis, MO, USA.

http://dx.doi.org/10.1016/j.fct.2017.08.020
0278-6915/

Boelsterli, 2011; Ramachandran et al., 2011; McGill et al., 2012a, 2014; Du et al., 2017). There is considerable evidence that the reactive metabolite of APAP binds to mitochondrial proteins (Tirmenstein and Nelson, 1989; McGill et al., 2012b; Xie et al., 2014, 2015) leading to altered mitochondrial morphology (Placke et al., 1987), inhibition of mitochondrial respiration (Meyers et al., 1988), mitochondrial oxidative stress and peroxynitrite formation (Jaeschke, 1990; Cover et al., 2005), loss of mitochondrial membrane potential (Kon et al., 2004; McGill et al., 2011; Xie et al., 2014) and release of mitochondrial proteins into the cytosol and plasma (Bajt et al., 2006; McGill et al., 2012a, 2014). In addition, several interventions aimed at preventing or reducing mitochondrial dysfunction have been shown to protect against APAP-induced liver injury, including post-treatment with the antidote GSH or N-acetylcysteine (NAC) (James et al., 2003; Knight et al., 2002; Saito et al., 2010) or SOD-mimetic Mito-Tempo (Du et al., 2017) to scavenge ROS, inhibition of the mitochondrial membrane permeability transition (MPT) (Kon et al., 2004; Ramachandran et al., 2011) and activation of autophagy to remove damaged mitochondria (Ni et al., 2012).

Mitochondrial biogenesis (MB) is the growth and division of existing mitochondria, resulting in increased mitochondrial mass within cells. The primary purpose of MB is to maintain or restore energy homeostasis during energy deprivation or following a mitochondrial insult. Several signaling mediators control this process, but PPARγ co-activator-1α (Pgc-1α) is thought to be the master regulator (Scarpulla, 2008). Although Pgc-1α itself does not bind to DNA, it interacts with other transcription factors in the nucleus to induce expression of genes that are important for MB (Scarpulla, 2008). In particular, induction of nuclear respiratory factor (Nrf) 1 by Pgc-1α controls the coordinate expression of other genes involved in MB in the nucleus and the mitochondria, especially those encoding subunits of the electron transport chain (ETC) complexes (Baker et al., 2007; Scarpulla, 2008). Importantly, Pgc-1α itself can be activated by the AMP-activated protein kinase (Ampk) and Sirtuin-1 (Sirt-1). It is also important to note that mitochondrial dynamics, involving mitochondrial fission and fusion are also carefully coordinated in order to ensure proper organization of the mitochondrial network (Chan, 2006a,b). In some cases, MB may determine whether a cell survives or dies (Jornayvaz and Shulman, 2010; Scarpulla, 2008), and in fact, impairment of MB is thought to contribute to several forms of tissue injury. In the liver, there is some evidence that the stress caused by chronic ethanol feeding can induce MB (Han et al., 2012), though it is not yet clear what role this plays in alcohol-induced liver injury. In extrahepatic tissues, kidney cells treated with pro-oxidants showed increased MB after the initial stress, and overexpressing Pgc-1α enhanced recovery of mitochondrial function (Rasbach and Schnellmann, 2007). Pharmacological induction of MB enhances regeneration and recovery in various rodent models of acute kidney injury (Rehman et al., 2013; Whitaker et al., 2013; Funk and Schnellmann, 2013; Jesinkey et al., 2014; Garrett et al., 2014; Khader et al., 2014) as well as other models of tissue injury (Finck and Kelly, 2007; St-Pierre et al., 2006; Funk et al., 2010).

Since mitochondrial dysfunction is a key factor in APAP-induced liver injury (Jaeschke et al., 2012), we hypothesized that MB could be affecting APAP hepatotoxicity. Therefore, the major objective of the present study was to characterize the time course of MB following APAP overdose and to determine whether or not induction of MB could be beneficial during APAP hepatotoxicity.

2. Methods

2.1. Animals

Male C57BL/6J mice (8–12 weeks old) were purchased from Jackson Laboratories (Bar Harbor, ME) and kept in an environmentally controlled room with a 12 h light/dark cycle and ad libitum access to food (LabDiet® PicoLab® Rodent Diet 20, #5053 Purina, Missouri, USA) and water. Mice were i.p. treated with 200 or 300 mg/kg APAP (Sigma-Aldrich) dissolved in warm saline after overnight fasting, and euthanized at the indicated time points between 0 and 96 h after APAP injection for collection of blood and liver samples. SRT1720 (EMD Millipore) was dissolved in 10% DMSO plus 2% Tween 20 and was i.p. administered at either 1.5 h or 12 h and 36 h post-APAP. All vehicle control mice received the same volume of DMSO (1 mL/kg) and Tween 20 (0.2 mL/kg). Blood was drawn from the caudal vena cava using a heparinized syringe. The liver was divided into several pieces some of which were used for mitochondrial isolation (Du et al., 2015), others for embedding in OCT medium for immunofluorescent staining or fixing in 10% phosphate-buffered formalin for histological analysis. The remaining pieces were snap-frozen in liquid nitrogen and stored at −80 °C for later analyses. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Kansas Medical Center and followed the criteria of the National Research Council for the care and use of laboratory animals.

2.2. Biochemical assays

Plasma ALT activity was determined using an ALT kit (Pointe Scientific, MI). Hepatic mitochondria were isolated, and submitochondrial particles were prepared by two cycles of freezing/thawing. Mitochondrial respiratory complex I & IV enzyme activity was assayed as described (Larosche et al., 2007; Kwong and Sohal, 2000). For complex I activity, the reaction mixture contained 25 mM potassium phosphate buffer (pH 7.4), 5 mM $MgCl_2$, 2 mM KCN, 2.5 mg bovine serum albumin, 100 mM NADH, 100 mM ubiquinone, and 2 mg antimycin. The reaction was initiated by addition of submitochondrial particles (20–50 mg of protein), and complex I activity measured by following the decrease in absorbance due to oxidation of NADH to NAD at 340 for 2 min. Complex I activity was then calculated as the difference between the total enzymatic rates and that obtained with the addition of rotenone (5 mg). For complex IV enzyme activity, the assay mixture had 10 mM potassium phosphate and 15 mM ferrocytochrome c, to which submitochondrial sample (1–5 mg protein) was added and the decrease in absorbance due to the oxidation of ferrocytochrome c followed at 550 nm for 30s. Complex IV activity was then calculated from the initial rate. GSH and GSSG levels were measured using a modified method of the Tietze assay as described (McGill and Jaeschke, 2015).

2.3. mtDNA levels

mtDNA was measured as previously described (Cover et al., 2005). Briefly, total hepatic DNA was isolated with Genomic-tip 100/G columns (QIAGEN GmbH, Hilden, Germany) then blotted onto Hybond-N nylon membranes (GE Healthcare). Membranes were first hybridized with a 10.9-kilobase mtDNA probe (nucleotides 4964–15,896) generated by long PCR and labeled by random priming, then stripped and hybridized with a mouse Cot-1 nDNA probe (Invitrogen, Cergy Pontoise, France). The levels were determined by densitometry analysis of autoradiographs and normalized to nuclear DNA levels.

2.4. Histology

Formalin-fixed tissue samples were embedded in paraffin and 5 μm thick sections were cut and transferred to glass slides. The slides were then stained with hematoxylin and eosin (H&E) for