Redox Biology 12 (2017) 264–273

Contents lists available at ScienceDirect

Redox Biology

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

Research Paper

Inhibition of Drp1 protects against senecionine-induced mitochondria-mediated apoptosis in primary hepatocytes and in mice

Xiao Yang[a,b], Hua Wang[b], Hong-Min Ni[b], Aizhen Xiong[a], Zhengtao Wang[a], Hiromi Sesaki[c], Wen-Xing Ding[b,*], Li Yang[a,d,**]

[a] The Key Laboratory of Standardization of Chinese Medicines of Ministry of Education, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai 2001203, China
[b] Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas 66160, USA
[c] Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD 21205
[d] Center for Chinese Medical Therapy and Systems Biology, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

ARTICLE INFO

Keywords:
Senecionine
Drp1
Mitochondria
Apoptosis
Liver injury

ABSTRACT

Pyrrolizidine alkaloids (PAs) are a group of compounds found in various plants and some of them are widely consumed in the world as herbal medicines and food supplements. PAs are potent hepatotoxins that cause irreversible liver injury in animals and humans. However, the mechanisms by which PAs induce liver injury are not clear. In the present study, we determined the hepatotoxicity and molecular mechanisms of senecionine, one of the most common toxic PAs, in primary cultured mouse and human hepatocytes as well as in mice. We found that senecionine administration increased serum alanine aminotransferase levels in mice. H & E and TUNEL staining of liver tissues revealed increased hemorrhage and hepatocyte apoptosis in liver zone 2 areas. Mechanistically, senecionine induced loss of mitochondrial membrane potential, release of mitochondrial cytochrome c as well as mitochondrial JNK translocation and activation prior to the increased DNA fragmentation and caspase-3 activation in primary cultured mouse and human hepatocytes. SP600125, a specific JNK inhibitor, and ZVAD-fmk, a general caspase inhibitor, alleviated senecionine-induced apoptosis in primary hepatocytes. Interestingly, senecionine also caused marked mitochondria fragmentation in hepatocytes. Pharmacological inhibition of dynamin-related protein1 (Drp1), a protein that is critical to regulate mitochondrial fission, blocked senecionine-induced mitochondrial fragmentation and mitochondrial release of cytochrome c and apoptosis. More importantly, hepatocyte-specific Drp1 knockout mice were resistant to senecionine-induced liver injury due to decreased mitochondrial damage and apoptosis. In conclusion, our results uncovered a novel mechanism of Drp1-mediated mitochondrial fragmentation in senecionine-induced liver injury. Targeting Drp1-mediated mitochondrial fragmentation and apoptosis may be a potential avenue to prevent and treat hepatotoxicity induced by PAs.

1. Introduction

Pyrrolizidine alkaloids (PAs) are the ester derivatives of necine base and necic acid that are found in more than 6000 plants [1,2]. PAs are potent hepatotoxins that can lead to liver injury, which over 8000 liver injury cases were reported worldwide to be associated with the use of PA-containing products such as herbal medicines [3,4]. In China, hundreds of people developed hepatic sinusoidal obstruction syndrome (HSOS) due to the consumption of Tusanqi (*Gynura segetum*), a Chinese medicine that contained high amount of PAs [3]. However, no effective therapies are currently available for hepatotoxicity induced by PAs.

Abbreviations: ALT, Alanine aminotransferase; ActD, Actinomycin D; CYP, Cytochrome P450; CQ, Chloroquine; Drp1, Dynamin-related protein 1; DHPAs, Dehydropyrrolizidine alkaloids; DHR, dehydroretronecine; EM, Electron microscopy; GSH, Glutathione; GAPDH, Glyceradehyde-3-phosphate dehydrogenase; HSEC, hepatic sinusoidal endothelial cells; HSOS, Hepatic sinusoidal syndrome; H & E, Hematoxylin and eosin; HM, Heavy membrane; HRP, Horseradish peroxidase; JNK, c-Jun N-terminal kinase; LPS, Lipopolysaccharide; MOMP, Mitochondrial outer membrane permeabilization; Mdivi-1, Mitochondrial division inhibitor-1; Mfn1, mitofusin 1; Mfn2, mitofusin 2; MMP, Mitochondrial membrane potential; MAPK, Mitogen-activted protein kinases; OPA1, optic atrophy 1; PAs, Pyrrolizidine alkaloids; PHH, Primary human hepatocyte; PFA, paraformaldehyde; pJNK, Phosphorylated c-Jun N-terminal kinase; ROS, Reactive oxygen species; Sene, Senecionine; Seph, Senephylline; TUNEL, Terminal deoxynucleotidyl transferase dUTP nick end labeling; TMRM, Teramethylrhodamine methyl ester; VDAC, Voltage-dependent anion channel; ZVAD-fmk, Carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone

* Correspondence to: Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, MS 1018 3901 Rainbow Blvd., Kansas City, Kansas 66160,.
** Corresponding author.
E-mail addresses: wxding@kumc.edu (W.-X. Ding), yl7@shutcm.edu.cn (L. Yang).

http://dx.doi.org/10.1016/j.redox.2017.02.020
Received 30 January 2017; Received in revised form 12 February 2017; Accepted 24 February 2017
Available online 02 March 2017

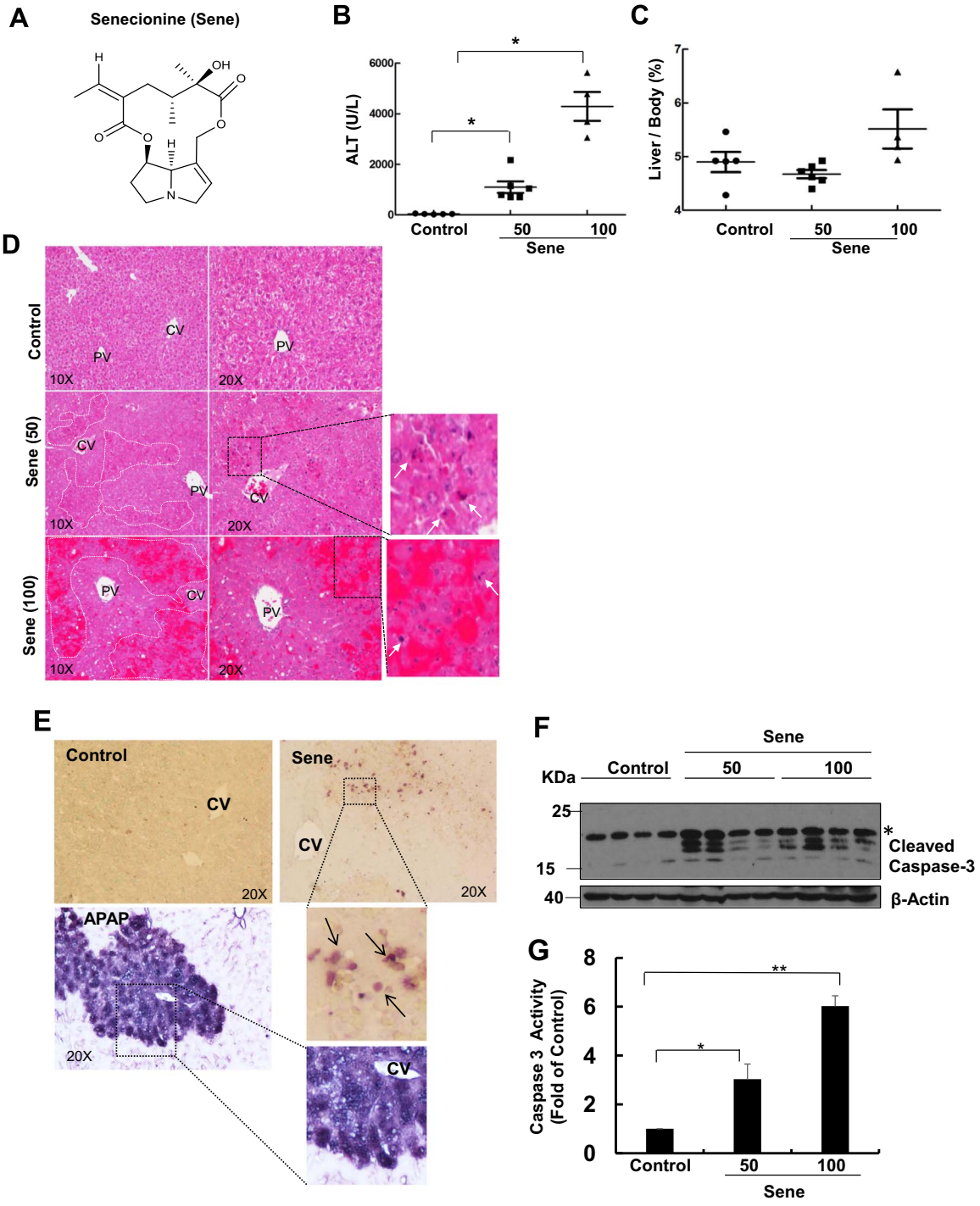

Fig. 1. Sene induces hepatocyte apoptosis and liver injury in mouse livers. The chemical structure of Sene (**A**). Male C57BL/6 J mice were treated with different doses of Sene (50 mg/kg, 100 mg/kg) or vehicle saline control by gavage and mice were sacrificed at 24 h after treatment. Serum ALT levels (**B**) and the ratio of liver to body weight (**C**) were analyzed and data are presented as means ± SE (n=4–6). * $p < 0.05$, one-way ANOVA analysis with Scheffé's post hoc test. Representative images of liver tissue H & E (**D**) and TUNEL staining (**E**). CV: Central vein; PV: Portal vein. Arrows denote apoptotic nuclei and dot line circled areas denote injury zones. APAP-induced TUNEL stained liver tissue used as a positive control for necrosis. Total liver lysates were subjected to western blot analysis (**F**) and caspase-3 activity assay (**G**). *: non-specific band. Data are presented as means ± SE (n=4–6). * $p < 0.05$. Student *t*-test.

PAs are predominantly metabolized in the liver by cytochrome P450 (CYP) enzymes such as CYP3A to generate reactive metabolites dehydropyrrolizidine alkaloids (DHPAs), which are further hydrolyzed to dehydroretronecine (DHR) [1,2]. DHPAs and DHR are highly reactive metabolites and bind to cellular glutathione (GSH) to form GSH-conjugates, which detoxify DHPAs and DHR. However, DHPAs and DHR can also bind to proteins to form pyrrole-protein adducts to initiate the hepatotoxicity in both parenchymal and non-parenchyma cells such as hepatic sinusoidal endothelial cells (HSEC) [1,2]. The damage to HSEC by PAs due to the depletion of the relative low level of GSH in HSEC often leads to HSOS, which is characterized by hepatomegaly, ascites and hyperbilirubinemia in human [2,5,6].

PAs can induce both apoptotic and necrotic/oncotic cell death in the livers of animals, cultured immortalized human hepatocytes and hepatoma cells [4,7–9]. Mechanistically, increased oxidative stress and mitochondrial pro-apoptotic BNIP3 expression and decreased anti-