Research Article

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Adiponectin protects against acetaminophen-induced mitochondrial dysfunction and acute liver injury by promoting autophagy in mice

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Background & Aims: Acetaminophen (APAP) overdose causes hepatic necrosis and acute liver injury by inducing mitochondrial dysfunction and damage. Although the biochemical pathways that mediate APAP-induced hepatotoxicity have been well studied, the body's defense mechanism to attenuate this disease remains elusive. This study investigated the roles of adiponectin, an adipocyte-secreted adipokine with pleiotropic protective effects against obesity-related metabolic dysfunction, in the pathogenesis of APAP-induced liver injury in mice.

Methods: Adiponectin knockout (ADN KO) and C57 wild type mice were treated with an overdose of APAP, followed by histological and biochemical evaluation of liver injury and activation of autophagy. The mechanism of adiponectin in APAP-induced hepatocytic toxicity was also explored in primary cultured hepatocytes.

Results: APAP overdose triggers a marked accumulation of adiponectin in injured liver tissues. ADN KO mice exhibit severely exacerbated mitochondrial dysfunction and damage, oxidative stress and necrosis and much higher mortality in response to APAP overdose, whereas these changes are reversed by a single injection of adiponectin. Mechanistically, adiponectin induces autophagosome formation by AMP-activated protein kinase (AMPK)-dependent activation of the Unc-51-like kinase 1, consequently leading to the removal of damaged mitochondria from hepatocytes. The protective effects of adiponectin against APAP-induced mitochondrial damage, oxidative stress and necrosis are abrogated by blockage of AMPK or pharmacological inhibition of autophagy.

Conclusions: Our findings suggest that the APAP-induced accumulation of adiponectin in liver tissues serves as an adaptive mechanism to ameliorate hepatotoxicity by promoting autophagy-mediated clearance of damaged mitochondria. Adiponectin agonists may represent a promising therapy for the drug-induced acute liver failure.

Introduction

Acetaminophen (APAP) is a widely used over-the-counter analgesic for pain relief and antipyresis. However, APAP overdose has become one of the most common causes of acute liver failure and intentional or accidental death in many countries [1]. APAP is metabolized by hepatic cytochrome P450 2E1 into a reactive intermediate N-acetyl-p-benzoquinoneimine (NAPQI), which is then detoxified by conjugation with glutathione (GSH) [2]. However, excessive NAPQI depletes hepatic GSH and causes mitochondrial dysfunction, ATP depletion and oxidative stress by covalently binding to cellular proteins. The resulting oxidative stress induces the formation of mitochondrial peroxynitrite, which in turn triggers mitochondrial DNA damage, nitration of mitochondrial proteins, consequently leading to the opening of the membrane permeability transition pore and necrotic cell death [3,4]. In addition, c-Jun-N-terminal kinase (JNK) exacerbates APAP-induced hepatotoxicity by amplifying mitochondrial oxidative stress and dysfunction. On the other hand, activation

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Abbreviations: APAP, acetaminophen; ALT, serum alanine aminotransferase; AST, aspartate aminotransferase; ROS, reactive oxygenic species; DCFH, 2′-7′-Dichlorofluorescein; H&E, hematoxylin-eosin; MTT, 3-(4, 5-dimethylthizaol-2-yl)-2, 5-diphenyltetrazolium bromide; AMPK, AMP-activated protein kinase; NAPQI, N-acetyl-p-benzoquinoneimine; GSH, glutathione; JNK, c-Jun-N-terminal kinase; ADN KO, adiponectin knockout; HMGB1, high-mobility group box 1; MDA, malondialdehyde; MRC, mitochondrial respiratory chain; mtDNA, mitochondrial DNA; LC3-II, microtubule-associated protein 1A/1B-light chain 3-II; p62, sequestosome 1; ULK1, Unc-51-like kinase 1; mTOR, mammalian target of rapamycin complex 1; LDH, lactate dehydrogenase; HMW, high molecular weight.

of autophagy, a process which involves the formation of autophagosomes for degradation of dysfunctional or damaged cellular components through the actions of lysosomes, has recently been shown to serve as a cellular adaptive mechanism to counteract APAP-induced hepatotoxicity [5,6]. However, despite the intensive research, there are currently few therapeutic options available for APAP-induced acute liver failure.

Adiponectin, one of the most abundant adipokines secreted from adipocytes, possesses multiple beneficial effects on obesity-related medical complications [7–9]. Many clinical studies have consistently demonstrated that a low level of circulating adiponectin is an independent risk factor for type-2 diabetes, hypertension, atherosclerosis and non-alcoholic steatohepatitis (NASH) [9–11]. Conversely, elevating plasma levels of adiponectin by either pharmacological or genetic approaches alleviates these disorders [7,12]. In addition to NASH, adiponectin potently alleviates several other forms of liver diseases, including alcoholic fatty liver and steatohepatitis [7], lipopolysaccharide/D-galactosamine-induced liver injury [13], carbon tetrachloride and bile duct ligation-induced cirrhosis [14]. The protective effects of adiponectin against these liver diseases are attributed to its direct actions in several types of cells in the liver tissues, including promotion of mitochondrial fatty acid oxidation in hepatocytes [7], inhibition of proinflammatory cytokine production in Kupffer cells [15], and suppression of stellate cell activation [16]. However, the precise mechanisms, whereby adiponectin exerts its hepato-protective effects, remain elusive.

In this study, we investigated the roles of adiponectin in APAP overdose-induced acute liver injury in mice. We found that an APAP overdose triggers a massive recruitment of adiponectin from the bloodstream into the injured liver tissues. By using adiponectin knockout (ADN KO) mice, *in vivo* supplementation of adiponectin, and *ex vivo* studies in primary hepatocytes, we have uncovered activation of autophagy as a key mechanism whereby adiponectin protects APAP-induced hepatotoxicity by alleviating mitochondrial damage and dysfunction.

Materials and methods

Relevant materials and methods

All relevant materials and methods are presented in the Supplementary materials and methods.

Statistical analysis

All analyses were performed with the Statistical Package for Social Sciences version 14.0 (SPSS, Chicago. IL). Data were expressed as mean ± SEM. Statistical significance was determined by Student's *t* test (for comparison of two experimental conditions) or analysis of variance (ANOVA) (for comparison of three or more experimental conditions). The survival ratio was analysed by Kaplan-Meier cumulative survival functions. In all statistical comparisons, a p value <0.05 was used to indicate a statistically significant difference.

Results

APAP overdose triggers accumulation of adiponectin in the liver tissues

In several models of acute and chronic organ injury, including myocardial infarction and atherosclerosis [17,18], adiponectin is recruited from the bloodstream to local damaged areas. In line with these observations, both our immunohistological and immunoblotting analysis detected a dramatic increase in adiponectin accumulation in the liver tissues of C57BL/6J as early as 2 h after administration with 500 mg/kg APAP (Fig. 1A and B). In contrast, circulating adiponectin levels were progressively decreased in response to APAP overdose (Fig. 1C). Quantitative real-time PCR analysis showed that mRNA expression of adiponectin was not detectable in liver tissues, and that APAP overdose had no impact on the mRNA abundance of adiponectin in either adipose tissues or liver (Fig. 1D). On the other hand, there was no obvious alteration in either mRNA or protein levels of adiponectin in skeletal muscle, heart, kidney, brain and pancreas (data not shown), suggesting that an APAP overdose leads to the selective recruitment of circulating adiponectin into liver tissues.

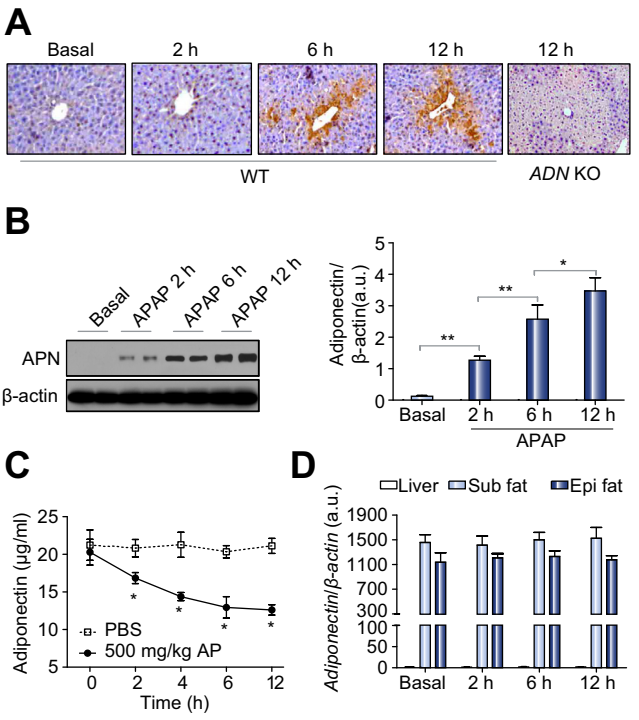

Fig. 1. APAP overdose causes accumulation of adiponectin in injured liver tissues. Eight-week-old C57BL/6J mice were intraperitoneally injected with APAP (500 mg/kg) or PBS as vehicle controls. Liver tissues were collected at various time points after APAP treatment. (A) Representative images of liver sections for immunostaining analysis with a rabbit anti-mouse adiponectin polyclonal antibody (magnification 40×). The liver sections from adiponectin knockout (*ADN* KO) were used as a negative control. (B) Immunoblot analysis of liver tissues with a rabbit anti-mouse adiponectin polyclonal antibody. (C) Serum levels of adiponectin quantified by ELISA. (D) The mRNA expression levels of *adiponectin* in subcutaneous and epididymal adipose tissues and liver at various time points after APAP treatment. a.u., arbitrary unit, n = 5–6, $^{*}p$ <0.05, $^{**}p$ <0.01.

Adiponectin deficiency promoted APAP-induced liver injury in mice

Previous studies from us and others have demonstrated the protective effects of adiponectin against several forms of liver diseases, including alcoholic and non-alcoholic steatohepatitis, and carbon tetrachloride-induced liver fibrosis [7,14,19]. To explore the role of adiponectin in APAP overdose-induced hepatotoxicity, adiponectin knockout (ADN KO) and wild type (WT) mice were treated with 500 mg/kg APAP by intraperitoneal injection, followed by biochemical and histological evaluation of the liver injury. As expected, serum levels of ALT and AST, both of which