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Upregulation of SIRT1-AMPK by thymoquinone in hepatic stellate cells ameliorates liver injury

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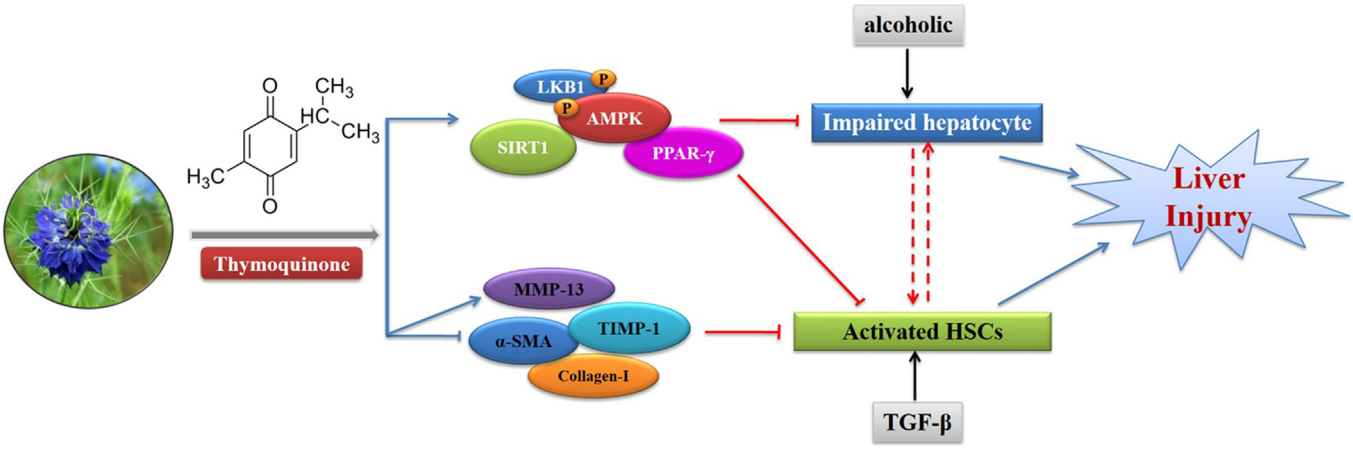
GRAPHICAL ABSTRACT



HIGHLIGHTS

- Thymoquinone efficaciously protects against liver injury.
- Thymoquinone inhibits TGF-β induced HSCs activation.
- Thymoquinone activates AMPK phosphorylation both in hepatocytes and HSCs.

Abbreviations: α-SMA, α-smooth muscle actin; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; ALD, alcoholic liver disease; ALT, alanine aminotransferase; AMPK, AMP-activated protein kinase; AST, aspartate aminotransferase; HSCs, hepatic stellate cells; LKB-1, liver kinase B-1; MMP-13, metallopeptidase 13; PPAR-γ, peroxisome proliferator activated receptor-γ; SIRT1, sirtuin 1; TIMP-1, tissue inhibitor of metalloproteinase-1; TG, triglyceride; TGF-β, transforming growth factor-β.

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ABSTRACT

Thymoquinone (TQ) is a biologically active compound isolated from the seeds of *Nigella sativa* L. (Ranuculaceae). This study investigated the hepato-protective effect of TQ on liver injury through AMP-activated protein kinase (AMPK) signaling in hepatic stellate cells (HSCs). *In vitro*, TGF-β time-dependently attenuated liver kinase B-1 (LKB1) and AMPK phosphorylation, which were blocked by pretreatment with TQ and AICAR (an activator of AMPK). TQ significantly inhibited collagen-I, α-SMA, TIMP-1 and enhanced MMP-13 expression, contributing to prevent TGF-β-induced human HSCs activation. Moreover, TQ induced peroxisome proliferator activated receptor-γ (PPAR-γ) expression, which was inhibited by genetic deletion of AMPK. *In vivo*, C57BL/6 mice were fed with ethanol diet for 10 days, then administering a single dose of ethanol (5 g/kg body weight) via gavage. TQ (20 or 40 mg/kg) were given by gavage every day. TQ attenuated the increases in serum aminotransferase and hepatic triglyceride in mice fed with ethanol, while significantly activated LKB1 and AMPK phosphorylation. In addition, TQ enhanced the sirtuin 1 (SIRT1) expression. In conclusion, we demonstrate that AMPK pathway is a key therapeutic target for controlling liver injury and TQ confers hepato-protection against TGF-β-induced the activation of HSCs and ethanol-induced liver injury.

1. Introduction

Liver injury is a basic pathological process in most hepatic diseases. Chronic liver injury causes death of hepatocytes and recruits inflammatory cells to the injured liver, leads to hepatic fibrosis, liver cirrhosis, and even liver cancer (Tu et al., 2015; Xu et al., 2014). Alcoholic liver disease (ALD) is one of the main causes of chronic liver injury worldwide (Gao and Bataller, 2011). At present, voluntary feeding with the liquid diet containing ethanol is the most commonly used animal model for alcoholic liver injury. Such chronic-plus-binge ethanol feeding synergistically induced steatosis, inflammation and liver injury in mice (Darakhshan et al., 2015; Zhou et al., 2007). Alcohol consumption causes hepatocytes damage, leading to the release of varieties mediators and subsequently induced hepatic stellate cells (HSCs) activation. Several studies have indicated that damaged hepatocytes may present as a major inflammatory stimulus for HSCs activation (Zhan et al., 2006). HSCs are the major extracellular matrix (ECM)-producing cells, activated HSCs express a variety of ECM proteins including α-smooth muscle actin (α-SMA), collagen, metallopeptidase (MMP), tissue inhibitor of metalloproteinas (TIMP) and transforming growth factor-β (TGF-β), which all contribute to liver fibrosis (Jin et al., 2011). They regulate hepatic sinusoidal blood flow and can also transdifferentiate into myofibroblasts during the process of liver fibrosis (Crispe, 2009). TGF-β is a member of the transformation growth factor family which is involved in the initiation and maintenance of fibrogenesis in liver. Once activated, TGF-β signals enhance procollagen I and procollagen III via its cognate receptors to Smad proteins, which are the transcription of target genes (Hernandez-Gea and Friedman, 2011).

AMP-activated protein kinase (AMPK) is a serine threonine kinase comprising a heterotrimeric complex and regarded as an energy sensor in most tissues (Rana et al., 2015). Liver kinase B-1 (LKB1) is a master serine/threonine kinase, directly activation of downstream kinases closely together with AMPK (Green et al., 2011). In the liver, AMPK has a central role in hepatic lipid homeostasis due to its phosphorylation and inactivation of key enzymes (Wu et al., 2014). In addition, activation of AMPK is important in anti-proliferative and anti-fibrotic effects in HSCs (Adachi and Brenner, 2008). Alcohol consumption exacerbated liver injury by down-regulating the activities of AMPK or sirtuin 1 (SIRT1). Hepatic SIRT1 is an important regulator of lipid homeostasis. The activation of SIRT1-AMPK in liver may increase the rates of fatty acid oxidation and repress lipogenesis largely by modulating activity of peroxisome proliferator activated receptors (PPARs) or sterol regulatory element-binding protein-1 (SREBP-1) through deacetylation and phosphorylation, respectively. PPARs belong to the superfamily of nuclear receptors. The PPARs family is composed of three members, PPAR-α, PPAR-β/δ and PPAR-γ, which show different physiological roles and a tissue specific expression pattern. PPAR-γ is one of the PPARs isoforms which controls growth and differentiation in different tissues. Introduction of exogenous PPAR-γ cDNA is sufficient to reverse the morphology of activated HSCs to the quiescent phenotype (Zhou et al., 2007). Hepatocyte-specific deletion of SIRT1 disturbs PPARs signaling, reduces fatty acid oxidation and causes aggravated liver steatosis and inflammation (Purushotham et al., 2009).

Thymoquinone (TQ) (2-isopropyl-5-methylbenzo-1, 4-quinone) is the most potent component of the volatile oil of *Nigella sativa* L. (Ranuculaceae) seeds (Ali and Blunden, 2003). Protective effects of TQ have been investigated including hepatoprotective, gastroprotective and neuroprotective activities. In addition, anti-inflammatory, anti-oxidant, anti-histaminic, anti-microbial and anti-tumor effects of TQ have been reported (Darakhshan et al., 2015; Johnson-Ajinwo and Li, 2014). In our previous study, TQ attenuated liver injury partially via blocking toll-like receptor 4 (TLR4) expression and phosphatidylinositol 3-kinase (PI3K) phosphorylation on the lipopolysaccharide (LPS) activated HSCs *in vitro* (Bai et al., 2013), inhibiting TLR4 signal and activating LKB1-AMPK signal induced by thioacetamide *in vivo* (Bai et al., 2014). In view of the researches, it is revealed that TQ possess hepatoprotective effects, so we further investigated the role of AMPK signaling targeting HSCs in liver injury.

2. Materials and methods

2.1. Materials

TQ was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The purity for GC of TQ reached 99%. Anti-AMPK (cs-2532), anti-P-AMPK (cs-2531), anti-LKB1 (cs-3047) and anti-P-LKB1 (cs-3482) antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-collagen-I (ab-34710), anti-α-SMA (ab-5694), anti-SIRT1 (ab-110304), anti-PPAR-γ (ab-19481) and anti-GAPDH (ab-8245) antibodies were purchased from Abcam (Cambridge, MA, USA). AMPKα1 siRNA (sc-29673) and control siRNA (sc-37007), horseradish peroxidase (HRP)-conjugated goat anti-rabbit and goat anti-mouse antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz Biotechnology, CA, USA).