Tetrahydropalmatine attenuates liver fibrosis by suppressing endoplasmic reticulum stress in hepatic stellate cells

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To the Editor: Liver fibrosis was characterized by the activation of hepatic stellate cells (HSCs) and extracellular matrix deposition. The pathogenesis of liver fibrosis involved many aspects. Studies have found that endoplasmic reticulum stress (ERS) in HSCs could lead to HSC activation and aggravate liver fibrosis.[1] Although liver fibrosis has been extensively studied, effective anti-fibrotic drugs are lacking at present. Tetrahydropalmatine (THP) was the active ingredient of Chinese herbs *Corydalis yanhusuo*. Previous studies have found the hepatoprotective effect of THP. Our study aimed to investigate the effects of THP on liver fibrosis and the underlying mechanisms.

C57BL/6J male mice (20–25 g) were randomly divided into five groups (8 mice for each group): Oil group, carbon tetrachloride (CCl_4) group, and three different dosages of THP groups (low dose group, THP-Low [THP-L], 20 mg/kg; middle dose group, THP-Middle [THP-M], 40 mg/kg; and high dose group, THP-High [THP-H], 80 mg/kg). The THP powder was suspended in 0.5% sodium carboxymethylcellulose (CMC-Na) solution. All the mice received intraperitoneal injection of Oil or 20% CCl_4 solution in oil (5 mL/kg; twice per week for 6 weeks). Meanwhile, CCl_4-induced mice were administrated intragastrically with THP daily. Detailed materials and methods are available in the [Supplementary File, http://links.lww.com/CM9/A843]. Hematoxylin-eosin staining showed marked necrosis, inflammatory cell infiltration, and disruption of tissue architecture in livers of mice receiving CCl_4, and these effects were significantly ameliorated by THP treatment [Figure 1]. Sirius red staining showed that THP treatment significantly attenuated collagen deposition, and histopathological examination showed that the expressions of α-smooth muscle actin (α-SMA) and collagen I were markedly downregulated in CCl_4-induced mice treated by THP [Supplementary Figure 1A, http://links.lww.com/CM9/A844]. In addition, serum alanine aminotransferase (ALT) levels were markedly increased in the CCl_4 group, while THP treatment significantly decreased the ALT level [Supplementary Figure 1B, http://links.lww.com/CM9/A844]. Quantitative real-time PCR (qPCR) assays showed that hepatic mRNA levels of profibrotic genes, such as *α-SMA*, *Collagen I*, platelet-derived growth factor, connective tissue growth factor, and tissue inhibitor of metalloproteinase-1, were significantly reduced by THP treatment in mice induced by CCl_4 [Supplementary Figure 1C, http://links.lww.com/CM9/A844]. Western blotting analysis also confirmed that THP downregulated α-SMA in mouse livers induced by CCl_4 [Supplementary Figure 1D, http://links.lww.com/CM9/A844]. These data indicated that THP attenuated chronic liver injury, inflammation, and fibrosis in mice induced by CCl_4.

Next, we used RNA-seq to identify significantly enriched signaling pathways and differentially expressed genes (DEGs). The DEGs were selected according to the cutoffs of P value <0.05 and $\log_2$ (Fold Change) >1. The main signaling pathways involved in liver fibrosis were investigated by gene ontology enrichment analysis, including MAPK pathways and 26 pathways related to ERS, inflammation, and fibrosis pathways (THP *vs.* CCl_4) [Supplementary Figure 1E, http://links.lww.com/CM9/A844]. The heatmap revealed that the genes related to ERS, inflammation, and fibrosis pathways were significantly downregulated by THP treatment [Supplementary Figure 1F, http://links.lww.com/CM9/A844].

ER stress has been shown to play a pivotal role in HSC activation. When the ER experienced an abundance of

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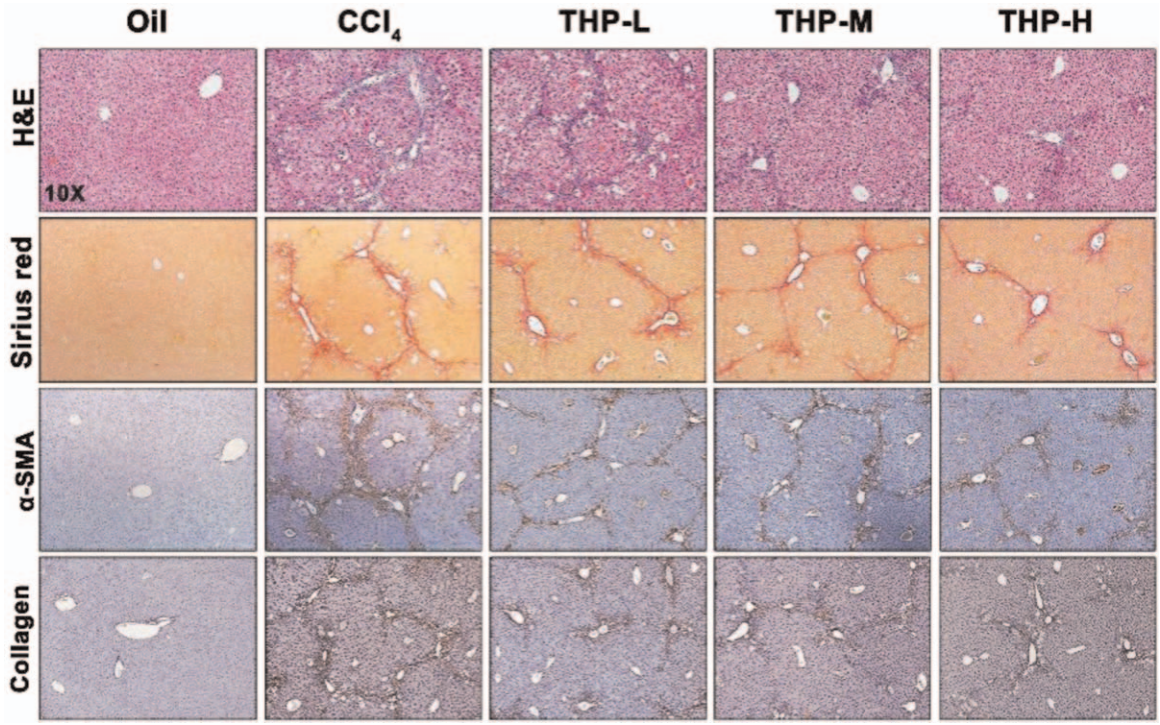

Figure 1: Representative histology of H&E, Sirius red, and immunohistochemical staining of α-SMA and collagen I (Original magnification ×10). H&E: Hematoxylin-eosin; α-SMA: α smooth muscle actin.

unfolded or misfolded proteins, protein kinase RNA-like ER kinase (PERK) experienced autotransphosphorylation via its kinase activity. The major substrate of PERK phosphorylation was eukaryotic translation initiation factor 2α (eIF2α). eIF2α phosphorylation caused global translation attenuation while permitting selective translation of activating transcription factor 4 (ATF4), which primarily acted through unconventional splicing of transcription factor X-box binding protein 1 (XBP1).[2] Spliced XBP1 (sXBP1) contributed to HSC activation through the facilitation of cargo secretion by expanding ER capacity and upregulating protein secretion.[2] C/EBP homologous protein (CHOP), as the downstream of ATF4, was implicated in the progression of liver disease. To verify the effect of THP on inflammation and ERS, qPCR assay was performed and hepatic mRNA levels of the pro-inflammatory genes C-C motif chemokine ligand 2 and C-X-C chemokine receptor 2, and ERS-related genes *Ddit3* (*Chop*), *Hspa5* (glucose-regulated protein 78, *Grp78*), *Atf4*, and *sXbp1*, were significantly downregulated by THP treatment in fibrotic mice [Supplementary Figure 1G, http://links.lww.com/CM9/A844]. To confirm the inhibitory effect of THP on ERS in HSCs, co-staining of CHOP/α-SMA was measured by immunofluorescence assay. Results indicated a significant reduction of positive area in the THP treatment groups [Supplementary Figure 1H, http://links.lww.com/CM9/A844].

Finally, we examined the effects of THP on ERS in LX-2 cells, an activated human HSC line. Results of qPCR and Western blotting analyses of *ATF4*, *CHOP*, and *α-SMA* showed significant downregulation after THP treatment for 24 h [Supplementary Figure 1I and 1J, http://links.lww.com/CM9/A844]. In addition, extracellular signal-regulated kinase 1/2 (ERK1/2) was implicated downstream of inositol-requiring enzyme 1α (IRE1α) kinase activity, which played important roles in HSC activation.[2] Yu *et al*[3] have reported that L-THP pretreatment alleviated hepatocyte injury caused by ischemia/reperfusion via inhibiting p-ERK1/2 and NF-κB. Therefore, we speculated that THP may also inhibit the downstream p-ERK1/2, and we supplemented co-staining of p-ERK1/2/α-SMA on the liver sections, which showed obvious downregulation in the THP treatment groups [Supplementary Figure 2, http://links.lww.com/CM9/A845]. Recently, it has been demonstrated that levo-tetrahydropalmatine prevented liver fibrosis through PPAR gamma/NF-kappaB and TGF-beta1/Smad pathway.[4] However, whether the inhibitory effects of THP on ERS were direct or through other signaling pathway, like TGF-β1/Smad pathway, still needed further study. Taken together, these data suggested that THP inhibited HSC activation and liver fibrosis by suppressing ERS in HSCs.

In summary, our study demonstrated a new mechanism for THP in the treatment of liver fibrosis. Furthermore, the inhibitory effect of THP on HSC activation was partially dependent on the suppression of ERS.

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Conflicts of interest

None.

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