Pharmacological Inhibition of Transient Receptor Potential Vanilloid 4 (TRPV4) Channel Alleviates Carbon Tetrachloride-Induced Liver Fibrosis in Mice

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Background: Transient receptor potential vanilloid 4 (TRPV4) is a member of the TRP channel family and is involved in diverse physiological and pathological processes. Accumulating evidence from in vitro studies indicates that TRPV4 has a potential role in liver fibrosis, but its precise role in the pathophysiological development of this condition is unclear. Exogenous interventions and endogenous reactions should be considered.

Methods: This study used a mouse model of carbon tetrachloride (CCl4)-induced liver fibrosis to investigate the effects of intraperitoneal injection of the novel TRPV4 channel selective agonist GSK1016790A (GSK) and antagonist HC-067047 (HC).

Results: As compared with the CCl4 group, collagen fiber deposition and alpha-smooth muscle actin (α-SMA) levels were markedly higher and hepatic lobule disorganization was worse in the CCl4+GSK group, while collagen fiber deposition was significantly lower and hepatic lobule disorganization was less severe in the CCl4+HC group.

Conclusions: The present findings suggest that activation of TRPV4 channels worsens liver fibrosis and that inhibition of TRPV4 channels may alleviate liver fibrosis in vivo.

Key words: TRPV4, liver fibrosis, in vivo

Introduction

Fibrosis is often caused by chronic liver injury due to infection, drugs, metabolic disorders, and immune disorders and is a leading cause of cirrhosis. In China, an epidemic of hepatitis B infection has increased the prevalence of liver fibrosis. The high risk of end-stage liver disease and hepatic carcinoma, and the fact that such outcomes are always costly and difficult to treat, makes identification of an effective treatment for liver fibrosis highly desirable.

The TRPV4 cation channel, a member of the TRPV subfamily, was first described in 2000. It exhibits cation permeability and participates in multiple cellular processes. Alteration of TRPV4 channel activity is believed to account for the development and progression of numerous diseases, and recent evidence from in vitro studies suggests that TRPV4 has a role in liver fibrosis. However, in vivo studies are required in order to determine the influence of TRPV4.

Using a mouse model of CCl4-induced liver fibrosis, and a selective agonist and antagonist of TRPV4, we investigated the relation between TRPV4 and liver fibrosis progression in histological and protein studies.

Materials and Methods

Animal model

Six-week-old male C57BL/6 mice were purchased from Hunan SJA Laboratory Animal Co. LTD (Changsha, China). All mice were housed in a specific pathogen-free facility and fed on a standard pellet diet and pure water at the animal experiment center of the Second Xiangya Hospital of Central South University. To simulate early
and advanced hepatic fibrosis, CCl4 was injected intraperitoneally for 5 and 8 weeks, respectively. At both time points, the mice were randomly distributed into four groups: a negative control group (NC group), CCl4-induced liver fibrosis group (CCl4 group), agonist group (CCl4+GSK group), and antagonist group (CCl4+HC group). Three mice were assigned to each group. The mouse in each group received intraperitoneal injections (5 μL/g body weight of CCl4/olive oil [1:4 vol/vol]) twice a week, except for the NC group (which received an equal volume of olive oil). The agonist group received intraperitoneal injections of GSK 10 μg/kg. The antagonist group received intraperitoneal injections of HC 10 mg/kg for 2 days during the last 2 weeks of CCl4 treatment. Drugs were dissolved in 0.5% dimethyl sulfoxide (DMSO) in a total volume of 50 μL. Each mouse in the NC and CCl4 groups received intraperitoneal injections of DMSO 50 μL. The mice were killed 24 hours after the final CCl4 injection, and liver tissues were harvested for further analysis. The experimental protocol was approved by the Central South University Animal Care and Use Committee.
Reagents
GSK1016790A, HC-067047, DMSO, and olive oil were obtained from Sigma Aldrich (USA). Carbon tetrachloride was purchased from Sinopharm (Beijing, China).

Chemical staining
The Hematoxylin-Eosin/HE staining kit, Masson’s trichrome staining kit, and Picro Sirius red (SR) solution were obtained from Solarbio Science and Technology (Beijing, China). All staining experiments were conducted according to the manufacturer’s instructions.

Western blotting
Whole-liver cell protein lysates were prepared by homogenizing 25 mg of liver tissue in lysis buffer. The procedure for the Western blot assay was reported previously. For Western blotting, the membrane was incubated in the primary antibody α-SMA (1:400 dilution; Boster Biological Technology, China). GAPDH (1:20,000 dilution; Proteintech) was used as an internal reference.

Statistical analysis
All experiments were performed in triplicate, and data are expressed as mean ± SD. Data analysis was per-
formed with the SPSS 19.0 software package. Statistical analyses were done by using the Student t-test, and a P value of <0.05 was regarded as statistically significant.

**Results**

Pharmacological activation or inhibition of TRPV4 channel activity can affect progression of advanced (8 weeks) and early (5 weeks) liver fibrosis in CCl4-treated mice.

To assess the effect of TRPV4 channel activation and suppression on liver fibrosis, we developed a model of advanced liver fibrosis in C57BL/6 mice (8 weeks). Liver fibrosis severity was determined by HE staining, Masson’s trichrome staining, and Sirius red staining. All mice treated with CCl4 (n=3) had liver fibrosis. As compared with the CCl4 group, deposition of collagen fiber and disorganization of liver lobules were more severe in TRPV4 activator-treated mice and less severe in TRPV4 inhibitor-treated mice (Fig. 1A).

We harvested liver tissue and performed Western blotting to analyze α-SMA, the actin isoform commonly used as a marker of fibroblast formation. α-SMA expression was upregulated in the activator-treated group and downregulated in the suppressor-treated group (Fig. 1B, C). This difference in α-SMA expression, along with the above histological findings, suggests that pharmacological activation or inhibition of TRPV4 channels regulates progression of advanced liver fibrosis.

The results for the early (5-week) liver fibrosis model were similar to those for the advanced model (Fig. 2).

**Discussion**

A number of chronic conditions, especially chronic liver disease, can lead to development and progression of liver fibrosis. However, although studies of the pathogenesis and treatment of hepatic fibrosis have made immense progress, there is currently no effective treatment. Interestingly, during liver injury, activated resident hepatic stellate cells differentiate into fibroblasts and secrete extracellular matrix components such as α-SMA and collagen I or III. This finding has been a dominant theme in research.

The TRPV ion channel family consists of several permeable nonselective members, which are distributed in multiple organs and tissues throughout the body. Activating factors include physical and chemical cues. The TRPV ion channel family is associated with multiple physiological and pathological processes, including calcium influx, intracellular environment stability, and maintenance of vascular function. Abnormal expression of TRPV and alteration of its channel activity are associated with many diseases. We previously reported that TRPV channels are important in the development of hepatocellular carcinoma, and recent studies noted that TRPV4 expression was significantly increased in liver fibrotic tissues. Blocking the TRPV4 channels of hepatic stellate cells with selective antagonists significantly inhibits their proliferation, perhaps because of autophagy-mediated apoptosis. However, the efficacy of drug-induced changes to the TRPV4 channel on liver fibrosis in vivo is unclear.

In the present study, we developed a mouse model of CCl4-induced liver fibrosis and treated the mice with a novel TRPV4 selective agonist, GSK1016790A, and antagonist, HC-067047. Our results suggest that activation of TRPV4 channels aggravates liver fibrosis and that inhibition of the TRPV4 channel alleviates liver fibrosis in vivo. Consistent with the results of previous in vitro experiments, the current findings indicate that TRPV4 is a latent target for treatment of liver fibrosis. Thus, the observed regulatory effects of TRPV4 on liver fibrosis suggest that our findings have broad implications for the study of TRPV4 as a therapeutic target for liver fibrosis in humans.

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**Conflict of Interest:** The authors have no conflicts of interest regarding the publication of this paper.

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