Answering Reviewers

Peer-Review considered that the authors aimed to determine the anti-fibrotic effects of Senicapoc (a KCa3.1 channel inhibitor) in different animal models. Senicapoc therapeutic effect seems to be mediated by a reduction in steatosis suggesting that fat accumulation in the hepatocytes might be directly related to the development of inflammation and in turn of fibrosis. The authors conclude that Senicapoc could be a good candidate for the treatment of NASH for the improvement of both steatosis and fibrosis.

Minor Peer–Review comments suggested to rewrite the abstract describing AIM, METHODS, RESULTS and DISCUSSION without exceeding the word count limits for publication, Other comments include to write Comments including Research frontiers, Innovations/breakthroughs and Applications, terminology and re-arranging references. Now, we have revised the manuscript according to all the peer-review comments for publication in the *WJG*.

ABSTRACT

AIM. To evaluate a calcium activated potassium channel (KCa3.1) inhibitor attenuates liver disease in models of non-alcoholic fatty liver disease (NAFLD).

METHODS. We have performed a series of *in vitro* and *in vivo* studies using the KCa3.1 channel inhibitor, Senicapoc. Efficacy studies of Senicapoc were conducted in toxin-, thioacetamide (TAA) and high fat diet (HFD)-induced models of liver fibrosis in rats. Efficacy and pharmacodynamic effects of Senicapoc was determined through biomarkers of apoptosis, inflammation, steatosis and fibrosis.

RESULTS. Upregulation of KCa3.1 expression was recorded in TAA-induced and high fat diet-induced liver disease. Treatment with Senicapoc decreased palmitic acid-driven HepG2 cell death. (*, $P<0.05$ vs control) supporting the finding that Senicapoc reduces lipid-driven apoptosis in HepG2 cell cultures. In animals fed a HFD for 6 weeks, co-treatment with Senicapoc, A) reduced

non-alcoholic fatty liver disease (NAFLD) activity score (NAS) (0-8 scale), B) decreased steatosis and C) decreased hepatic lipid content (Oil Red O *, P<0.05 vs. vehicle). Randomization of TAA animals and HFD fed animals to Senicapoc was associated with a decrease in liver fibrosis as evidenced by hydroxyproline and Masson's trichrome staining (p<0.05 vs vehicle). These results demonstrated that Senicapoc mitigates both steatosis and fibrosis in liver fibrosis models.

CONCLUSION. These data suggest that Senicapoc interrupts more than one node in progressive fatty liver disease by its anti-steatotic and anti-fibrotic activities, serving as a double-edged therapeutic sword.

COMMENTS

Background

Nonalcoholic steatohepatitis (NASH), a potentially serious form of the disease due to liver inflammation, which may progress to scarring and irreversible damage. This damage is similar to the damage caused by heavy alcohol use. In severe cases NASH can progress to cirrhosis and liver failure risk factors being obesity, diabetes and aging. Nonalcoholic fatty liver disease (NAFLD) is increasingly common around the world, especially in Western nations. In the United States, it is the most common form of chronic liver disease, affecting an estimated 80 to 100 million people.

Research frontiers

Given the large numbers of people with fatty livers and the generally increased life expectancy even relatively indolent steatosis can result in a significant population progressing to NASH, NASH with fibrosis and finally cirrhosis. The development of new therapies that prevent the transition from steatosis and inflammation to fibrosis would have substantial clinical value.

Innovations and breakthroughs

We report for the first time that a KCa3.1 channel inhibitor exerts an anti-steatotic effect in the setting of fatty liver disease which can be harnessed for the treatment of liver fibrosis. Second, we are the first to report that Senicapoc, a drug that has been through Phase III clinical trials, can be repurposed for the treatment of fatty liver disease and potentially for the treatment of other lipid disorders.

Applications

Based on its selectivity for the KCa3.1 channel, its half-life in humans, it's highly favorable safety profile and its activity against steatosis, Senicapoc is an ideal candidate to be repurposed for NASH or other lipid-related disorders.

Terminology

The continuum between steatosis, hepatitis, fibrosis and cirrhosis lends itself to therapeutic intervention at any one of these nodes. This study is the first to demonstrate that Senicapoc, a KCa3.1 channel inhibitor, exerts an anti-fibrotic effect in the liver and that in diet-induced liver disease this anti-fibrotic effect is mediated via a reduction in steatosis.