

Specific Comments to Authors: This is the first report that Fasudil's inhibitory effect on liver damage is due to the apoptosis of HSC. There is novelty in that respect. In the future, clinical introduction is desired for the improvement of liver fibrosis of Fasudil. Based on that, please add some notes.

1. Please explain as much as possible the direct causal relationship between NK activation and HSC suppression. To rule out epiphenomenon associated with fasudil administration, have you also investigated whether inhibition of NK activity abolishes the HSC suppression effect? If so, please add the results and a discussion.

Response: Thanks for your comments. As we know, HSCs play a vital role in the pathogenesis of liver fibrosis, they can secrete fibrogenic factors and then encourage several kinds of cell types including portal fibrocytes, fibroblasts, and bone marrow-derived myofibroblasts to produce collagen and thereby propagate fibrosis. More and more evidence suggests that NK cells generally display antifibrotic properties, including killing of activated HSCs by natural-killer-cell-derived IFN γ and induction of apoptosis in HSCs by expressing death receptor ligands such as TRAIL and FASL. NK cells also help clear senescent activated HSCs, thereby facilitating the resolution of fibrosis (*Fasbender F, et al. Natural killer cells and liver fibrosis. Front Immunol. 2016; Tsuchida T, et al. Mechanisms of hepatic stellate cell activation. Nat Rev Gastroenterol Hepatol. 2017*).

In this study, we found that Fasudil treatment promoted hepatic NK cell activation with the elevation of CD69 and NKG2D (Fig.3 & Fig.4), while NK cell depletion or inhibition was not performed. Furthermore, we observed that Fasudil treatment increased the lysis activity of NK cells against cultured LX2 cells (Fig.4B). Precious studies have found that inhibition of cytotoxicity of NK cells promoted the accumulation of LX2 cells (*Okwor CIA, et al. iScience. 2020; Peng H, et al. Sci. China Life Sci. 2018*). We have added these comments in **Introduction** and **Discussion** in revised manuscript.

2. Why is Fasudil specific for the suppression of HSCs? Also, please explain why Fasudil specifically induces apoptosis in HSCs.

Response: Thanks for your comments. Previous studies suggested that RhoA/ROCK signaling pathway activation is necessary for activated HSC, and it plays an important role in the development of hepatic fibrosis (*Gortzen J., et al. Rho-kinase inhibition is beneficial in fibrosis. Hepatology 2017*). Fasudil, as the first-generation selective Rho/ROCK inhibitor in the clinic, is clinically used to improve brain microcirculation and promote nerve regeneration, the viability of Fasudil for liver fibrosis needs to be investigated. In this study, we found that Fasudil treatment induced apoptosis in HSCs *in vitro*. The possible mechanism is that RhoA/ROCK inhibition could induce apoptosis via inhibition of ERK1/2, this phenomenon was also observed in other models (*Shi J. et al. Front Physiol. 2020; Jia Y. Int J Mol Med. 2018; Zhang Z., Life Sci.,2019*). We have added these comments in **Introduction** and **Discussion** in revised manuscript.

3. If you have studied the relationship between IL13, IL33 and other cytokines, please add it.

Response: In this study, the relationship between IL13, IL33 and other cytokines was not involved during Fasudil's inhibitory effect on liver damage. While according to the literature, IL-13, a multifunctional Th2 cytokine has been widely confirmed to be profibrotic mediator, IL-33 is a critical mediator of inflammation, is also involved in the development of liver fibrosis (*Gieseck RL., et al. Nat Rev Immunol.,2018; Tan Z. et al. Cell Mol Immunol.2018*). We added this point in **Introduction** in revised manuscript, we will implore the exact roles of these cytokines during Fasudil treatment in further study.

4. Some letters are incorrect or missing, and the abbreviation descriptions for TAA and HSC are in the wrong place. Please change them.

Response: We are very sorry for our careless. I have changed abbreviation descriptions in proper place.

