Reviewer's report

Title: Fluvastatin attenuates hepatic steatosis-induced fibrogenesis in rats through inhibiting paracrine effect of hepatocyte on hepatic stellate cells

Version: 2  Date: 10 September 2014

Reviewer: Paola Dongiovanni

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Fluvastatin attenuates hepatic steatosis-induced fibrogenesis in rats through inhibiting paracrine effect of hepatocyte on hepatic stellate cells
Chong LW et al.

In this study Chong et al. aimed to investigate the in vitro and in vivo anti-fibrotic properties of fluvastatin (Flu). In vitro, Flu inhibited PA-induced free-radical production, NADPH oxidase gp91phox expression and NFkB nuclear translocation in HepG2 cells and rat primary hepatocytes (PRHs). Moreover, conditioned medium-induced #-SMA protein expression in HSC-T6 cells was significantly suppressed in Flu-pretreatment group compared to those without treatment. In vivo, Flu reduced steatosis and fibrosis scores, #-SMA protein expression, mRNA expression of pro-inflammatory and pro-fibrogenic genes in livers of CDAA rats. The authors concluded that PA-induced HSC activation through paracrine effect of hepatocytes in vitro was significantly suppressed by Flu-pretreatment. In CDAA rats Flu reduced hepatic steatosis and fibrosis by mitigating inflammation and oxidative stress suggesting a possible therapeutic role of Flu in NASH treatment.

Major comments:

1. In the paragraph "Histopathological examination," the authors described that liver sections were stained with hematoxylin-eosin (H&E) or Sirius Red to evaluate collagen distribution. The authors didn’t show representative images of liver sections stained with H&E. Moreover, it seems that the images of sections stained with Sirius Red have been acquired with different magnifications. Therefore it is difficult to compare the images of the different treatments at 4 and 8 weeks. In table 1, authors reported a steatosis score of 3.00 in rats maintained at CDAA diet for 4 and 8 weeks. However, the degree of steatosis seems greater in rats fed CDAA diet for 8 weeks. Similarly the amount of steatosis does not seem the same in rats treated with the low dose of Flu at 4 weeks compared to untreated rats. Moreover, it is difficult to compare the degree of fibrosis between rats treated with CDAA+Flu [5] and CDAA+Flu [10] at 8 weeks.

2. Authors asserted that half of the animals from each group were sacrificed after 4 weeks, while the rest were sacrificed after 8 weeks. Does CDAA diet result in weight loss? During the first weeks rats fed CDAA lose weight (about 67 g) compared to controls. We should expect a further loss of weight at 8 weeks.
3. Authors have conducted in vitro experiments treating primary rats hepatocytes with Flu at different concentrations. It would be interesting to isolate primary hepatocytes from rats fed CDAA diet plus Flu at 4 and 8 weeks.

4. Have the authors tested higher concentrations of Flu in the in vivo model as described in literature?

5. RT-PCR is a semiquantitative method. It would be better to assess gene expression by qRT-PCR.

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests