Reviewer's report

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

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Reviewer: Stefania Grimaudo

Reviewer's report:

The manuscript is interesting because it is aimed at clarifying the effects of statins on NASH mediated fibrogenesis. Even if the current standard of care for the treatment of patients with NAFLD/NASH is focuses on lifestyle interventions, particularly weight loss and exercise, pharmacological therapies will still be required for the majority of patients with NASH. However there is a lack of consensus regarding the most effective and appropriate treatment for NAFLD/NASH.

In this work the paracrine effect of hepatocyte on HSCs activation is investigated and an appreciable in vitro and in vivo integrate approach is used. However the animal model for NAFLD/NASH chosen (Choline-deficient L-amino acid defined diet) is able to cause rapid hepatic accumulation of lipids and development of fibrosis due to an acute damage. This condition is deeply different from the human’s progression from NAFLD to NASH, so be careful in extending the results to human NASH.

Moreover the methods are generally appropriate and well described and the discussion and the conclusions are adequately supported by the data. For these reasons the writing is acceptable.

However the following Minor Essential Revisions are required:

• ABSTRACT - Results paragraph: “Flu-pretreated cells” instead of “Flu-treatment group”.

• INTRODUCTION: “nuclear factor kappa (NFκB) comprises a family of inducible transcription factors consisting among others of p65 and p50 subunit of Rel protein family”. Remember that NFκB is a dimeric transcription factor comprised of five family members RelA (p65), RelB, c-Rel, p50 and p52.

• MATERIALS AND METHODS – The title of Third paragraph must be “Measurement of reactive oxygen species (ROS) production”.

• MATERIALS AND METHODS – Third paragraph- “Performed at indicated time points by the excitation” instead of “Performed with”.

• MATERIALS AND METHODS – The title of Fourth paragraph must be “Cytotoxicity assay”.

• MATERIALS AND METHODS – Fourth paragraph- Cellular plating concentration must be indicate.

• MATERIALS AND METHODS – Fourth paragraph- “The absorption intensity at
540 nm” instead of “The A540 absorption intensity”.

• MATERIALS AND METHODS –Seventh paragraph- “levels were measured following standard procedures using a colorimetric analyzer (Dri#Chem 3000).

• RESULTS –First paragraph, first sub- paragraph: “after 24 hours of exposure” instead of culturing.

• RESULTS –First paragraph, second sub- paragraph: I disagree with the assertion “Flu (1-20 µM) concentration-dependently attenuated NF#B p65 nuclear translocation in both cell types (Figure 2A)”. In fact, even though it is visible that Pre-treatment with Flu for 2 hr reduced the NF#B p65 nuclear translocation in PA-treated HepG2 cells and PRHs at 6 hr after treatment, it is not evident a dose-dependent effect. The same assertion is found in the Discussion section.

• RESULTS –First paragraph, second sub- paragraph: “Flu treatment inhibited the mRNA expression levels of pro-inflammatory gene transcripts (ICAM-1, IL-8, TNF-#) in both PA-treated HepG2 cells and PRHs”. It is not clearly visible in the electrophoresis the difference between the line 2 and the others especially for TNF-# (Figures 2B and 2C).

• FIGURE 1 (A): On the ordinate axis of the HepG2 diagram “% of control group” must be use instead of “Fold of control group”.

• FIGURE 1(B),1(C): the figure must be more similar for HepG2 and PRH cells: the istograms –PA, -Flu and 200 uM PA, -Flu must be repeat twice in HepG2 -6hr and -12hr.

• FIGURE (2A): On the ordinate axis of the HepG2 diagram “P65/PCNA” must be used instead of “P65/#-actin”.

• NOX could be added to abbreviations.

• Finally, I suggest the following Discretionary Revision:
The method used to evaluate mRNA expression is the Reverse Transcription and semi-quantitative PCR. I think that an approach of relative quantification using Reverse Transcription Real Time PCR could be more appropriate. It can provide better sensitivity and precision respect to the results shown in FIGURE 2B and 2C.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**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’ below