Reviewer's report

Title: Dissolved molecular hydrogen (H2) in Peritoneal Dialysis (PD) solution preserve mesothelial cells and peritoneal membrane integrity

Version: 0 Date: 16 Feb 2017

Reviewer: Sandra Rieger

Reviewer's report:
The manuscript by Zhu et al. investigates the potential positive effects of dihydrogen (H2) on mesothelial injury induced by peritoneal dialysis. Peritoneal dialysis can cause oxidative injury and H2 is thought to act as an antioxidant to reduce injury. In this study, the authors tested this idea. They find that H2 supplementation in dialysis bags has various positive effects on the mesenthelium leading to reduced mesothelial thickening and infiltration of the mesothelium by macrophages, and H2 can also normalize cell death and proliferation.

Comments:

1) The authors make the point that H2 acts as anti-oxidant, however no evidence is shown that it does. In the discussion, first paragraph the statement saying that the authors studied increased oxidative stress induced by FeCl3 is not supported. Could the authors assess oxidative stress markers by qPCR or use their microarray data to identify any stress-related genes that might be differentially regulated?

2) The images in Figure 2 are too small and one cannot clearly distinguish individual cells with expression of the indicated genes. Please generate zoomed images and show them side-by-side.

3) It is unclear how cells were counted. In the Materials and Methods section it was mentioned that 5 randomly selected fields were quantified (how large are they?) but the graphs show that cells were counted per image (if "pic" means picture?). A standard way would be to express the cell numbers per area (e.g. cm2).
4) Perhaps the authors could be more explanatory why PD increases proliferation and apoptosis at the same time? Is there evidence that the same cell type is affected or are these different cell types?

5) It is unclear how the shedding cell analysis in Fig. 6c was performed? There is no mentioning in the text.

6) The legend for Figure 6 does not match what is shown in Figure 6.

7) It is unclear how the array data was evaluated. What does the percentage difference between the two data sets mean in terms of gene expression and biology?

8) The authors state "combined pathologies of EMT.....". It is unclear what that means. If it means that EMT is increased after PD, the authors can perhaps comment on the observation that the pro-EMT gene Snail is downregulated in PD rats, and is not rescued by H2. How does the H2 improve the phenotypes?

9) It would be helpful to know why the authors assessed the expression of the chosen genes, especially aSMA and cytokeratin.

10) How were the dialysis solutions administered to rats?
11) Please also indicate the statistical significance of the PD and P/H2 groups in Figure 3A-D, if there is any?

**Are the methods appropriate and well described?**

If not, please specify which controls are required in your comments to the authors.

No

**Does the work include the necessary controls?**

If not, please specify which controls are required in your comments to the authors.

Yes
Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.
Yes

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