Reviewer's report

Title: 1 MAC Isoflurane Preconditioning Induce Protective Effects of Heme oxygenase-1 on Hepatic Ischemia Reperfusion in Rats

Version: 1 Date: 10 May 2010

Reviewer: Greg Barritt

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Major Compulsory Revisions

1. Effects of intervention on HO-1 enzymatic expression, Results, paragraph 1 and Fig. 1. The effects of IR and IR plus isoflurane compared to sham are relatively small. IR has induced an approximately 1.5-fold increase, and IR plus Iso in approximately 2-fold increase, compared to Sham. Given the magnitude of the error bars, it is not completely clear whether there should be or is a significant difference between IR and Sham. Again, taking account of the size of the indicated error bars, it is not completely clear whether there should be or is a significant difference between IR plus isoflurane and IR. Since this is really the central point of the whole paper, more convincing evidence for these changes should be provided.

2. Relating further to the above, the effect of Znpp. The bar indicated Iso plus Znpp. The authors claim that Znpp added prior to isoflurane pre-treatment completely blocks the action of isoflurane. It should be made clear in this graph that isoflurane treatment after Znpp involves both isoflurane treatment and IR. There is no control for the effect of Znpp on the effect of IR in absence Iso. Therefore it seems that the authors cannot conclude that Znpp has reduced the isoflurane effect. The decrease in the bar marked Iso plus Znpp could be due to an effect of Znpp on the IR action and not on isoflurane.

3. Related further to the above comments, the description of the groups in the Methods under “4. Experimental groups” should be stated in list form much more clearly than it presently is. As mentioned, it seems that it would be necessary to add an additional group which is Znpp and IR without isoflurane pre-treatment.

4. Western blots, Fig. 2, Results, paragraph 2, effect of the different conditions on HO-1 protein experiment analysed by Western blot. I believe two things are required for these experiments. Firstly, data on the quality of the primary antibody employed in Western blot should be shown with a full scale gel showing the position of the HO-1 band and molecular weight markers. How specific is the antibody? Secondly, the data should be quantitated by measuring the intensity of the spots by image analysis, number of experiments given and mean values for each condition given. The argument above related to the effects of Znpp and the interpretation of these results also applies for Fig. 2 Western blot, Group D, Iso plus Znpp Group compared to Group C which is IR plus isoflurane.

5. Fig. 3, immunohistochemistry results and Results text, paragraph 2. The data
should be scored quantitatively using an accepted form of scoring for injury (grading score), quantitative results expressed and statistics undertaken. Serafin et al. (2002) Am. J. Path. 161, 587 is one example of a grading system.

6. Fig. 4, plasma ALT and AST concentrations, Results, Section 2. The changes in marker enzymes for liver damage, apart from the effect of IR compared to Sham, are small. The trend is there (as for also Figs. 5 and 6), but again the changes are very small. A confirmation on the statistical analysis is important. As indicated by others, in other studies effects of ischemic preconditioning, for example, or intermittent ischemia are not usually revealed as changes in liver marker enzyme concentration until about 6 h post beginning of reperfusion. (The same comment also may apply to histological changes.) With respect to liver marker enzymes, there is no data for effect of Znpp alone. Since Znpp plus Iso increases liver marker enzymes compared to IR plus Iso, it would be important to check that Znpp alone does not increase compared to Sham.

7. Title and relationship of this study to Reference 16. As indicated by the present authors, Schmidt et al. have previously shown that isoflurane will induce HO-1 in liver. The potential value of the present studies is the effects of isoflurane at clinically-relevant doses. It would be better to change the title to reflect this more explicitly. The term “1 MAC” may not be readily understandable to many readers. Something like “isoflurane preconditioning at clinically relevant doses induces protective effects of HO-1 ...”. (There may be better wording.) Also in this connection a paper of Beck-Shimmer et al., Annals of Surgery, 248, December 2008, could also be cited. This refers to clinical trials and possibly induction of HO-1.

Minor Essential Revisions

1. Methods, paragraph 2, hepatic ischemia reperfusion. Rats were anaesthetised with sodium pentobarbital and remain under anaesthesia for at least 5 h. Some comment on effectiveness of anaesthesia during this time period should be included.

2. Methods, hepatic HO-1 activity assay, homogenisation. More details of ultrasonic homogenisation should be given. Also a reference for measurement of bilirubin, the principle.

3. Methods, Section 9, transaminases TNF and myloperoxidase. Where appropriate, principle of the assay should be given, rather than just saying, for example, “using a kit from ....”.

4. Results, paragraph 2, histology, Fig. 3. The statement “based on morphological characteristics HO-1 was expressed mostly in hepatocytes”. This requires more detail in order for the reader to be convinced that HO-1 is actually expressed principally in hepatocytes.

5. Results, Section 3, effects of intervention on cell morphology, Fig. 7, second to last paragraph before Discussion. The statement “integrity of the overall structure of hepatic lobules were seriously compromised” should be clarified and explained better.

6. In the Discussion, first three paragraphs. To a great extent these paragraphs
refer to reasons why the experiments were performed, especially relating to Schmidt et al. reference 16 and the aim of the present study which is to use lower clinically-relevant doses. This material should be expressed more concisely that it is now but in the Introduction.

7. Discussion, paragraph 4, referring to results relating to “As Znpp is not the specific inhibitor of HO-1, it completely blocked the increase of HO-1 activity”. As mentioned, cannot really see clearly from the data the effects of Znpp. Also the differentiation between effects of Znpp on activity of the enzyme which is present in the cells and effects of Znpp on gene expression of the enzyme should be more clearly described. These issues should be made more clear.

8. Later in Discussion, two paragraphs on “HO-1 ubiquitous enzyme and among the three mammalian isoforms of HO-1 etc”. Should be expressed more concisely in Introduction and Discussion shortened.

9. Second to last paragraph of Discussion, comment that “IR damage through phenomenon called preconditioning”. This should be modified to say “through the same mechanism as preconditioning”. The phenomenon, although it is a form of preconditioning, is not the same.

10. Fig. 1, for some statistics, there is no symbol described in the legend.

Discretionary Revisions

1. Methods, Section 7, Western blot, chemiluminescence agents, source should be indicated.

2. Immunochemistry, reference for the principle of measurement of HO-1 immunoreactivity should be given.

3. Methods, Section 8, towards the end of the paragraph, the word “polymerise” in relation to anti-rabbit IgG is probably not correct. Possibly covalently-linked.

4. Discussion, beginning of paragraph 2, the term “hepatic flux” possibly means blood flow?

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.