Author's response to reviews

Title: Pretreatment of parecoxib attenuates hepatic ischemia reperfusion injury in rats.

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Author's response to reviews: see over
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Dear Editor,

Thank you very much for your consideration of our manuscript. We have provided point-by-point responses to yourself and the reviewers concerning the comments.

Responses to reviewer 1

1) According to your comments, we have revised the clerical error between 12th and 13th references.

Responses to reviewer 2

1) With regard to the time of reperfusion selected in this study, we used 6 hours after reperfusion to do all the measurements in this study, because some previous studies have shown that at 6 hours after reperfusion, the tissue damage, serum levels of liver injury markers and inflammation can already be well detected \(^{(1,2)}\). Some previous studies also showed that the peak levels of serum ALT and AST and inflammatory cytokines could be detected within 6 h of reperfusion \(^{(3,4)}\). We observed a significant reduction of liver injury and inflammation at 6 h after reperfusion by parecoxib pretreatment, so we did not further pursue the effects on liver at later time points, such
as 24 hours.

2) According to your comments, we have confirmed the expression of TNF-α and IL-6 at the protein level by ELISA using the serum samples stored at −80°C in this experiment.

3) According to your suggestion, we describe the mechanism of the IR injury suppression by parecoxib in more details by comparing the expression of iNOS and nitrotyrosine among the three groups using the liver samples. We observed that parecoxib administration lead to decreased iNOS and nitrotyrosine levels in hepatic ischemia/reperfusion (I/R), which suggested that parecoxib decreased the nitrosative stress caused by I/R. This result is consistent with the decrease in number of apoptotic cells (see result of TUNEL staining). Nitrosative stress is increasingly being recognized as playing an important role in the cellular damage associated with I/R injury (5).

When iNOS is upregulated in I/R injury the excessive nitric oxide (NO) produced is free to react with superoxide anion (O2 −), creating peroxynitrite (ONOO−). Peroxynitrite then contributes to injury through lipid peroxidation, apoptosis, and necrosis by nitration of tyrosine residues on tissue proteins (6). Nitrotyrosine is a marker for peroxynitrite (ONOO−).

4) Concerning the animal model used in the survival study, most techniques of hepatic ischemia/reperfusion (I/R) in rats or mice
include segmental rather than total clamping of the hepatic blood supply to prevent mesenteric congestion.

In order to be comparable to previous hepatic I/R studies, we used this 70% ischemia model in this study. However, partial hepatic ischemia is not a method from which animal survival can be assessed. When using a 70% I/R model, I/R-mediated deterioration of liver function is in part compensated for by the residual 30% of unaffected liver, precluding accurate functional assessment of the post-ischemic liver parenchyma. Resection of the residual 30% liver at the onset of reperfusion constitutes a more relevant model, in which only post-ischemic liver parenchyma is retained \(^{(7,8)}\). This model allows the assessment of survival.

5) According to your comments, we found that we had made a mistake in describing the result of MPO. We apologize for the mistake and we have corrected it.

6) On the basis of your comments, we have revised the discussion segment and have discussed more mechanism about the effects of parecoxib.

7) According to your comments, we have corrected some grammatical errors in the results and discussion.

We hope that we have satisfactorily answered the comments and
concerns. Please do not hesitate to contact me if you have any further questions. Thank you again for your consideration.

Yours sincerely,

Wen-Qi Huang

Reference


