Reviewer's report

Title: Protection of Early Phase Hepatic Ischemia-Reperfusion Injury by Cholinergic Agonists

Version: 1 Date: 28 October 2005

Reviewer: Ian Hines

Reviewer's report:

General
The article entitled "Protection of Early Phase Hepatic Ischemia-Reperfusion Injury by Cholinergic Agonists" by Crockett et al. describes the ability of cholinergic agonists to improve the acute phase of hepatic ischemia and reperfusion injury possibly through modulation of pro-inflammatory cytokine expression. The methods of the current study are sound and adequately described. The conclusions made from the data derived in this study are appropriate. Overall, the manuscript is well written.

The findings of the current study are relatively novel and provide a new avenue for exploration into the treatment of post-ischemic liver injury. Previous studies have identified the protective effect of cholinergic nerve activation in other organ systems and in other inflammatory models. The current manuscript builds on a potential role for cholinergic signaling to affect hepatic IR injury. In the current series of studies, cholinergic nerve activation appears to protect, at least acutely, the post-ischemic liver. Perhaps most significantly, the findings presented here separate the early acute phase of reperfusion (3-6 hours post-ischemia) injury from the later subacute phase as agonist treatment failed to reduce the later IR-induced injury. Specifically, the early cytokine response associated with hepatic ischemia and reperfusion injury appears to play little or no role in the later sub-acute phase. Unfortunately, the mechanisms for this disconnect are not explored.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Neutrophil accumulation is a hallmark of ischemia and reperfusion injury to the liver. In figure 4, neutrophil accumulation is presented by immunohistochemistry. Appropriate control antibodies are used to demonstrate staining specificity. However, when stained with the anti-neutrophil antibody, it appears that the majority of the necrotic tissue is also stained. Demonstration of the massive neutrophil infiltration using another method such as the biochemical myeloperoxidase assay is strongly suggested.

2. This manuscript suggests a disconnect between hepatic mRNA and serum protein levels for TNFa, IL6, and MIP1a. The presentation of routine rtPCR results by gel is not convincing. In the next figure, you present real time amplification curves and their respective cT values. Real-time PCR is far superior to standard end-point analysis by traditional rtPCR. A more convincing demonstration of liver cytokine message expression by real-time PCR expressed relative to the sham operated control value is strongly suggested. Use of the comparative ct method will allow for quantitative determination of liver message expression.

3. Blockade of cholinergic nerve stimulation to the liver is associated with increased perfusion following short-term ischemia in the rat (Am J Physiol Heart Circ Physiol. 2000 May;278(5):H1565-70). These data would suggest that activation of cholinergic nerve pathways may decrease sinusoidal perfusion and thus delay the onset of reperfusion-induced tissue injury. Are
there any direct data available to assess the impact of DMPP or nicotine on sinusoidal perfusion?

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Recheck the format of all references. Several errors in format were noted.
2. Check all figures and legends for completeness.

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Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.