ANSWERING REVIEWERS

Regulatory effect and mechanisms of carbon monoxide-releasing molecule II on hepatic energy metabolism in septic mice

Feng Liang, Jie Cao, Wei-Ting Qin, Xu Wang, Xue-Feng Qiu, Bing-Wei Sun

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 7264

Responses to Reviewers

Reviewer #1

1. In the abstract under results, actual data should be presented, including SD and p values.

Yes, the actual data including SD and p values have been included in the Results paragraph of Abstract in the revised manuscript.

2. Results and Fig. 1, p values are to be presented.

Yes, the p values have been included in Fig 1 in the Results section of the revised manuscript.

3. Discussion, first sentence. Please provided reference(s). Second sentence, please compare the deaths by sepsis to death by other diseases in the US.

Yes, the reference has been cited in Discussion section first sentence of the revised manuscript.

4. A detailed discussion in the text is necessary how your data may be applicable in human sepsis.

Yes, the benefit effects of CORM-2 on improving survival during sepsis and application prospects were discussed in Discussion section. Exogenous CO has significant physiological functions that could be exploited as a potential therapeutic agent. More detailed molecular mechanisms need to be further studied and clarified from a more

comprehensive perspective.

Minor points:

1. In the title and the first sentence of the abstract, please give some information about CORM-2 to those readers not familiar with this product.

Yes, detail information about CORM-2 has been provided in the Discussion section as followed:

Recently, transition metal carbonyls have been identified as potential CO-releasing molecules (CORMs) with the potential to facilitate the pharmaceutical use of CO by delivering it to the tissues and organs of interest. Studies elucidated that CORM-2 suppresses LPS-induced inflammatory responses in human umbilical vein endothelial cells (HUVECs), peripheral blood mononuclear cells (PBMCs) and macrophages. Similarly, many results have confirmed that CO derived from CORMs rescues mice from lethal endotoxemia and sepsis induced by LPS or cecal ligation and puncture (CLP) models. Our previous studies have shown that CORM-2 inhibited over-expression of adhesion molecules, attenuated leukocyte sequestration in the organs of CLP or burn-induced septic mice, decreased intracellular oxidative stress and NO production in LPS-stimulated HUVECs.

2. Abstract, aim: sepsis should read experimental sepsis.

Yes, the inappropriate expressions have been revised in Abstract section in the revised manuscript.

3. In the abstract, all abbreviations should be expanded when first mentioned. Enzymes such as ALT, AST and others should be evaluated as activities not as levels, this applies also to the text of the manuscript.

Yes, the inappropriate expressions have been revised in the revised manuscript.

4. Introduction, second sentence requires reference(s).

Yes, the reference has been cited in Introduction section second sentence of the revised manuscript.

5. Materials, first line. Please give details of the origin of CORM-2, from what is was prepared. Last sentence, German should read Germany.

Tricarbonyldichlororuthenium(II) dimer (CORM-2) has been provided in Materials section, first line of the revised manuscript. The inappropriate expression (German) has been corrected.

Reviewer #2

What is a main mechanism of CORM-2 on improving survival? Please explain this mechanism in discussion.

Yes, the benefit effects of CORM-2 on improving survival during sepsis were discussed in Discussion section as followed:
Recently, transition metal carbonyls have been identified as potential CO-releasing molecules (CORMs) with the potential to facilitate the pharmaceutical use of CO by delivering it to the tissues and organs of interest. Studies elucidated that CORM-2 suppresses LPS-induced inflammatory responses in human umbilical vein endothelial cells (HUVECs), peripheral blood mononuclear cells (PBMCs) and macrophages. Similarly, many results have confirmed that CO derived from CORMs rescues mice from lethal endotoxemia and sepsis induced by LPS or cecal ligation and puncture (CLP) models. Our previous studies have shown that CORM-2 inhibited over-expression of adhesion molecules, attenuated leukocyte sequestration in the organs of CLP or burn-induced septic mice, decreased intracellular oxidative stress and NO production in LPS-stimulated HUVECs. In the present study, release of CO molecules by CORM-2 protects mitochondria and maintains the stable level of hepatic glucose metabolism. CORM-2 thus improves liver function and survival in septic mice.

Reviewer #3

1 In materials and methods section, the authors should provide the chemical structure of CORM-2.

Our published paper regarding chemical structure of CORM-2 has been cited in MATERIALS AND METHODS section of the revised manuscript:
Sun BW, Zhiwei Sun, Qin Jin, et al. “CO-releasing molecules (CORM-2)-liberated CO attenuates infiltration of leukocytes in the renal tissue of thermally injured mice. “ International J Biological Sci, 4（3）：176-83, 2008.

2 A dose of 8 mg/kg of intravenous CORM-2 was administered to each mouse in the treatment group. But why you selected the dose of 8 mg/kg?

The concentration of CORM-2 used in the present study was based on our previous report of the use of this compound in mice and the preliminary experiments in our laboratory by

measuring dynamic COHb levels and peak levels, which did not exceed 15±5% above normal levels.

1）Liu DM，et al, “Suppression of inflammatory cytokine production and oxidative stress by CO-releasing molecules-liberated CO in the small intestine of thermally-injured mice,” Acta Pharmacol Sin, 29 (7) : 838 - 846, 2008.

2） Sun BW， et al. “CO-releasing molecules (CORM-2) -liberated CO attenuates leukocytes infiltration in the renal tissue of thermally injured mice,” Int J Biol Sci, 4 (3) : 176 -183, 2008.

3） Sun BW，et al, “Attenuation of leukocytes sequestration by carbon monoxide-releasing molecules: liberated carbon monoxide in the liver of thermally injured mice,” J Burn Care Res, 28(1) : 173 - 181, 2007.

4） Sun BW， et al, “Role of CO-releasing molecules liberated CO in attenuating leukocytes sequestration and inflammatory responses in the lung of thermally injured mice,” J Surg Res, 139 (1) : 128 -135, 2007.