August 12, 2016

Dear Editor and Reviewers:

Thank you very much for reviewing our manuscript. We carefully read the comments and revised our manuscript accordingly, as described below:

Reviewer's comments:

Reviewer #1 (03051573): Decision: Major revision
This is a very interesting manuscript that reviews the role of nitric oxide administration during ischemia/reperfusion injury in patients undergoing liver transplantation. The manuscript is well written although the text needs some improvement. A list of potential modifications to the manuscript has been attached for author's evaluation to be included.

1. Abbreviation of nitric oxide should be changed from NO to NO ·

   We changed from NO to NO· according to the reviewer's comments.

1. Additional pathways for nitric oxide generation in the body should be included. In addition to enzymatic generation of NO ·by Nitric Oxide Synthases, there is a mechanism for chemical NO ·generation in the stomach by nitrite acidification [Gago Bet al., 2007 Free Radic Biol Med 43: 1233-1242]; Rocha et al., 2012 Free Radic Biol Med 52: 693-698] The nitric oxide generated in the stomach by this pathway can diffuse from gastric lumen ·to the gastric vasculature and outside the stomach after cardiac arrest. The need of a nitric oxide supplementation during liver transplantation could indicate the importance of the nitric oxide generated in the stomach for liver function in normal individuals.

   We agreed the reviewer's opinion and added eNOS independent NO production in stomach in the discussion. Also we revised the figure according to reviewer's recommendation.

1. Special care should be empathized in cases where liver transplantation is needed and patient suffers from methemoglobinemia. There are several cases reported in the bibliography where methemoglobin formation is found when nitric oxide is administered by inhalation (these references should be included in the review). This results correlates with the presence of nitrite and nitrate in blood described in the manuscript. Since methemoglobin reductase plays an important role on hemoglobin recycling, patients with a deficit on this activity could suffer fatal consequences after nitric oxide administration.

   We agreed the reviewer's opinion and added nitric oxide induced methemoglobinemia in the discussion.

1. Generation of peroxynitrite by rapid reaction between superoxide anion and nitric oxide is expected. The rate constant for the reaction between NO ·and O2 ·- to produce peroxynitrite is 6.7x109 M -1 s -1 and production of peroxynitrite is a diffusion-controlled reaction. Indeed lung damage has been reported after inhaled NO ·administration. For this reason when NO ·has been administered by inhalation other gasses has been used to be co-administered to avoid formation of peroxynitrite, i.e. hydrogen. For this reason not only the amount of NO ·should be indicated but also time that the procedure took to administrate NO ·. The percentage administered in relationship to other gasses (O2 or others) should also be included.

   We agreed the reviewer's opinion and added nitric oxide induced peroxynitrite production and co-administration of hydrogen gas to ameliorate peroxyniterite-associated lung injury in the discussion.

The last paragraph of the manuscript about recommendation of nitric oxide use for routine administration should be avoided, until more studies and clinical trials are performed.

We deleted the last sentences according to reviewer's advice and added following sentence: "Therefore nitric oxide has a potential to be a good therapeutic option for organ resuscitation in liver transplantation, especially for the extended criteria (marginal quality) donors, but further investigation is still warranted for routine clinical use." Thank you for all of your advice. I appreciate your time and effort to make this manuscript more valuable.

Reviewer #2 (3042420) Decision: Minor revision
In this review, authors recapitulated biological characteristics and therapeutic application of nitric oxide (NO) in liver transplantation. In general, this manuscript is well prepared although there are several revisions that need to be addressed.

1. Major points: As shown previously and discussed by the authors, the exact role of NO in organ repair/regeneration is quite controversial. The main determinants of the effects of NO on liver protection are its concentration, dosage, and duration. In this regard, the authors should address the potential risk on the therapeutic use of NO for liver transplantation in the animal experiments and clinical translation.

   Potential risk for methemoglobinemia and generation of peroxynitrite were added to the text according to the reviewer's advice.

2. Minor points: Grammar and typo errors: "These pulmonary effects include decrease pulmonary and systemic vascular resistance with resultant improvements in tissue oxygenation, …"

   Errors are corrected according to reviewer's advice. Thank you for all of your advice. I appreciate your time and effort to make this manuscript more valuable.

Reviewer #3 (0502871) Decision: Major revision
The manuscript is a well performed literature review of studies of nitric oxide.

1. It would be an appropriate introduction for a grant proposal for a randomized control trial of use of nitric oxide in liver transplantation. Such a study would be useful, but until it is performed, it is premature to recommend this as a change in standard of care.

   We deleted the last sentences according to reviewer's advice and added following sentence: "Therefore, nitric oxide has a potential to be a good therapeutic option for organ resuscitation in liver transplantation, especially for the extended criteria (marginal quality) donors, but further investigation is still warranted for routine clinical use." Thank you for all of your advice. I appreciate your time and effort to make this manuscript more valuable.

Reviewer #4 (3536011) Decision: Accept
This review article is well written with a wide area of experimental and clinical results involved in NO and NOS of liver IR injury. In inflamed liver, iNOS is expressed in Kupffer cells and hepatocytes, followed by

high levels of NO production. Proinflammatory cytokines including TNF-α and IL-1β and NO produced by iNOS have been implicated as factors in liver injury [Inflammation & Allergy- Drug Tagets, 2015;14:77-83]. In general, NO produced by eNOS in liver sinusoidal endothelial cells protects against injury, whereas NO produced by iNOS contributes to pathological processes. However, NO produced during various types of liver injury may have either detrimental or beneficial effects depending on the insults and cell types involved. Reviewer agree authors' conclusion; Accumulated evidence indicates that inhaled NO may have protective-effects on the donor liver graft against IR injury in patients undergoing liver transplantation.

Thank you for all of your advice. I appreciate your time and effort to make this manuscript more valuable.

Thank you very much for your constructive comments. We appreciate your time and effort to review our manuscript, and ultimately make it more informative to the readership.

If you have any additional questions about our manuscript, please contact John Lang, MD at the addresses listed below. We look forward to your favorable review of our revisions.

Sincerely,

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