

RESPONSE TO EDITORS AND REVIEWERS
of “Ultrastructural Changes in Porcine Liver Sinusoidal Endothelial Cells of
Machine Perfused Liver Graft Donated after Cardiac Death”
by Hiroki Bochimoto, Yo Ishihara, Nur Khatijah Mohd Zin, Hiroyoshi Iwata,
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July 5, 2021

On behalf of my co-authors, I would like to express our gratitude to the editors and reviewers for their thoughtful review of the manuscript. They raise important issues and their comments are very helpful for improving the manuscript. We agree with almost all their comments and we have revised our manuscript carefully.

We are including all editors' and reviewers' suggestions and clarifying the text when needed. We are confident that the new version of the manuscript will be greatly improved.

We respond below in detail to each of the comments. In addition, we include how we have revised things, or if we have slightly disagreed with something, we stated why. We hope that the editors and reviewers will find our responses to their comments satisfactory, and we would be glad to respond to any further suggestion and comments that may be raised.

Please, find below the editors' and reviewers' comments repeated in italics and our responses inserted after each comment.

We fully understand the COVID-19 pandemic is now having a big impact on you, but we look forward to hearing from you at your earliest convenience.

Sincerely,
Hiroki Bochimoto M.D., Ph.D.

Response to comments from Company editor-in-chief

We are grateful to company editor-in-chief for sparing your precious time to review our manuscript. As indicated in the responses that follow, we have taken all the comments and suggestions into account in the revised version of our manuscript.

Comment

I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.

Response:

We really appreciate your wonderful decision that our manuscript have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. We follow your advice to revise our manuscript according to the peer review report, Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.

Response to comments from Science editor

We are grateful to science editor for the scientific comments that our manuscript is conditionally accepted for the publication in World Journal of Gastroenterology. As indicated in the responses that follow, we have taken all the comments and suggestions into account in the revised version of our manuscript.

1 Scientific quality:

The manuscript describes a basic study of the ultrastructure of liver sinusoidal endothelial cells after machine perfusion. The topic is within the scope of the WJG.

1 Scientific quality – (1) Classification:

Grade B and Grade B;

Response:

We really appreciate the science editor for judging that our research topic is within the scope of the WJG and the very good rating to the scientific quality of our research manuscript. The editor has a good understanding of the goal of our manuscript. The positive comments to scientific quality of our manuscript from the science editor really encourage us.

1 Scientific quality – (2) Summary of the Peer-Review Report:

Reviewer 05433027 point out that the findings of the authors are very interesting. It is suggested that MMP is more effective than HMP in alleviating graft injury after DCD, in pictures 2-5, there are groups A, B, C, and D. can you put A, B, C, and D together. The activity of hepatocyte should be reevaluated by deepening method. Reviewer 03660289 point out that the manuscript is well written and interesting and provides insight into ultrastructural changes of DCD livers after machine perfusion. The HMP used in group A is similar to dual-HOPE except for the portal line, which is not oxygenated. Can the authors comment on this?;

1 Scientific quality – (3) Format:

There are 5 figures;

Response:

We appreciate the science editor that summarize the reviewer's comments. We taken all reviewers comments and suggestion, and revised our manuscript and figures as described

in Response to comments from Reviewer #1 and #2. Please see our revised manuscript.

1 Scientific quality – (4) References: *A total of 58 references are cited, including 7 references published in the last 3 years;*

1 Scientific quality – (5) Self-cited references:

There are 10 self-cited references. The self-referencing rates should be less than 10%. Please keep the reasonable self-citations (i.e. those that are most closely related to the topic of the manuscript) and remove all other improper self-citations. If the authors fail to address the critical issue of self-citation, the editing process of this manuscript will be terminated; and

Response:

We appreciate the science editor's comment that make us realize too much self-cited reference. Following your comment, we deleted the 5 self-citation described below from revised manuscript.

7. Matsuno N, Sakurai E, Uchiyama M, Kozaki K, Miyamoto K, Kozaki M, Nagao T. Role of machine perfusion preservation in kidney transplantation from non-heartbeating donors. Clin Transplant [Internet] 1998 [cited 2015 Oct 13];12:1–4 [PMID: 9541415]Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9541415>
15. Matsuno N, Obara H, Watanabe R, Iwata S, Kono S, Fujiyama M, Hirano T, Kanazawa H, Enosawa S. Rewarming preservation by organ perfusion system for donation after cardiac death liver grafts in pigs. Transplant Proc [Internet] 2014;46:1095–8 [PMID: 24815137 DOI: 10.1016/j.transproceed.2013.12.035]Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24815137>
16. Meng L, Matsuno N, Watanabe K, Furukori M, Obara H, Bochimoto H, Watanabe T, Fukukawa H. Scanning Electron Microscopy Findings of Machine Perfused Liver Graft After Warm Ischemia Between Hypothermic and Rewarming Machine Perfusion in Pigs. Transplant Proc [Internet] 2016;48:2467–70 [PMID: 27742324 DOI: 10.1016/j.transproceed.2016.03.059]Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0041134516302767>
31. Matsuno N, Uchiyama M, Iwamoto H, Hama K, Narumi Y, Kikuchi K, Degawa

- H, Kozaki K, Nagao T. Machine perfusion preservation for liver transplantation from non-heart-beating donors with agonal stage. *Transplant Proc* [Internet] 2002;34:2610–1 [PMID: 12431543 DOI: 10.1016/s0041-1345(02)03444-9]Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12431543
54. Shigeta T, Matsuno N, Obara H, Kanazawa H, Tanaka H, Fukuda A, Sakamoto S, Kasahara M, Mizunuma H, Enosawa S. Impact of rewarming preservation by continuous machine perfusion: improved post-transplant recovery in pigs. *Transplant Proc* [Internet] 2013;45:1684–9 [PMID: 23769024 DOI: 10.1016/j.transproceed.2013.01.098]Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0041134513001887>

1 Scientific quality – (6) References recommendations:

The authors have the right to refuse to cite improper references recommended by the peer reviewer(s), especially references published by the peer reviewer(s) him/herself (themselves). If the authors find the peer reviewer(s) request for the authors to cite improper references published by him/herself (themselves), please send the peer reviewer's ID number to editorialoffice@wjgnet.com. The Editorial Office will close and remove the peer reviewer from the F6Publishing system immediately.

Response:

We really appreciate the science editor for your kindness. Here, we declare that the peer reviewers did NOT recommend to cite improper references.

2 Language evaluation – Classification:

Grade B and Grade B.

3 Academic norms and rules:

The authors provided the Biostatistics Review Certificate, the Institutional Review Board Approval Form, and the Institutional Review Board Approval Form. The authors should provide the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement. The authors need to fill out The ARRIVE Guidelines with page numbers. No academic misconduct was found in the Bing search.

4 Supplementary comments:

This is an invited manuscript. The study was supported by JSPS KAKENHI. The topic has not previously been published in the WJG.

Response:

We really appreciate the science editor's comment that make us realize to have to provide the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement. Moreover, we filled out the ARRIVE Guidelines with page number. Following your comment, we make and revised, and upload the three forms described above in this revision. In addition, we are grateful to the science editor's positive evaluation to academic quality and topic of our manuscripts.

5 Issues raised – (1)

The title is too long, and it should be no more than 18 words;

Response:

We appreciate the science editor's comment that make us realize to the title is too long. We changed our manuscript title "Ultrastructural Changes in Porcine Liver Sinusoidal Endothelial Cells of Machine Perfused Liver Graft Donated after Cardiac Death" to "Ultrastructural Changes in Porcine Liver Sinusoidal Endothelial Cells of Machine Perfused Liver Donated after Cardiac Death".

5 Issues raised – (2)

The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

Response:

We appreciate the science editor's comment that make us realize to have to provide the approved grant application forms. Following your comment, we upload the forms of two our JSPS KAKENHI Grant Numbers JP17K10503 (to Matsuno N) and JP 20K11539 (to Bochimoto H) in this revision.

5 Issues raised – (3)

The authors did not provide original pictures. Please provide the original figure

documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; and

Response:

We agree with the science editor that we should provide the original figure documents. We prepared the original figure documents of this manuscripts by Photoshop, not PowerPoint. Therefore, in addition to ordinary “Image File”, we upload the PSD files of our original figure documents as “Supplementary Material” to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

5 Issues raised – (4)

The “Article Highlights” section is missing. Please add the “Article Highlights” section at the end of the main text.

Response:

We appreciate the science editor's comment that make us realize the “Article Highlights” section is missing. Following your comment, we added the “Article Highlights” section at the end of the main text of the revised manuscript.

In addition, we have revised the formatting of our manuscript carefully following the “Guidelines and Requirements for Manuscript Revision: Basic Study” and “Format for Manuscript Revision: Basic Study”, including described below:

- adding page no.
- adding manuscript no (page 1)
- revision of Author contribution (page 2)
- truncation of Abstract CONCLUSION to 30 words (page 5)

6 Recommendation:

Conditionally accepted.

Response:

We really appreciate your wonderful recommendation that our manuscript have been

conditionally accepted to publish in the World Journal of Gastroenterology. The positive recommendation to our manuscript from the science editor really encourage us.

Response to comments from Reviewer #1

We are grateful to reviewer #1 for the critical comments and useful suggestions that have helped us to improve our paper considerably. As indicated in the responses that follow, we have taken all these comments and suggestions into account in the revised version of our paper.

Specific Comments to Authors:

The manuscript is well written and interesting and provides insight into ultrastructural changes of DCD livers after machine perfusion. I have only a few issues:

Response:

We really appreciate the positive and cheerful comment from the reviewer. We follow the advice in your comments described below to modify the section in our manuscript.

Comment 1.

The HMP used in group A is similar to dual-HOPE except for the portal line, which is not oxygenated. Can the authors comment on this?

Response:

We really appreciate the reviewer's comment. We agree with the reviewer that our circuit of HMP used in group A is similar to dual-HOPE. Thanks to you, we can recognize our mistake of description of machine perfusion systems. We revised the sentence "A gas blender attached to the oxygenator mixes air and oxygen is installed to the HA circuit." in the "Machine Perfusion Preservation" section of MATERIALS AND METHODS as "A gas blender attached to the oxygenator mixes air and oxygen is installed to the PV and HA circuit." in this revised version (Page 8). Moreover, the schema in Figure 1 was also corrected.

Comment 2.

Euro-collins solution was used for cold flush, while UW solution was used for HMP/MMP. Can the authors comment on the change of perfusion solution?

Response:

We are grateful to you for your important question. We considered that the UW solution is the optimal solution for liver preservation. However, there are concerns over the cost and viscosity of the solution, with potentially cause of inadequate perfusion and hepatic circulation. Therefore, flushing with Euro-Collins solution that is less viscous before infusing the UW solution may wash the blood out of the graft, and facilitate that the UW can be distributed in the microcirculation level effectively. Thus, we corrected the part “euro-Collins” as “Euro-Collins solution that is less viscous” in the revised manuscript (Page 10).

Comment 3.

The authors say that they used an unpaired two-tailed t-test to compare groups A and B, but I cannot find any p-value in the text and figures.

Response:

We are terribly sorry that the p-value was not mentioned in the text and figures. Following your comment, we added the p-value to the text in the “Comparison of ultrastructural changes in LSEC after HMP or MMP” section of RESULTS as “However, there was a trend of improvement in MMP (Fig. S1 (HA); HMP_4h vs. MMP_4h, 38.33 ± 8.88 vs. 26.00 ± 3.46 ng/ml, $P = 0.27$ vs HMP and MMP).” (Page 14). Moreover, the p-value was also inserted to Figure S1.

Furthermore, we are grateful to reviewer for your comment that make us realize to our wrong description of sample size. We revised the sentence "The grafts in group A were perfused at a constant temperature of 8°C as HMP (n = 4), on the other hand, the grafts in group B were gradually warmed from 8°C to 22°C during perfusion as MMP (n = 5), as described previously[9]." in the Machine Perfusion Preservation section of MATERIALS AND METHODS as "The grafts in group A were perfused at a constant temperature of 8°C as HMP (n = 3), on the other hand, the grafts in group B were gradually warmed from 8°C to 22°C during perfusion as MMP (n = 3), as described previously[14]." in this revised version (Page 10).

Response to comments from Reviewer #2

We are grateful to reviewer #2 for the critical comments and useful suggestions that have helped us to improve our paper considerably. As indicated in the responses that follow, we have taken all these comments and suggestions into account in the revised version of our paper.

Specific Comments to Authors:

The superiority of the machine perfusion preservation (MP) to simple cold storage was reported in kidney and liver preservation donated after cardiac death(DCD), the MP of the DCD grafts has been discussed about the optimal conditions including perfusion temperature, oxygenation, flow rate and pressure, steady or pulsatile flow, oxygen and nutrition-containing solution have also been reported to have numerous advantages to liver transplantation, but there are few reports about fusion temperature, especially midthermic machine perfusion (MMP). The authors comparatively analyzed the ultrastructural changes in the LSEC and sinusoids around them at four hours after HMP or MMP by using OM-SEM. MP alleviated the ER damage of LSEC caused by warm ischemia, MMP temperature conditions restore the metabolism of LSEC via the normalization of cristae of mitochondria and prevent the damage of the liver graft. The findings of the authors are very interesting. It is suggested that MMP is more effective than HMP in alleviating graft injury after DCD; However, there is still a long way for clinical application, which is also the direction of the author's efforts today

Response:

We really appreciate the reviewer for having an interest in our research. The reviewer has a good understanding of the goal of our manuscript. The positive comments to our findings from the reviewer really encourage us.

Comment 1.

In the abstract, the author only describes how to group, but does not describe the use of electron microscopy to evaluate the ultrastructures.

Response:

We apologize the omission of the use of electron microscopy in the former abstract of the manuscript, and we agree with the reviewer that it should be definitively included in the

new version of the manuscript. Following your comment, we describe that the use of electron microscopy to evaluate the ultrastructure in the METHODS section in Abstract as “Then the ultrastructural changes in the LSEC and sinusoids in Group A and B were comparatively analyzed by using OM-SEM with complementary TEM methods.” (Page 4).

Comment 2.

In pictures 2-5, there are groups A, B, C, and D. can you put A, B, C, and D together, so that you can see the differences visually through horizontal comparison in one picture.

Response:

We are very grateful to the reviewer for helpful suggestion. However, in this research, we would like to emphasize the correlative analysis on the intracellular ultrastructure of liver sinusoidal endothelial cells by using immunohistochemistry, OM-SEM and TEM. For this aim, we would appreciate it if you could let us arrange the images of immunohistochemistry, OM-SEM and TEM of same experimental group in each figure.

Comment 3.

The ultrastructural damage of liver was confirmed by electron microscope. Can the activity of hepatocyte be reevaluated by deepening method.

Response:

We really appreciate the reviewer’s thoughtful comments. We agree with the comment that it is important to reevaluate the activity of hepatocyte by this deepening method by using OM-SEM. Actually, we previously evaluated the ultrastructural damages of hepatocytes in the liver preserved by HMP, and MMP with which relatively low level of the LDH and AST in perfusate, by using the OM-SEM and TEM in advance of the present research (Bochimoto H, et al. PLoS One 2017;12:e0186352). We could demonstrate the hepatocyte preserved by HMP has strongly swollen mitochondria, in contrast, MMP could preserve the functional appearance of mitochondria in hepatocytes in this previous research. This is consistent with the finding of the present study that MMP temperature conditions restore the metabolism of LSEC via the normalization of cristae of mitochondria. Thus, we added the sentence “This is consistent with the finding of the previous study that the hepatocyte preserved by HMP has strongly swollen mitochondria,

in contrast, MMP could preserve the functional appearance of mitochondria in hepatocytes.” to discussion paragraph in this revised manuscript (page 18).