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Prof. Andrzej S Tarnawski, MD, PhD
Editors-in-Chief
World Journal of Gastroenterology

Resubmission - Manuscript NO: 80399

Dear Editors:

Thank you for providing us an opportunity to submit a revised draft of our manuscript for publication in *World Journal of Gastroenterology*, titled "Impact of Endothelial Nitric Oxide Synthase Activation on Accelerated Liver Regeneration in a Rat ALPPS Model: Basic Study".

Your comments, as well as those of the reviewer, were highly insightful and enabled us to greatly improve the quality of our manuscript. We have amended the attached manuscript in accordance with the detailed suggestions you have graciously provided. Please refer to the underlined text in the revised manuscript. In the following pages, I have provided our point-by-point responses to all comments and have quoted the revised portions of the manuscript. The changes in the attached revised manuscript have been indicated using underlined text.

I hope that you will find our revised manuscript suitable for publication in *World Journal of Gastroenterology*. I look forward to hearing from you at your earliest convenience.

Sincerely,

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Response to Reviewers

Thank you for your comments on our manuscript. We sincerely appreciate these insightful comments, which we have used to revise our manuscript. We believe that our manuscript has greatly improved as a result. Our point-by-point responses to the comments are presented below.

Response to the comment of Reviewer 1

Comment:

The subject of the paper is actual and exists in the focus of liver surgeons. The title reflects the main subject/hypothesis of the manuscript. Abstract is well written and reflects well the work described in the manuscript. The background is provided clearly and is based on the data of modern literature. The used methods are well selected and the animals number is adequate to the aim and objective of the study. The main result showing that in case of portal vein branch occlusion, if it will be supplemented by the activator of Akt-eNOS pathway, might give the same results similar to ALPPS, have to be considered as important and interesting. The figures, illustrations and tables are clear and well designed. Statistical analysis is done correctly. The paper is written with correct English and organized in accordance with journal requirements.

Response:

Thank you very much for your positive feedback on our paper. We sincerely hope that our research will be published in *World Journal of Gastroenterology*.

Response to the comment of Reviewer 2

Comment:

As we all know, ALPPS is an effective method to grow the future liver remnant before performing extended hepatectomy. However, its mechanism remains unclear. Hitoshi et al. presented an interesting manuscript on the mechanism of promoting liver regeneration by ALPPS. The results indicate that activation of the Akt-eNOS signaling pathway may contribute to accelerated regeneration of the FLR after ALPPS. The authors make a good description of the mechanism. It is a useful and interesting work for hepatobiliary surgeons.

Response:

Thank you very much for your positive feedback on our paper. We sincerely hope that our research will be published in *World Journal of Gastroenterology*.

Response to the comment of Reviewer 3

Comment:

First of all, this is a very interesting study, starting from clinical problems and using animal models as research objects, to profoundly explain the factors related to liver regeneration of ALPPS and PVE. The research idea of the manuscript is clear, the result is reliable and the conclusion is appropriate. However, I have some questions which need to be answered by the author. The details are as follows : 1. In the Surgical Procedures and Study Design section, the authors divided the rats into two groups. Should a control group be set up? The control group only received open and closed abdomen. Is it necessary? 2. The process of liver regeneration is a complex process, never a single factor or a single pathway plays a key role. So the discussion section and the conclusion don't have to be too absolute. In a word, this manuscript is novel, full of content and correct in research methods, and can be published after revision.

Response to comment 1:

Thank you for your suggestion. As the reviewer pointed out, the purpose of this study was to clarify the mechanism of hepatic enlargement in ALPPS by comparing PVL and ALPPS. We would like to answer the reviewer's question associated with the need for the control group, in which open and closed abdomen was performed.

In Figures 1, 2, 3, and 4, the comparison of PVL and ALPPS was considered important, so the control group was not referred. In Figure 5 (Western blot analysis), we used the control group because we thought it was better to compare PVL and ALPPS based on protein expression in the control group. Similarly, in Figure 7 (Volumetric Blood Flow Analysis), we thought it would be better to compare changes in flow velocity between PVL and ALPPS based on hepatic blood flow in the control group. However, in Figure 6, the control group was removed because the control group is not necessary for comparing the changes in liver weight of PVL and ALPPS after drug administration. The definition of the control group was not described in the Methods section, so we have added the following text to our revised manuscript.

Page 10, lines 164-166:

In western blotting analysis and volumetric blood flow analysis, PVL and ALPPS groups

were compared based on the control group, in which only open and closed abdomen was performed.

Response to comment 2:

As the reviewer pointed out, the process of liver regeneration is complex and involves the combined action of various factors and pathways. Therefore, the Akt-eNOS pathway, which we have reported in this study, might be a factor that partially explains the mechanism of liver regeneration in ALPPS. The text has been revised as follows to avoid absolute expressions.

Page 20, lines 332-334 (revised manuscript):

Second, activation of the Akt-eNOS pathway might be an important factor in promoting liver regeneration after ALPPS.

Page 21, lines 361-364 (revised manuscript):

These results suggested that the induction of inflammatory cytokines in the early phase after ALLPS was not necessarily a major factor in accelerating liver regeneration of ALPPS.

Page 25, lines 424-425 (revised manuscript):

Activation of the Akt-eNOS pathway in ALPPS might be an important factor in promoting early liver regeneration.

Response to the comment of Reviewer 4

The manuscript entitled "Impact of Endothelial Nitric Oxide Synthase Activation on Accelerated Liver Regeneration in a Rat ALPPS Model" by Dr. Hitoshi Masuo, et al. uses an interesting APPLS model to explore the mechanism of liver regeneration. Although the effect of endothelial nitric oxide synthase on liver hyperplasia was previously discussed in papers, but the authors focused on "Associating Liver Partition and Portal vein Ligation for staged hepatectomy (ALPPS)", which has certain academic value. In addition, the author's conclusion, that is "Early introduction of inflammatory cyclines might not be positive for accelerated FLR regeneration after ALPPS, where as Akt eNOS path activation may contribute to accelerated regeneration of the FLR", is somewhat innovative.

Response:

Thank you very much for your positive feedback on our paper. We sincerely hope that our research will be published in *World Journal of Gastroenterology*.

Although there were no suggestions from the reviewer in this regard, we deleted the reference to Enzyme-linked Immunosorbent assays because ELISA is a commonly used abbreviation.

Page 10, line 167 (revised manuscript):

ELISAs of Serum Inflammatory Cytokines and Hepatocyte Growth Factor