Format for ANSWERING REVIEWERS

July 23, 2013

Dear Editor,

Title: Platelet therapy; a novel strategy for liver regeneration, anti-fibrosis, and anti-apoptosis

Author: Kazuhiro Takahashi, Soichiro Murata, and Nobuhiro Ohkohchi

Name of Journal: *World Journal of Surgical Procedures*

ESPS Manuscript NO: 3946

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer
Answers to reviewers

Reviewer1

1) Does this platelet therapy affect hepatic progenitor cell mediated liver regeneration?

   →Thank you very much for giving us an interesting question. To the best of our research, we could not find any report of hepatic progenitor cells to be affected by platelets. In our lab, we have been studying the role platelets in liver regeneration for a decade, but we still have not studied the role of platelets in hepatic progenitor cells during liver regeneration. We would like add experiments to access the relationship between platelets and hepatic progenitor cells.

2) Please mention about disadvantage of platelet therapy if this therapy has any? Does this therapy cause formation of blood clot or something?

   →I appreciate reviewer's comments. At this moment, we consider platelet therapy consists of thrombopoietin, eltrombopag administration, and platelet transfusion. In our animal experiments, we did not recognize any side effects such as allergic reactions, thromboembolic events, or damages to vital organs by thrombopoietin administration or platelet transfusions[1-3]. However, in our clinical study, despite the lack of thromboembolic events, there were some patients who experienced mild allergic reactions or inductions of auto-antibodies such as anti-HLA or anti-HPA antibodies by platelet transfusion[4]. Furthermore, it is reported that thrombopoietin injection can cause severe thrombocytopenia by induction of anti-thrombopoietin antibody[5]. On the other hand, despite some incidence of side effects including thromboembolic events and liver enzyme elevation, eltrombopag does not compete with endogenous thrombopoietin and have no reported incidence of induction of auto-antibody, and is safe and well tolerated for long usage [6]. Therefore, we consider eltrombopag to be an ideal goal for platelet therapy, and we are now preceding another clinical study to

evaluate its effect on liver regeneration and anti-fibrosis.

[1] **Murata S**, Ohkohchi N, Matsuo R, Ikeda O, Myronovych A, Hoshi R. Platelets promote liver regeneration in early period after hepatectomy in mice. *World J Surg* 2007; **31**: 808-816 [PMID:17354025 DOI: 10.1007/s00268-006-0772-3]

[2] **Matsuo R**, Nakano Y, Ohkohchi N. Platelet administration via the portal vein promotes liver regeneration in rats after 70% hepatectomy. *Ann Surg* 2011; **253**: 759-763 [PMID:21475016 DOI: 10.1097/SLA.0b013e318211caf8]

[3] **Takahashi K**, Kozuma Y, Suzuki H, Tamura T, Maruyama T, Fukunaga K, Murata S, Ohkohchi N. Human platelets promote liver regeneration with Kupffer cells in SCID mice. *J Surg Res* 2013; **180**: 62-72 [PMID:23260232 DOI: 10.1016/j.jss.2012.11.030]

[4] **Maruyama T**, Murata S, Takahashi K, Tamura T, Nozaki R, Ikeda N, Fukunaga K, Oda T, Sasaki R, Ohkohchi N. Platelet transfusion improves liver function in patients with chronic liver disease and cirrhosis. Tohoku J Exp Med. 2013;229(3):213-20.

[5] **Li J**, Yang C, Xia Y, Bertino A, Glaspy J, Roberts M, Kuter DJ. Thrombocytopenia caused by the development of antibodies to thrombopoietin. Blood. 2001 Dec 1;98(12):3241-8.

[6] **Saleh MN**, Bussel JB, Cheng G, Meyer O, Bailey CK, Arning M, Brainsky A; EXTEND Study Group. Safety and efficacy of eltrombopag for treatment of chronic immune thrombocytopenia: results of the long-term, open-label EXTEND study. Blood. 2013 Jan 17;121(3):537-45. doi: 10.1182/blood-2012-04-425512.

3) Please mention about the advantage and disadvantage of platelet therapy compared with other candidate of therapy for liver regeneration such as bone marrow infusion therapy (Terai et al. Stem Cells 2006 Oct 24,(10) 2292-8) or macrophage therapy (Thomas et al. Hepatology, 2011, Jun; 53 (6) 2003-15).

→Thank you very much for an excellent indication. We think platelet therapy has advantage in its convenience and cost-efficiency, and especially, eltrombopag is well-guaranteed for its safety [1]. Platelet transfusion has disadvantage in its short-term availability after collection (72 hours), allergic reactions, and induction of auto-antibodies (anti-HLA, anti-HPA, ect) [2]. And, thrombopoietin has possibility of inducing autoantibody [3]. However, eltrombopag is an oral medicine, which is proven for its long-term safety, and is well tolerated, and effective [1]. On the other hand, regarding bone marrow cell infusion therapy and macrophage therapy, although the efficacy for the resolution of liver fibrosis and induction of hepatocyte mitosis are excellent, bone marrow has to be harvested from the ileum, femurs, or tibias under general anesthesia, and stem cells has to be isolated by complicated process [4,5]. Furthermore, long-term safety has not been investigated, yet [4]. We add these descriptions in conclusion.

[1] **Saleh MN**, Bussel JB, Cheng G, Meyer O, Bailey CK, Arning M, Brainsky A; EXTEND Study Group. Safety and efficacy of eltrombopag for treatment of chronic immune thrombocytopenia: results of the long-term, open-label EXTEND study. Blood. 2013 Jan 17;121(3):537-45. doi: 10.1182/blood-2012-04-425512.

[2] **Maruyama T**, Murata S, Takahashi K, Tamura T, Nozaki R, Ikeda N, Fukunaga K, Oda T, Sasaki R, Ohkohchi N. Platelet transfusion improves liver function in patients with chronic liver disease and cirrhosis. Tohoku J Exp Med. 2013;229(3):213-20.

[3] **Li J**, Yang C, Xia Y, Bertino A, Glaspy J, Roberts M, Kuter DJ. Thrombocytopenia caused by the development of antibodies to thrombopoietin. Blood. 2001 Dec 1;98(12):3241-8.
[4] **Terai S**, Ishikawa T, Omori K, Aoyama K, Marumoto Y, Urata Y, Yokoyama Y, Uchida K, Yamasaki T, Fujii Y, Okita K, Sakaida I. Improved liver function in patients with liver cirrhosis after autologous bone marrow cell infusion therapy. Stem Cells. 2006 Oct;24(10):2292-8.
[5] **Thomas JA**, Pope C, Wojtacha D, Robson AJ, Gordon-Walker TT, Hartland S, Ramachandran P, Van Deemter M, Hume DA, Iredale JP, Forbes SJ. Macrophage therapy for murine liver fibrosis recruits host effector cells improving fibrosis, regeneration, and function. Hepatology. 2011 Jun;53(6):2003-15. doi: 10.1002/hep.24315.

Reviewer2

I would just suggest adding references about the decreased amount of platelets in the patients with liver cirrhosis, where liver regeneration is severely impaired.

→I appreciate the reviewer's comments. We added description and references regarding decreased amount platelet count in the cirrhotic liver and impairment of liver regeneration in the second paragraph in the introduction.

Reviewer3

There was no suggestion for revision.

Reviewer4.

1. There are typos and grammatical errors. The authors need to ask to native speakers for English proof reading.

   →Thank you very much for indicating our faults. We had our English corrected by native speaker and attached English proof reading.

2. Abstract and Core tip sections are almost identical. The authors should change the description or delete the Core tip section.

   →We appreciate the reviewer's comment. We changed description of the Abstract and Core Tip.

3. Some of the references are inappropriate. The authors should avoid papers written in Japanese (Refs 20, 25, 56). I could not find and retrieve Refs 30 and 46. The authors should replace these references to other relevant papers from more common journals.

   →Thank you for indicating our fault. We replaced Japanese written papers (Ref, 20, 25, 46, 56) from English written papers, and deleted Ref 30.

4. Many Greek characters did not show correctly in the Word file (TNF-?, TGF-?, etc...).

→Thank you for indicating our faults. We changed our Greek characters to normal Alphabet.

5. There are typos in figures. Hepatpcyte should be hepatocytes in Figure 1. "Sinusoidal endothelial cells" and "the space of Disse" would be appropriate instead of liver endothelial cells and Disse's space in Figure 1 and 2. In Figure 2 and 3, hepatic stellate cell and hepatocyte should be hepatic stellate cells and hepatocytes, respectively.

   →Thank you for indicating our mistakes. We corrected all typos in figures and manuscripts. We changed "Liver endothelial cells" and "Disse's space" to "Sinusoidal endothelial cells" and "the space of Disse", respectively in figure 1. We changed "hepatic stellate cell" and "hepatocyte" into "hepatic stellate cells" and "hepatocytes" in figure 2 and 3.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Surgical Procedures.*

Sincerely yours,

Kazuhiro Takahashi

Kazuhiro Takahashi, MD, PhD
Department of Surgery,
Division of Gastroenterological and Hepatobiliary Surgery,
and Organ transplantation, University of Tsukuba
Tennoudai 1-1-1, Tsukuba, Ibaraki
305-8575 Japan
Fax: +81-29-853-3222
E-mail: kazu1123@hh.iij4u.or.jp