ANSWERING REVIEWERS

August 11th, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 12001-review.doc).

Title: Effects of Oral Tacrolimus as a Rapid Induction Therapy in Ulcerative Colitis

Author: Ken Kawakami, Takuya Inoue, Mitsuyuki Murano, Ken Narabayashi, Sadaharu Nouda, Kumi Ishida, Yosuke Abe, Koji Nogami, Nobuyuki Hida, Hirokazu Yamagami, Kenji Watanabe, Eiji Umegaki, Shiro Nakamura, Tetsuo Arakawa, Kazuhide Higuchi

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Thank you for your letter dated July 28, 2014. We consider the manuscript has been improved significantly largely as a result of the many thoughtful comments of the reviewers. I have listed our responses to the reviewer's comments below.

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

Answers to the comments of the reviewers
To Reviewer 02941672:

Thank you very much for your thoughtful comments. Your comments were very helpful for enhancing our manuscript. We consider our manuscript has been greatly improved.

1) Both Fig.1a and Fig.1b is not needed because these two figures indicate same result. I suggest removing Fig.1a.

Thank you for your comment. We have deleted Figure 1a in accordance with your suggestion.

2) ΔLichtiger scores in Fig.4 seem unnecessary because the similar data is shown in Fig.3.

Thank you for this suggestion. We have deleted the ΔLichtiger scores from Figure 4.

3) Adverse effects should be presented in additional table for easy understanding.

In accordance with your suggestion, we have added Table 3 that shows the adverse responses. We have also added the following description of these effects in the Results section and Figure 5 to explain the blood glucose levels during treatment with oral tacrolimus. Although 48.6% (18/37) of the patients had at least one elevated glucose (>120 mg/dL) measured while on tacrolimus treatment, mean fasting blood glucose level was significantly lower at day 21 compared with that on day 0 (86.0±21.4 mg/dL and 107.3±22.9 mg/dL, respectively; p=0.012) (Figure 5). Other documented clinical reactions and laboratory abnormalities thought to be related to tacrolimus included tremors (35.7%, 15/42), headache (9.5%, 4/42), nausea (7.1%, 3/42), and hypomagnesemia (74.1%, 20/27, 1.56±0.26 mg/dL) (Table 3) (Revised manuscript page 9).

4) Adverse effects comparing with that of clinical trial until approval and post-marketing investigation needs to be discussed if there are some differences.

Thank you very much for this constructive suggestion. In a medical package insert of prograf® Astellas Pharma Inc reported that the adverse effects associated with tacrolimus treatment in patients with UC included tremor (29.2%), hypomagnesemia (16.8%), hyperglycemia (7.3%), and nausea (6.6%). In this study, it was likely these adverse effects were increased compared with that described in the Astellas Pharma Inc report and previous reports. However, no significant clinical symptoms were observed during treatment, and no patient discontinued oral tacrolimus therapy due to adverse effects. In accordance with your suggestion we have therefore added several sentences in the Discussion section as follows: With regard to other adverse effects, many patients developed hypomagnesemia (74.1%, 1.56±0.26 mg/dL), at a frequency similar to that reported in previous studies (33.3-87.5%) [7,16]. Although 48.6% (18/37) of the patients had at least one elevated glucose measured during this study, the mean fasting blood glucose level was significantly lower at day 21 compared with that on day 0. Benson also reported that 62.5% of patients had elevated glucose and most of them were on corticosteroid therapy at that time [16]. We therefore consider that it was likely that the hyperglycemia observed was not related to tacrolimus treatment. Tremor appeared to be increased (35.7%) compared with that reported previously (9.4-19.0%) [7,8,16,25] (Revised manuscript page 12).

5) Change of dosage increase or starting of new treatment just before this trial should be confirmed and mentioned.

As you mentioned, to obtain trough whole-blood levels of 10-15 ng/mL as soon as possible, a final dose of oral tacrolimus (0.15-0.16 mg/kg/day) was needed in our study. Because no significant clinical symptoms were observed during treatment, and no patient discontinued oral tacrolimus therapy due to adverse effects, oral tacrolimus at an initial dose of 0.15 mg/kg/day may be more suitable for patients with refractory UC. According to your suggestion, we added the following description in the Discussion section. Regarding the starting dose of oral tacrolimus and dose adjustment, 29.1%-36.4% of patients needed to have their dose adjusted from day 1 to day 4 (data not shown), with a final daily dose of oral tacrolimus of 0.15-0.16 mg/kg needed to achieve appropriate trough levels. We therefore, consider that oral tacrolimus at an initial dose of more than 0.1 mg/kg/day may decrease the number of times dose adjustment is needed and be more suitable for patients with refractory UC (Revised manuscript page 12).

To Reviewer 02548913:

Thank you very much for your comments and suggestions. We truly appreciate your concerns, and your comments were very helpful to us for improving our manuscript.

1) and 2) How much time was necessary to obtain the results of the blood level? Probably, this method was limited to the institution where the measurement of the blood level is possible to obtain on the day. There was lacking in versatility, because there was a few hospital which was available for the measurement in the own institution. Criteria of the blood level adjustment are written in Table1. How many percent was an observance rate of these criteria actually?

Thank you very much for your constructive comments. As you mention, there are only a few hospitals where measurements are available on the same day. Therefore, rapid induction therapy with oral tacrolimus may lack versatility. This is a limitation of this therapy. Because this study showed that an oral tacrolimus dose of 0.15-0.16 mg/kg/day was needed to obtain appropriate trough levels and many patients (29.1-36.4%) needed to have their dose adjusted from day 1 to 4, oral tacrolimus at an

initial dose of more than 0.1 mg/kg/day may decrease the number of times dose adjustment is required and be more suitable for the patients with refractory UC. We added the following description in the Discussion section. Regarding the starting dose of oral tacrolimus and dose adjustment, 29.1%-36.4% of patients needed to have their dose adjusted from day 1 to day 4 (data not shown), with a final daily dose of oral tacrolimus of 0.15-0.16 mg/kg needed to achieve appropriate trough levels. We therefore, consider that oral tacrolimus at an initial dose of more than 0.1 mg/kg/day may decrease the number of times dose adjustment is needed and be more suitable for patients with refractory UC (Revised manuscript page 12).

3) Exclusion criteria include (2) prior abdominal surgery and (5) history of previous abdominal surgery. What were these two contents different in?

Thank you for this comment. We have deleted (5) history of previous abdominal surgery from the Methods section.

4) Figure 1a seem unnecessary.

We have deleted Figure 1a in accordance with your suggestion.

5) Either figure 3 or ΔLichtiger scores (in Figure 4) should be removed.

We have removed ΔLichtiger scores from Figure 4 in accordance with your suggestion.

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

Sincerely yours,

Ken Kawakami, MD
Second department of internal medicine，
Osaka Medical College
2-7 Daigakumachi, Takatsuki city,
Osaka 569-8686, Japan
FAX: +81-72-684-6532
E-mail: in2090@poh.osaka-med.ac.jp