

Format for ANSWERING REVIEWERS



July 8, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3385-edited.doc).

Title: Fucoidan enhances intestinal barrier function by upregulating the expression of claudin-1

Authors: Atsushi Iraha, Hiroshi Chinen, Akira Hokama, Takumi Yonashiro, Tetsu Kinjo, Kazuto Kishimoto, Manabu Nakamoto, Tetsuo Hirata, Nagisa Kinjo, Futoshi Higa, Masao Tateyama, Fukunori Kinjo, Jiro Fujita

Name of Journal: *World Journal of Gastroenterology*

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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.

(1) Answers to comments of referee #37549

A1. With significant effects of fucoidan for enhancing the intestinal barrier of Caco-2 cells in this study, we are planning to evaluate these effects on rodent intestine in the next paper.

A2 & 9. As indicated by prior reports, our preliminary study has confirmed that low concentration of fucoidan caused little apoptosis to the cells and no effects to the barrier function. Therefore, we have performed the study with high concentration of fucoidan, which enhances the barrier function without the apoptotic effects. This high concentration may be a potential one when we plan to apply fucoidan for enema use of colonic inflammation in the next paper.

A3. The description of the subtraction has been added.

A4. The description of the subtraction has been added.

A5. The primers have been added.

A6. The procedures of immunofluorescence have been added.

A7. Direct effects of fucoidan vs. effects of fucoidan on the cells are not easy to be distinguished. The title and discussion were revised.

A8. As mentioned by the reviewer, the conclusion that fucoidan prevented destruction at the late phase is not supported. We have changed the discussion about it.

(2) Answers to comments of referee #49305

A1-6. We have revised the description.

A7 & 8. We are planning to investigate quantitative studies and other TJ proteins in next paper. We have changed the title and discussion to describe the results only from the present study.

(3) Answers to comments of referee #160164

Major points.

A1. As mentioned by the reviewer, we have asked a statistician to check the results and applied

Student's *t*-test to Figure 3.

A2. Because of the results in PCR, we have conducted Claudin-1 (sealing TJ) only in the immunohistochemistry.

A3. Although PCR results were clearly demonstrated following 24 hours incubation, immunohistochemistry staining showed less clear pictures, possibly due to the cell viability-related immunogenicity. Therefore, we have conducted the staining following 6 hours incubation.

A4. We have addressed why fucoidan prevents claudin but not occludin down regulation.

Minor points.

A5. We understand that ion passage would provide a more complete picture of barrier function. However, TER and flux are incomplete but enough measures for *in vitro* barrier functional study.

A6. Our preliminary study has confirmed that low concentration of fucoidan caused little apoptosis to the cells and no effects to the barrier function. Therefore, we have performed the studies with high concentration of fucoidan, which enhances the barrier function without the apoptotic effects.

A7. We have conducted transmembrane proteins (claudins, occludin) in this study. We are planning to study scaffold proteins (ZO-1, ZO-2) which link claudins to the actin cytoskeleton in the next study.

A8. According to the prior reports, we have chosen the concentration of hydrogen peroxidase.

A9. Omitted information has been added.

(4) Answers to comments of referee #227549

A1. As mentioned by the reviewer, our data have different time course in various experiments. We have conducted different time course because of suitable conditions in each experiment. We have added the description about it.

A2. As mentioned by the reviewer, the changes on claudin could be the loss of cellular integrity. We have changed the title and added the description about it.

3. References and typesetting were corrected.

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

Sincerely yours,

Akira Hokama, MD, PhD.

Department of Infectious, Respiratory, and Digestive Medicine,

Faculty of Medicine, University of the Ryukyus,

207 Uehara, Nishihara, Okinawa 903-0215, Japan.

hokama-a@med.u-ryukyu.ac.jp

Telephone: +81-98-8951144 Fax: +81-98-8951414