

ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 3385

Title: Fucoidan upregulates the expression of tight junction proteins and enhances barrier function in human intestinal epithelial cells

Reviewer code: 00037549

Science editor: Wen, Ling-Ling

Date sent for review: 2013-04-26 20:19

Date reviewed: 2013-05-21 01:07

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of	<input type="checkbox"/> No records	
<input checked="" type="checkbox"/> Grade D (Fair)	language polishing	BPG Search:	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Minor revision
		<input type="checkbox"/> No records	<input checked="" type="checkbox"/> Major revision

COMMENTS TO AUTHORS

The subject is of interest, since seaweeds are abundant and can have potent biological effects, as with carrageenan. The paper is carefully written and well-expressed. Several recommendations follow: 1. It would be informative to perform similar experiments in sections of either human or rodent intestine, or at least another polarized cell line, and measure the impact of the combination of H₂O₂ and fucoidan on membrane potential, permeability, and expression of the junctional proteins of interest. 2. The concentration of fucoidan (0.1 - 2.5 mg/ml) seems high, based on other studies in the literature (e.g. Kim BMC GE) on apoptosis in which 0-20 ug/ml quantities were used. 3. It is not clear that baseline measurements of transepithelial electrical resistance with or without fucoidan in unseeded inserts were performed and that these were subtracted from the readings. 4. Also, unseeded control values for FITC flux with or without fucoidan need to be measured and used to correct the observed values. 5. Please include the primers for the PCR studies. 6. The procedure for immunofluorescence is unclear, since in the chamber slides there is no access to the basolateral side to administer H₂O₂. Please clarify. 7. Direct effects of fucoidan vs. effects of fucoidan on the cells need to be distinguished in the experiments. Analysis of H₂O₂-induced changes directly on the cells, as in measurements of SOD, will be helpful, with varying concentrations of H₂O₂ and of fucoidan, as well as the unseeded, control experiments, suggested above. 8. In Figure 2, the TER declines about 40% from the baseline with H₂O₂ alone, and declines about 40% with fucoidan 2.5 mg/ml and H₂O₂, but starts from the higher 2 hour peak. The conclusion that fucoidan prevented destruction at the late phase is not supported. It would be useful to explain the increase at 2 hours. 9. Since fucoidan has been reported to induce apoptosis in human intestinal epithelial cell lines, it would be helpful to have measurements of the effect of fucoidan and of H₂O₂ on cell number of the Caco2 cells. (Please see the uploaded comments below.)

ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 3385

Title: Fucoidan upregulates the expression of tight junction proteins and enhances barrier function in human intestinal epithelial cells

Reviewer code: 00049305

Science editor: Wen, Ling-Ling

Date sent for review: 2013-04-26 20:19

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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input checked="" type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)		BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

In this study, authors studied the role of fucoidan in protecting intestinal epithelial barrier function through the upregulation of claudin-1. The following points should be concerned: 1. In Page 10, lines 9-11, please indicate the dose of fucoidan including low dose and high dose. 2. In Page 10, second paragraph, lines 5-6, "As expected, ...", please indicate how long pretreatment with fucoidan has been undergone. 3. In Page 11, line 10, please change "some TJ proteins" into "claudin-1 and -2". 4. In Page 23, figure 3 legend, please indicate the dose of FD4 in the culture medium. 5. In Page 23, figure 4 legend, please indicate how long pretreatment with fucoidan has been performed. 6. In Figures 1 and 2, please change "hour" into "hours". 7. In figure 4, it is better to show the results for claudin-1, -2 and occludin expression using quantitative RT-PCT. 8. In Figure 5, it is better to show staining results for claudin-2 and occludin, in addition to claudin-1.

ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 3385

Title: Fucoidan upregulates the expression of tight junction proteins and enhances barrier function in human intestinal epithelial cells

Reviewer code: 00160164

Science editor: Wen, Ling-Ling

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Date reviewed: 2013-05-28 15:00

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)		BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

Iraha A et al describe the protective effects of a fucodian, an extract of brown algae, on tight junctions using an intestinal epithelial cell model under oxidative stress. This is an interesting study, the aims are clearly stated, the manuscript is appropriately written and easy to follow. However there are a number of issues that should be addressed. Major points ? Some of the statistics presented are unclear and inappropriate. For example in Figure 2 it is not clear which groups are being compared: Tukey multiple comparison follows from an ANOVA, the ANOVA results should also be presented. Figure 3, a paired t-test is inappropriate. This manuscript would benefit from a review by a statistician. ? Statistical results should also presented in the text and not just figure legends ? The assessment of claudin 1,2 and occludin mRNA but only claudin 1 immunohistochemistry provides an incomplete picture. Claudin 2 and occludin should also be included in the immunohistochemistry analysis ? There is inconsistency with study time points. Figure 4 is following 24 hours incubation while figure 5 is following 6 hours incubation ? The authors did not address why fucoidan prevents claudin but not occludin down regulation? Minor points ? The lack of a fucoidan but no peroxidise group for all measures was a disappointing omission ? TER and dextran flux is an incomplete measure of barrier function. Including additional measure such as ion passage would provide a more complete picture of barrier function ? It would have been helpful to include a measure of cell toxicity and/or apoptosis ? Zona occludens-1 is commonly used as a marker of tight junction integrity. This study would of benefitted from also analysing ZO-1 ? Why was 500µM hydrogen peroxidise chosen, has this been established as relevant to the clinical situation in IBD? ? Introduction page 5. "investigation of in vivo intestinal epithelial barrier.." should read "investigation of in vitro intestinal epithelial barrier.." ?



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Material and Methods page 6. Company information should be provided for penicillin and streptomycin ? Material and Methods page 8. Should company information be provided for DNA Engine? ? Figure 4 Legend. The statement "The data are expressed as means +/- SEM of 3 independent experiments" is in appropriate. The authors should clarify if the PCR was conducted 3 times and the results presented are representative of those 3 experiments ? Figure 4 legend. "occluding" should be "occludin"

ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 3385

Title: Fucoidan upregulates the expression of tight junction proteins and enhances barrier function in human intestinal epithelial cells

Reviewer code: 00227449

Science editor: Wen, Ling-Ling

Date sent for review: 2013-04-26 20:19

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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)		BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

The manuscript's title and abstract imply that the up-regulation of tight junction protein by fucoidan leads to an enhancement of barrier function in a human intestinal cell model, Caco2. While some part of the conclusion is supported by the data, the other part is not convincing. Alternative interpretation of data does exist and additional experiments are needed to strengthen or dispel the current conclusion. A major problem is that various experiments presented did not have a consistent time course that supports the causal relationship. After carefully examining all data, my own conclusion is that fucoidan as a protein probably directly prevents the hydrogen peroxide toxicity by chelation. Other properties may also exist but more experiments are needed. The hydrogen peroxide-induced loss of barrier function was clearly demonstrated (Figs. 2, 3). This can be observed at 2 hours after the hydrogen peroxide addition to the culture and persisted for 4 more hours (Figure 2). At 6 hours, an increase in dextran permeability has also been observed (Figure 3). At 6 hours after the H₂O₂ treatment, a change in the pattern of distribution of claudin-1 was observed (Fig. 5 middle). The loss of Claudin-1 and 2 was shown at 24 hours after the H₂O₂ treatment. The authors did not have data that indicated if any changes in claudin happened at 2 hours after the treatment. Based on the data provided, hydrogen peroxide appears to affect the cellular integrity quickly and the changes on claudin could be secondary to the loss of cellular integrity. Fucoidan increased TER in Figure 1 in the absence of any treatment. The results are interesting but are not consistent with the results in Figure 2 where the increase in TER by fucoidan was greatest at 2 hours after the treatment. The experiment was not followed to 24 hours. Figure 3 did not include the fucoidan only group which should have a lower dextran permeability compared to the control based on Figure 1. Figure 3



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should also be performed at 2 hours after the H₂O₂ treatment. To support the conclusion as indicated in the title of the manuscript, Figure 4 and 5 each needs a fucoidan only group and in addition, the 2-hour time point, when fucoidan showed the most protective effect in Figure 2 should be used.