April 23, 2015

Dear Editor, Dr. Jing Yu

Please find enclosed the edited manuscript in Word format (file name: 16997-Review.docx).

Title: Local corticosterone production and ACE shedding in a mouse model of intestinal inflammation

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 16997

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

1. We agree, that the question on intestinal corticosterone production and angiotensin converting enzyme (ACE) and their possible connection should be tested *in vivo* in animal studies and secondly in clinical pilot studies. Both of these are in our "program" in the near future. Some patient samples are already collected and analyzed for ACE existence. In the present study (our manuscript) we used *ex vivo* incubation, which has been used in previous studies of other groups (referred in the text).
2. In previous studies, simultaneous measurements of corticosterone production, ACE expression and their possible connections have not been reported. Therefore we wanted to publish our data as soon as possible for the priority in your highly regarded Journal.
3. The Reviewer asks about repetition of the experiments. We have made pilot studies to confirm the "proof of concept" on the basis of which the doses, treatment periods and incubation times have been decided and the results have been consistent. In Finland there are very strict rules and limitations in use of animals in preclinical experiments, and therefore it is not possible to repeat experiments which furthermore are expensive and time consuming. However, this question will be explained better in the revised version in the Methods.

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Detailed comments:

1-3. Legends to Figures 1-3 are changed according to the Reviewer´s suggestions to more detailed ones. We thought that giving detailed data in the text (Results) were enough and not to add them in the Legends of the figures to avoid unnecessary repetition. We agree that Figure 1 might not be of great importance for an experienced reader, but in this type of studies it is good to convince the non-experts of the grade of inflammation which can be achieved with these concentrations of DSS (3 and 5 %). Furthermore the use of two concentrations of DSS is also a positive aspect of pharmacological studies.

4. The error in page 9 (Figure 3E,F) has been corrected (Figure 2E,F).
5. The rationale to use Angiotensin II, captopril and metyrapone in the incubation studies was to try - by using pharmacological tools which act systemically given *in vivo* - find out whether similar regulatory mechanisms act in the intestine. The components of RAS and glucocorticoids are so abundant and important in systemic circulation that we wanted to first use *ex vivo* studies to distinguish the effects in intestine and circulation. The present findings support the idea that different regulation than in the adrenal glands (corticosterone) exist in the intestine and ACE inhibition does not influence by a negative feedback mechanism the expression of ACE protein.

Improvements made to the manuscript according to the suggestions of editor:

- All changes are marked with red font
- Added references. However, due to intestinal corticosterone production being studied by only few groups and most studies describing ACE shedding are very old, the requirement for 30 relevant references from the past 4 years could not be met.
- Added a comments section
- Added missing statements as separate PDF files

General improvements were made to the manuscript:

- Reduced font size according to instructions and fixed a typo
- Changed SEM values to SD values to reflect those in the figures in some cases

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Thank you very much for giving us the possibility to publish our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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