Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road,
Wan Chai, Hong Kong, China

ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 8646
Title: Localizationandvasopressinregulationofthe Na+-K+-2Cl-cotransporter (NKCC2)inthe distal colonic epithelium
Reviewer code: 00592428
Science editor: Qi, Yuan
Date sent for review: 2013-12-31 21:52
Date reviewed: 2014-01-09 06:26

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[Y] Grade A: Priority Publishing	Google Search:	[ ] Accept
[Y] Grade B (Very good)	[ ] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[ ] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	[ ]Rejection
[ ] Grade D (Fair)	[ ] Grade D: rejected	BPG Search:	[ ] Minor revision
[ ] Grade E (Poor)		[ ] Existed	[Y] Major revision
		[ ] No records	

COMMENTS TO AUTHORS
Xue et al investigated the expression and the regulation of NKCCs in the distal colon of the mouse. Although the paper is very well-written and the conclusions are of interest, the following points still remain to be addressed, in my opinion: Specific Comments / Concerns: 1. Please ascertain that all abbreviations are defined on their first usage throughout the text. 2. Animals and tissue preparations: Anesthesia and final disposition of the experimental animals is not reported. 3. Statistical Analyses Please justify the selection of the employed statistical tests, and define the level of significance. Did the authors employ a post hoc test for multiple comparisons? For each study, were numbers of measurements pre-specified? For instance, for ANOVA, one should normally pre-specify an effect size and the number of required subjects and/or measurements for an alpha value of 0.05 and a power of 0.80. Presentation of the data as mean +/- SD might be preferable. 4. Results, Paragraph 1: "…consistent with observations in rat and human colonic tissues." Please provide appropriate references. 5. In Figure 1: The number of animals used for the localization experiment is unclear. Figures 2-4: Please specify the number of mice and total number of measurements for each one of the compared variables. 6. Throughout the manuscript: "Not shown data" should be presented. 7. Discussion, first sentence of first paragraph: please provide appropriate references for "previous studies."

Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road,
Wan Chai, Hong Kong, China

ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 8646
Title: Localizationandvasopressinregulationoftheа+-K+-2Cl-cotransporter (NKCC2)inthe distal colonic epithelium
Reviewer code: 02876566
Science editor: Qi, Yuan
Date sent for review: 2013-12-31 21:52
Date reviewed: 2014-01-16 14:41

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[ ] Grade A: Priority Publishing	Google Search:	[ ] Accept
[Y] Grade B (Very good)	[Y] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[ ] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	[ ]Rejection
[ ] Grade D (Fair)	[ ] Grade D: rejected	BPG Search:	[Y] Minor revision
[ ] Grade E (Poor)		[ ] Existed	[ ] Major revision
		[ ] No records	

COMMENTS TO AUTHORS
Hong Xue et al. investigated localization and regulation of NKCC2 in the distal colon. The results clearly demonstrate that vasopressin regulate membrane trafficking of NKCC2 by immunohistochemistry and Western blotting. Major comments 1. Fig. 3 clearly confirms that dDAVP increases membrane trafficking of NKCC2. Western blot of NKCC2 and GAPDH was performed in the different gels? Since the molecular weights of these two proteins are different, the same gels should be used for the detection. The authors showed whole and membrane NKCC2 expressions by Western blot. Low-density membrane fraction, which predominantly contains intracellular vesicles, would be useful to compare the expression in the high-density membrane fraction, which predominantly contains plasma membrane. 2. NKCC2 is present in the thick ascending limbs of the kidney, which lacks water channels. In contrast, NKCC1 is present in the collecting ducts. The author mentioned that NKCC1 and NKCC2 are present in the different types of cells. However, immunohistochemistry did not reveal that the two isoforms are present in the different types of cells. Heterogeneity of the cells is very strong in the kidney. How about the colon? Is the presence of NKCC2 and AQP2 in the different cells? Minor comments 3. Page 8, The antibody against NKCC2 is not sc-21547. 4. To investigate the differences among more than three groups, multiple comparison should be performed after ANOVA. ANOVA can be used only for the detection of heterogeneity among the groups.

Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road,
Wan Chai, Hong Kong, China

ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 8646
Title: Localizationandvasopressinregulationoftheа+-K+-2Cl-cotransporter (NKCC2)inthe distal colonic epithelium
Reviewer code: 00353947
Science editor: Qi, Yuan
Date sent for review: 2013-12-31 21:52
Date reviewed: 2014-01-21 04:40

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[ ] Grade A: Priority Publishing	Google Search:	[ ] Accept
[ ] Grade B (Very good)	[Y] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[ ] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	[ ]Rejection
[Y] Grade D (Fair)	[ ] Grade D: rejected	BPG Search:	[ ] Minor revision
[ ] Grade E (Poor)		[ ] Existed	[Y] Major revision
		[ ] No records	

COMMENTS TO AUTHORS
The manuscript is describing the effect of VP on both the intracellular trafficking and activation of NKCC2 in mouse colons. They are using RT-PCR, western blot immunofluorescence and short circuit current assays. Base on figure 1b, we can not conclude that NKCC1 is not expressed in the apical pole due to the resolution. The staining seems to be both subapical and apical. Dapi staining should be visible in Fig 1 d and H. The reader is not able to know if Fig 1F show apical or cells staining. Provide a better image. The figure 2 show an increase of NKCC2 in the subapical area. In order to conclude to an increase of NKCC2 into the apical membrane, authors should perform gold particles labeling NCKK2 quantification. GAPDH is a cytosolic protein marker. The western blot of the membrane fraction should be performed with a membrane protein marker such as b-actin or other proteins cited in the literature. The Western blot should show more than 3 tissue samples by condition. Accordingly to Fig. 1, the mRNA in the kidney is more abundant than the mRNA in the colon. This difference is not apparent in the western blot where all lines were loaded with 20 ug of protein. Mastropaolo et al. (Regul pept 2013) showed that mouse colon has V1R receptor. The author should perform their short-circuit assay with dDAVP in the condition that will only affect V2R. They should confirm the effect with V1 and V2 antagonist, which are commercially available. An increase of calcium via V1R activation may result in the reduction of endocytosis of NKCC2 and subsequently an increase in NKCC2 in the apical pole. Minor: 1) Verify to cite the correct references example; the number 16 is wrong. 2) In Materiel and method, add a reference for the previously described method 3) in fluorescence image Analysis specify the statistical method in addition to the software. 4) Can not found Boogo company manufacturer instruction on the web.

Please describe the method with more details. 5) Fig 2D, authors should add arrows to determined punctated vesicles.