May 12, 2015

Dear Editor,

Please find enclosed the revised manuscript in Word format (file name: 17574-revised.doc) titled "Calcium Glycerophosphate preserves Transepithelial **In**tegrity in the Caco-2 Model of Intestinal Transport " for publication in World Journal of Gastroenterology.

We thank the reviewers for the comments, which have been taken into account for the revision of the manuscript.

Author: Palika Datta, Margaret T. Weis

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 17574

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

Sincerely,

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The manuscript has been improved and the changes are highlighted according to the suggestions of reviewers:
Reply to the reviewers' comments:

Reviewer 2529364:

Calcium glycerolphosphate preserves transepithelial integreity in the Caco-2 model of intestinal transport. The authors examined the effects of Calcium glycerolphosphate on intestinal epithelial barrier under different experimental conditions. They used Caco-2 cell model of transpithelial transport and measured transepithelial electrical resistance (TEER) and mannitol flux. I feel that the authors have not provided enough details about their experiments,

which makes it difficult to interpret some of the results. My comments are listed below.

1. **Has dissolving different concentrations of CGP in 0.1% citric acid changed the pH of the solutions? The pH of solutions containing different concentrations of CGP need to be provided, which will allow to see whether the effects of CGP in reversing 0.1% citric acid induced damage of epithelial integrity were due to the true effects of CGP or were due to the increase of pH in solutions containing different concentrations of CGP.**

Response: Thanks for your comment. The pH was checked and we observed no change after dissolving different concentrations of CGP on 0.1% of citric acid. We have added this detail in the revised manuscript.

2. **Measurement of TEER. The details of how TEER was measured should be provided. For example, what were the time points when TEER was measured, were MEM media completely removed from wells when TEER was measured? In Table 1, the results of TEER and mannitol flux were from time 0 to 4 hours. What does time 0 mean? Does this refer to the TERR measured immediately after the addition of 0.1% citric acid and CGP solutions or before the addition of these solutions? The authors measured mannitol flux at time 0, 4 hours and 24 hours in Figure 1 (these should be described in materials and methods section), but measured TEER at time 0 and 4 hours in Table 1. Was TEER also measured at 24 hours?**

Response: TEER was measured using the Voltohmmeter purchased from World precision Instruments. The resistance was measured by placing the electrodes directly in the transwell inserts using with media present. The lengths of the electrodes are unequal allowing the longer (external) electrode to touch the bottom of the dish containing the external culture media while preventing the shorter (internal electrode) from reaching the bottom of the tissue culture insert. This ensures proper positioning between the electrode and the cell layer in the cup during the resistance measurement.

In Table 1, time 0 indicates that TEER was measured immediately after addition of CGP concentrations and 0.1% citric acid. Subsequent measurements were taken at various hours up to 24 hours.

How was TEER % baseline change calculated?

Response: The time 0 TEER was measured for each well (baseline). Subsequent TEER measurements for that well were expressed as a percentage of the baseline measurement. Rate of TEER and mannitol flux were calculated by taking the

slope of the linear portion of the TEER or mannitol flux vs time curve (0 to 4 hours).

3. **In the materials and methods section, the authors simply wrote that E-cadherin expression was measured using Western-blot. The authors also need to tell how the expression levels were determined.**

Response: The protein E-cadherin expression was measured using the Western Blot analysis. The cells were collected from the transwell inserts after the treatments and were lysed in the cell lysis buffer. The proteins were then separated according to the molecular weight through gel electrophoresis. They were then transferred to a membrane producing a band for each protein. The membrane was then incubated with E-Cad antibody and developed later with secondary antibody. The expression was compared to the control that had no treatment and no significant change in the expression was observed.

4. **In Tables and Figures, there was a "control", what was it? The authors need to provide this information in materials and methods section.**

Response: The experiments done had a set of cells represented as control, which were not subjected to any kind of treatment of Calcium Glycerophosphate, cytokines and peptides. All the results were compared to control.

5. **Please revise the figure legends. The contents of current figure legends are not sufficient for readers to interpret and to understand the significance of the data.**

Response: The contents of the figure legends have been revised.

6. **In the discussion part, please cite published references but not information obtained from websites.**

Response: Thanks for your remark on that, in the revised manuscript, the information from the website has been removed.

7. **What are the possible mechanisms by which CGP maintains the epithelial barrier function in the experimental conditions used in this study? The authors should discuss this in the discussion part.**

Response: The hypothesis was made that CGP is able to increase the Sphingosine 1 Phosphate, thereby inhibiting the Alkaline Phosphatase enzyme thus maintaining the epithelial integrity. We were able to show the results in

that direction but additional research on this mechanism is required to further strengthen our hypothesis.

9. In the title of this paper, please change "transepithial" to "transepithelial".

Response: Thanks for your suggestion; the title has been corrected to "Transepithelial".

Reviewer 00001391:

I have no comments on the experimental protocol. Indicate in Methods the statistics you have used to test the differences (perhaps better than in the legends of figure) At the end of the discussion you that calcium glycerophosphate had been used as a dietary supplement. Can you give reference, dosage and, if so results?

Response: We appreciate your comments and have incorporated the details on the statistics in revised manuscript.
Calcium Glycerophosphate has been used as the dietary supplement mainly as a palliative in interstitial cystitis, prostatitis and overactive bladder. They are being sold under the brand name Prelief by AK Pharma Company. Each tablet contains 345mg calcium glycerophosphate (65 mg of elemental calcium). Two tablets are recommended per day. Though the mechanism of action is not very clear but it appears to reduce cellular inflammation in the urinary bladder.
Here is the reference:
Dietary consumption triggers in interstitial cystitis/bladder pain syndrome patients. Bassaly R(1), Downes K, Hart S. Female Pelvic Med Reconstr Surg. 2011 Jan;17(1):36-9.

Reviewer 00070280:

This is an interesting article. The authors should be congratulated.

Response: We really appreciate reviewer's positive comment.