Author's response to reviews

Title: Effects of oral adenosine 5'-triphosphate and adenosine in enteric-coated capsules on indomethacin-induced permeability changes in the human small intestine: a randomized cross-over study

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Author's response to reviews: see over
Dear Editor,

Thank you for reviewing our manuscript, entitled:

Effects of oral adenosine 5′-triphosphate and adenosine in enteric-coated capsules on indomethacin-induced permeability changes in the human small intestine (ID: 1122177090126326, submitted December 21 2006).

Below, we provide the requested power calculation by the editor and also reply on the comments of the reviewers. Please find enclosed the revised manuscript, in which all requests/comments made by the editor/reviewers have been incorporated; changes in the revised manuscript are underlined.

We thank the editor/reviewers for their valuable comments and suggestions. The request by the editor and the comments of the reviewers are answered below. All page numbers refer to the revised manuscript.

Power calculation as requested by the editor:

As requested, we have included the power calculation for the present study, which was based on the results of our previous randomized cross-over experiment. The following text has been included on p. 8-9 of the revised manuscript:

“Sample size calculation for the present randomized cross-over study was based on the results of our previous study (Bours et al. Eur J Gastroenterol Hepatol 2007;19(3):245-50), in which subjects showed an average attenuation in the indomethacin-induced increase in L/R ratio of 0.016 (i.e. a reduction of 33%) in response to topical ATP. In the cross-over experiments of our previous study, a standard deviation of 0.021 and correlation between paired measures of 0.74 were observed.

To be able to detect at least half of the previously observed effect, that is ~15% reduction in L/R ratio, based on the standard deviation of 0.021 and a correlation between paired measures of 0.60 (i.e. a conservative estimate relative to the correlation of 0.74 observed in our previous study), it was calculated that 31 subjects would be sufficient to detect a significant effect of ATP/Ado on an indomethacin-induced increase in L/R ratio with a power of 90% and two-tailed alpha of 0.05. Accounting for potential dropout during experiments, a total of 35 participants were recruited for the present study.”
any relevant effects of ATP and adenosine, showing that our negative results are not a problem of low power, but a real negative finding.

Comments reviewer #1:
Minor essential revisions:
1. “Comment should be made in the introduction regarding intestinal permeability in celiac disease, along with Crohn’s disease, since celiac disease is then mentioned twice later (in exclusion criteria pg 7 and in results page 11)”
We have incorporated the following text in paragraph 3 of the Introduction (p. 5 of the revised manuscript): “It has been shown that increased mucosal permeability of the small intestine is associated with several gastrointestinal disorders, including inflammatory bowel disease and celiac disease.”
2. “Check spelling dipyridamole”
“Dipyridamole” has been changed to “dipyridamole” on p. 8 of the revised manuscript.

Discretionary revisions (which the author can choose to ignore)
3. “Abstract – Conclusions include the expression “untimely opening” which has an ambiguous meaning which could be interpreted as premature opening”
The conclusion in the abstract has been changed to the following text (p. 4 of the revised manuscript): “The observed lack of effect of encapsulated ATP/adenosine may have been caused by opening of the enteric-coated supplement at a site distal from the indomethacin-inflicted site.”
4. “Probabilities are often expressed as p=0.00, except for the discussion on pg 14 using p is less than 0.01, which is the more usual term”
The expression of probabilities has been changed accordingly in “p<0.01” (see p. 4, 13, 14, 28, 29 of the revised manuscript).
5. “Introduction – Clarify that paracellular pathway is being used as a synonym for intercellular pathway”
In paragraph 2 of the Introduction (p. 5 of the revised manuscript), the following change has been made: “In general, it has been proposed that there are two distinct pathways in the intestine through which translocation occurs, that is, a transcellular and a paracellular (i.e. intercellular) pathway.”
6. “Methods – The comments on pg 8 protocol on alcohol and drugs could be moved to pg 7 under exclusions based on drug use”
Participants were requested to abstain from alcohol and caffeine-containing beverages and foods for 4 days preceding and during the experiments (see paragraph 2 of the Protocol section on p. 9). As this requested four-day abstinence was not an exclusion criterion per se, but a regimen to be followed by subjects during their participation in the experiments, it would be incorrect to move it to the exclusion criteria (criterion 3 in the Subjects section on p. 8), since exclusion criteria are restricted to subject characteristics which represent inherent (“absolute”) contraindications for participation.
7. “Discussion – Comments on pg 15 regarding ATP stability could be moved to results”
To our surprise, we observed no effect of encapsulated ATP on the indomethacin-induced permeability changes. Several explanations for this lack of effect were considered and are put forward in the Discussion. One potential explanation was possible degradation of ATP within the enteric-coated supplement during storage. In an attempt to verify this possibility, we performed HPLC analyses to obtain post-hoc data on the stability of ATP. These post-hoc data were merely used to substantiate the discussion on potential explanations for the lack of effect of encapsulated ATP, and would therefore, in our opinion, be somewhat inappropriate for the Results, in which only primary outcomes are presented.

Comments reviewer #2:
8. A. More detail on the preceding study in the Introduction; B. When is peak of permeability following indomethacin expected; and C. Delete paragraph 4 in the Introduction or move to the Discussion
Paragraph 4 of the Introduction has been deleted (comment C) and replaced by the following text on p. 5-6 of the revised manuscript, which provides more detail on the preceding study (comment A): “Frequent use of NSAIDs is associated with an elevated risk of damage to the mucosal epithelium that lines the gastrointestinal tract lumen, thereby compromising integrity of the mucosal barrier. One of the earliest events in NSAID toxicity is uncoupling of oxidative phosphorylation within enterocytes resulting in depletion of cellular energy stores in the form of adenosine 5’-triphosphate (ATP), which leads to an increase in mucosal permeability in the intestine. It has been demonstrated in previous experiments by Bjarnason and co-workers that mucosal permeability of the small intestine
is increased within 8-10 hours after ingestion of two subsequent doses of the NSAID indomethacin (75 and 50 mg); the permeability increase is rapidly reverted, being no longer evident 48 hours after indomethacin ingestion (comment B). Utilizing this human model of increased intestinal permeability induced by short-term challenge with indomethacin, we recently showed that topical administration of ATP into the upper small intestine attenuated the indomethacin-induced increase in intestinal permeability in healthy human volunteers. In this randomized cross-over study, fasting subjects received two subsequent indomethacin dosages (75 and 50 mg) concomitant with administration of ATP or placebo directly into the upper small intestine via a naso-intestinal tube. Intestinal permeability was measured by the lactulose/rhamnose (L/R) sugar absorption test, which is a widely used and sensitive permeability measure of the small intestine. Results showed that indomethacin induced an approximately two-fold increase in median urinary L/R excretion ratio relative to the basal L/R ratio in the control condition (i.e. no indomethacin, no ATP). Administration of ATP concomitant with indomethacin ingestion completely prevented the indomethacin-induced increase in L/R ratio. This finding suggested that ATP might be a beneficial compound in alleviating detrimental NSAID effects in the small intestine.”

Comments reviewer #3:
9. Relevance of the study:
Unfortunately, we were unable to detect an effect of encapsulated ATP/adenosine on the indomethacin-induced permeability changes in the human small intestine. However, results of this study provide additional evidence that short-term administration of indomethacin, which is one of the most frequently used over-the-counter NSAIDs, compromises epithelial integrity in the small intestine. In combination with the results of our previous study, in which ATP was delivered in the proximal small intestine where it attenuated the indomethacin-induced increase in mucosal permeability, the present results suggest that the early-phase mucosal damage inflicted by indomethacin appears to occur quite locally, probably in a limited area of the proximal small intestine where levels of indomethacin are highest during absorption. Because of this local character, targeted intestinal delivery by means of enteric-coated capsules proved to be difficult in the present study. This indicates that it is crucial to realize that the selection of a proper type of enteric coating to be used for targeted drug delivery in a specific region of the small intestine is a pivotal step in drug evaluation since an improper coating could substantially interfere with intended drug effects, as demonstrated in our case for ATP, which was previously shown to completely prevent indomethacin-induced small intestinal permeability changes.

Sincerely, on behalf of all co-authors,

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