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Re: Celecoxib induced gastrointestinal, liver and brain lesions in rats, counteraction by BPC 157 or L-arginine, aggravation by L-NAME (No. 32256)

Dear Editor Yuan Qi,

Thank you very much for your kind response, and we highly appreciate all suggestions given by the reviewers. The manuscript is accordingly revised, and we strongly hope that you will find the improved version to be suited for final presentation in your distinguished journal.

Thus, we are sending to you the revised version of our manuscript No.32256 according to suggestion given by your reviewer.

The following comments were raised by the reviewer:

The manuscript reports a potentially interesting data. However, the paper has several notable weaknesses and errors. Specific comments are as follows: 1. The major concern is experimental model. The Authors used unusual high dose (1g/per kg body weight) of celecoxib to induce gastrointestinal, liver and brain lesions in examined rats. It means that human (adult) weighing approximately 70 kg has to intake approximately 70 g of celecoxib per day to induce similar lesions (or to treat inflammation). Usually 200 mg of celecoxib is used (maximal dose 400 mg). The Authors should examine much lower concentration of celecoxib to induced described lesion. Eventually this issue should be commented/discussed in the discussion section. 2. I wonder why the effect of 10 ng and 10 µg of BPC 157 on celecoxib induced liver lesion (microscopic assessment and ALT activity) are essentially similar. It seems to me that lower amount of BPC 157, let say 1 ng (or even lower) should be studied. Why only 10 µg but not 10 ng of BPC 157 was examined in the presence of L-NAME? 3. Please check manuscript throughout for writing.

To these requests see our specific arguments and reply

The manuscript reports a potentially interesting data.

We appreciate this point of the reviewer.

Specific comments are as follows: 1. The major concern is experimental model. The Authors used unusual high dose (1g/per kg body weight) of celecoxib to induce gastrointestinal, liver and brain lesions in examined rats. It means that human (adult) weighing approximately 70 kg has to intake approximately 70 g of celecoxib per day to induce similar lesions (or to treat inflammation). Usually 200 mg of celecoxib is used (maximal dose 400 mg). The Authors should examine much lower concentration of celecoxib to induced described lesion. Eventually this issue should be commented/discussed in the discussion section.

Acknowledged. This point is now fully emphasized. This was specifically pointed out in Introduction (see last paragraph),

Furthermore, we should emphasize the previous high-dose regimens used for toxicity demonstration of all non-selective NSAIDs^[5-8], versus μg - ng regimens BPC 157 counteracting potential as a likely antidote^[1], and celecoxib safety profile. Thereby, we consistently used selective NSAID in a particular very high dose that consequently markedly exceeds maximal dose used in the patients^[10]. Likewise, in celecoxib rats, both BPC 157 regimens, μg and ng , were also validated against NO-agents, L-NAME and L-arginine, given either alone and/or combined.

and in Discussion (see first and last paragraph)

This study argues the celecoxib-stomach, liver and brain lesions extended COX-2 inhibition as a particular NO-system dysfunction, in the worst conditions after a high-over dose application, in particular (note, considering advanced safety celecoxib profile^[10], lower celecoxib regimens such as 200 mg/kg and 500 mg/kg given intraperitoneally were without notable effect on gastric, liver or brain lesions (thereby, not specifically shown)). These lesions could be all influenced by NOS-substrate L-arginine, and in particularly with the stable pentadecapeptide BPC 157, as the agent known to counteract non-selective NSIDs own ulcerogenesis, and liver and brain lesions and particularly interact with NO-system^[1,9]. The additional

support comes from the similar therapy effects obtained with correspondingly high dose range of BPC 157 therapy, which was similarly used in other studies as well^[33,34].

Conclusively, L-arginine but more BPC 157 may provide a particular therapy that may alleviate likely gastrointestinal, liver and brain lesions and readdress NSAIDs' post-surgery application and NO-system involvement. The particular point, however, remains, a single huge over-dose challenge versus considerably lower regular patient regimens throughout markedly more prolonged treatment duration.

Namely, it should be mentioned that most of the animal data were also obtained with the markedly higher NSAIDs regimens. And thereby, in support, we emphasized our previous reports dealing with over-dose of paracetamol, diclofenac and ibuprofen. The additional emphasize is that these data were obtained with one single application of a huge over-dose, while the human data would consider markedly lower dose regimens, but markedly longer therapy duration.

We hope that this explication would satisfy reviewer's comments.

2. I wonder why the effect of 10 ng and 10 µg of BPC 157 on celecoxib induced liver lesion (microscopic assessment and ALT activity) are essentially similar. It seems to me that lower amount of BPC 157, let say 1 ng (or even lower) should be studied. Why only 10 µg but not 10 ng of BPC 157 was examined in the presence of L-NAME?

Acknowledged. This point is now fully emphasized. In principle, the original regimen 10 µg and 10 ng per kg was used in most of our studies, while the higher dose was regularly challenged with L-NAME and L-arginine application, and thereby this regimen was accordingly used in the present study. However, to satisfy this reviewer's request additional data were included in the text (Table 1, Table 2, Table 3). These data evidence that 10 ng/kg i.p. would have an essentially similar effect to the effect of the higher dose. In additional the additional dose of the 1 ng/kg was also tested, and it demonstrated a beneficial effect as well (Table 1, Table 2, Table

3). In principle, these findings are along with the findings obtained in other studies, and in support, that point was emphasized in our revised text.

3. Please check manuscript throughout for writing.

Acknowledged.

Thus, once again, thank you very much for your kind effort in improving our manuscript

Looking forward to hearing from you

Sincerely

Predrag Sikiric, MD, PhD

Professor