Answers to reviewer's queries

Reviewed by 00035901

Manuscript Number	30721
Manuscript Title	Investigation of mitigating effect of colon-specific prodrugs of boswellic acid on TNBS-induced colitis in Wistar rats: Design, kinetics and biological evaluation
Review Time	2016-11-15 11:38
Comments To Authors	The authors demonstrated the efficacy of colon-specific prodrugs of BA on TNBS-induced colitis in mice. The present study was well organized and well investigated, and will give us important information especially in the field of IBD. To improve the quality of the present paper, the authors should revise it according to our suggestions, 1) The introduction is extremely too long, especially for general concept for the treatment of IBD. The authors should shorten the introduction. 2) The authors use the "colon-specific" prodrug. However, the prodrug was changed into BBA by the action of N-acyl amidase of microbiota. The authors should clarify the distribution of N-acyl amidase (+) microbiota in the GI tract. 3) In the patients with IBD, the dysbiosis in intestinal microbiota has been reported recently in animal model as well as human. Does these dysbiosis affect on the activation of pro-drug to BBA?

Answers to reviewer's comments

1) The introduction is extremely too long, especially for general concept for the treatment of IBD. The authors should shorten the introduction.

We have shortened the introduction as per your suggestion.

2) The authors use the "colon-specific" prodrug. However, the prodrug was changed into BBA by the action of N-acyl amidase of microbiota. The authors should clarify the distribution of N-acyl amidase (+) microbiota in the GI tract.

Clarification: I am quoting from literature in support of distribution of N-acyl amidase (+) microbiota in the GI tract. We have added reference no 41 to that effect in the manuscript.

"Over 400 distinct bacterial species have been found residing in high numbers in the colon 20 - 30% of which are of the genus bacteroids. The most important anaerobic bacteria are bacteroides, bifidobacterium, eubacterium, peptococcus, peptostreptococcus, ruminococcus, and clostridium which secrete various enzymes (Krishnaiah YSR., Satyanarayana S. In: Jain NK (Ed.) Advances in Controlled and Novel Drug Delivery New Delhi, India. 2001; 89- 119.).

These enzymes are used to degrade coatings or matrices as well as to break bonds between an inert carrier and an active agent (i.e., release of a drug from a prodrug). Activation of prodrugs is based on the exploitation of the specific enzymatic activity of the microflora (enterobacteria) present in the colon. The colonic bacteria are predominately anaerobic in nature and secrete enzymes that are capable of metabolizing substrates such as carbohydrates and proteins that escape the digestion in the upper GIT (1. Sinha VR., Kumria R. Microbially triggered drug delivery to the colon. Eur J Pharm Sci 2003; 18: 3.
2. Gurpreet K., Subheet J., Ashok KT. Investigations on microbially triggered system for colon delivery of budesonide. Asian J Pharm Sci 2010; 5: 96.).

Predominant species in colon are such as *Bacteroides, Bifidobacterium and Eubacterum* etc., whose major metabolic process occurring in colon are hydrolysis and reduction. The enzymes present in the colon are:

Reducing enzymes: Nitroreductase, Azoreductase, N-oxide reductase, sulfoxide reductase, Hydrogenase etc.,

Hydrolytic enzymes: Esterases, N-acyl amidases, Glycosidases, Glucuronidase, sulfatase etc (Colon Specific Drug Delivery Systems: A Review on Various Pharmaceutical Approaches Prasanth V.V, Jayaprakash. R, Sam T. Mathew Journal of Applied Pharmaceutical Science 02 (01); 2012: 163-169)

3) In the patients with IBD, the dysbiosis in intestinal microbiota has been reported recently in animal model as well as human. Does these dysbiosis affect on the activation of pro-drug to BBA?

We have not studied the effect of dysbiosis on the activation of pro-drug to BBA. But we do agree with the reviewer's suggestion and will definitely incorporate this in our study protocol in future.
