To the Editor of *World Journal of Gastroenterology*,

The Reviewers' comments were all valuable, and they helped greatly in revising and improving our paper. We have studied them carefully and revisions are highlighted in red throughout the paper. We appreciate the Editor and Reviewers' work and hope that the revised version of our manuscript will meet with approval.

Point by point responses to the Reviewers' comments

Reviewer 1 (Code: 02411089)

Congratulations on your work and paper. They are both valuable. I'd just suggest the review of the study by a pharmacologist.

We gratefully thank the Reviewer for having appreciated our work. We hope that the revision of the paper is approved by the Reviewer.

Reviewer 2 (Code: 02439036)

Dianzani *et al* reported the effects of co-loaded dexamethasone and butyrate in SNL on the in vitro inflammatory response and in an in vivo DSS-IBD model. Manuscript is well written, figures are precise and the idea is of great interest in the field. However, further clarifications should be approached to improve the quality of the manuscript.

Major comments:

1) **It is intriguing that same authors have previously analyzed the effect of dexamethasone-SNL and butyrate-SNL in a clinical trial (Serpe et al. European Journal of Pharmaceutical Sciences: 39, 2010) but they have not done the same comparison here. According to their experience and also the material availability, the comparison between DX-SNL, Butyr-SNL and DX+Butyr-SNL could have provided a more relevant conclusion. Otherwise, the combination of DX and Butyrate without nanoparticles could have also provided interesting information. In the current manuscript, it is not clear whether the effect is due to the combination of the two drugs or the presence of the nanoparticle.**

We thank the Reviewer for this comment. In our previous work, mentioned by the Reviewer, we performed only *in vitro* experiments on PBMC derived from inflammatory bowel disease (IBD) patients to study whether the nanoformulation of anti-inflammatory drugs such as dexamethasone and butyrate might influence mRNA and protein expression of cytokines. We showed that the incorporation of dexamethasone or butyrate into solid lipid nanoparticles (SLN) significantly improved the *in vitro* anti-inflammatory effect, even if only at the higher concentration tested. We agree with the Reviewer's comment about the usefulness of testing *in vivo* also the dexamethasone-SNL, butyrate-SNL and dexamethasone+butyrate-SNL. However, according to our previous results and the *in vivo* 3Rs principles (replacement, reduction and refinement), we investigated *in vivo* only the potential benefit of the dexamethasone+butyrate-SNL. Indeed, we decided to test *in vivo* if the association in a single nanoparticle of low doses of dexamethasone and butyrate was able to exert improved anti-inflammatory effects, reducing their known dose-related side effects. This study design allowed us also to evaluate the potential priming effect of butyrate on gene expression modulation as suggested by our previous work (Serpe L et al. *Eur J Pharm Biopharm*, 2004).

We agree with the Reviewer that our design does not allow full elucidation of the synergism between butyrate and dexamethasone, but our aim was to investigate the potential benefit of this nanoparticle formulation in IBD. Therefore, we consider our results to be an interesting first step toward further investigations of the underpinning mechanisms through molecular pharmacology and pharmacokinetic studies. We have added these limitations of our study in the "Discussion" section according to the Reviewer's suggestion.

2) **The *in vitro* and *in vivo* effects of DX, Butyrate and DX+Butyrate-SNL on cytokines is not the same. Could they discuss further this point.**

In response to the Reviewer's comment, we have extended the discussion about the different effects of dexamethasone, butyrate and dexamethasone+butyrate-SNL on cytokines in *in vitro* and *in vivo* models.

3) **It would be of interest to show gut-tissue images from the DSS-mice treated with DX, with Butyrate and with the DX+Butyrate-SNL**

Unfortunately, our approved *in vivo* procedure did not include histological analyses. Hence we had no images of tissue sections for the different treatments.

4) **Do the authors have any further information about the modulation of CAM proteins in HUVEC?**

Actually, we already evaluated the expression of CAM proteins in HUVEC after cholesteryl butyrate treatment (Minelli R et al. *Br J Pharmacol*, 2012). Notably, the drug at 100 μM for 24 h was not able to affect different CAM, as well as other adhesion molecule, expression in HUVEC. Therefore, the association proposed adds to the cholesteryl butyrate properties the ability of dexamethasone to modulate CAM expression as described in the background.

Minor comments:

1) **Please correct some misspelling words.**

A careful English language and spelling revision has been performed by an English native language colleague (these changes have not been highlighted in red throughout the manuscript).

Reviewer 3 (Code: 02984371)

General comments:

This is a very interesting article discussing a novel drug delivery using of dexamethasone cholesteryl butyrate solid lipid nanoparticles in in vitro and in vivo models. In vitro analysis of cell adhesion of inflammatory cells and cytokine production was performed. In vivo analysis of mice with colitis was performed.

Abstract/core tip/introduction:

1) **Extensive English language editing needed.**

A careful English language and spelling revision has been performed by an English native language colleague (these changes have not been highlighted in red throughout the manuscript).

Introduction:

1) **Important to discuss budesonide MMX and how this delivery mechanism is different, including advantages and disadvantages.**
   We have modified the "Introduction" section accordingly.

2) **It is not common knowledge that Butyrate monotherapy is effective in IBD. Please elaborate on the data behind this statement (i.e. Butyrate use in clinical trials in human subjects)**
   We have modified the "Introduction" section accordingly.

3) **Unclear statement, please elaborate "SLN have been proposed as a rational, effective and economic system to improve butyrate therapy[28-30]. Their use in cancer therapy has been already figured out in preclinical and clinical trials"**
   We have modified the sentences accordingly.

4) **Statement of study aim: would make separate paragraph within introduction Would be more specific that just "in vitro" "in vivo". What type of in vitro/in vivo models?**
   In response to the Reviewer's suggestion, we have modified the statement of study aim.

Methods:

1) **Extensive English language and spelling errors must be corrected.**
   A careful English language and spelling revision has been performed by an English native language colleague (these changes have not been highlighted in red throughout the manuscript).

2) **Meaning of abbreviations HUVEC and PBMC must be outlined in the text.**
   In response to the Reviewer's suggestion, we have reported the meaning of these abbreviations when they first appear in the manuscript.

3) **What type of patients were HUVEC extracted from. Their characteristics should be outlined.**

HUVEC were isolated from healthy parturients aged between 18-35 years undergoing a natural birth. The umbilical cord was collected, after obtaining the informed consent, at birth and stored at 4°C until the isolation procedure. This information has been added to the manuscript.

4) **Where were the experiements done? and over what time period?, which years? Must be explicit in text.**

In response to the Reviewer's suggestion, we have added this information in the "Scientific research process".

5) **In vitro cell adhesion assay: Please explain logic for using to incubate cells with IL-1B instead on TNF. TNF is well know to be implicated in IBD and its blockade is, so far, a cornerstone of therapy.**

We completely agree with the reviewer about the pivotal role of TNF in IBD; indeed, its blockade is considered the standard therapy for IBD in the clinic. However, various papers underline that also IL-1β have a detrimental role in this disease. As reported by M. Neurath (*Nat Rev Immunol*, 2014) in IBD there is an increased IL-1 system activation. This cytokine especially contributes to the initiation phase of the disease (Vounotrypidis P et al. *Autoimmun Highlights*, 2013). On this basis we decided to use IL-1β to better reproduce the initial phase of the disease. This consideration has been added to the "Discussion" section.

6) ***In vivo*** **model of colitis: It is not clear why only the colitis induced mice received sterile phosphate-buffered saline solution. Please clarify.**

We have clarified this issue in the "Materials and Methods" section. We administered sterile phosphate-buffered saline solution in one of the groups with DSS-induced colitis mice as a sham treatment, since using a protocol otherwise identical to that used with the drug enables the effects of the supposedly "active" treatment to be assessed objectively.

7) **How many mice in each group?**

We have added this information in the "Materials and Methods" section. Specifically, we treated at least five animals for each group.

Results:

1) **Language editing needed.**

A careful English language and spelling revision has been performed by an English native language colleague (these changes have not been highlighted in red throughout the manuscript).

2) **Must explain jurkat cells relevance in the methods. Jurkat is a continuous cell line of T-lymphocytes from acute T-cell leukemia.**

Although they derived from tumor cells, they are widely used as T-cell continuous line, which guarantees a more standardized response compared to primary fresh-isolated cells.

3) **Cell adhesion: should use comparative statistical analysis between different drugs used for continuous variable (% inhibition of cell adhesion).**

The comparative statistical analysis between different drugs has been added in Figure 2, accordingly.

4) ***In vitro* cytokine production: should provide P values for statistical comparison**

In response to the Reviewer's suggestion, we have added *p* values in the "Results" section.

5) ***In Vivo* models: Should mention numbers when discussing comparisons of reductions in DAI (i.e X% vs Y% p=z)**

In response to the Reviewer's suggestion, we have added these data in the "Results" section.

6) **When animals were sacrificed, were colonic specimens examined? This would be interesting to compare.**

Unfortunately, our approved *in vivo* procedure did not include histological analyses. Hence we were not able to investigate colon tissue sections for the different treatments.

Discussion:

1) **Language editing needed**

A careful English language and spelling revision has been performed by an English native language colleague (these changes have not been highlighted in red throughout the manuscript).

2) **Should discuss why no significant differences in *in vitro* assays was seen at higher concentrations**

We have discussed this issue in the "Discussion" section.

3) **Please discuss limitations of study**

We thank the Reviewer for this comment. We have added the limitations of the study in the "Discussion" section.