Author's response to reviews

Title: Black tea extract prevents lipopolysaccharide-induced NF-kappaB signaling and attenuates dextran sulfate sodium-induced experimental colitis

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Version: 2 Date: 16 June 2011

Author's response to reviews: see over
Editorial requests

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Ethical approval? You have indicated that the experiments were carried out according to the local guidelines, however, could you please explicitly document ethical approval for the study by a local ethics committee, and include the details of the committee that granted approval.

Reply: All methods used in this study were approved by the guidelines of the institutional Animal Care and Use Committee of the Chonnam National University Hospital, Gwangju, Korea and conformed to US National Institutes of Health (NIH publication No. 86-23 revised 1985) guidelines. We added this red color sentence in the method section of manuscript.

Conflict of interest - Please rename this section? Competing interests? an place it between the Conclusions and Authors? contributions. If there are none to declare, please write? The authors declare that they have no competing interests?

Reply: We renamed this section as recommended.

Authors' contributions? Please include an Authors? contributions section after the Competing interests. For the Authors' contributions we suggest the following kind of format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.
Reply:

Author Contributions
YAS, YLP, KYK and CYC carried out the study and designed the experiments. GHL, DHC, HSK and KJP contributed reagents/materials/analysis tools. SBC and WSL analyzed data. NK and BWA supervised work and corrected the manuscript. YEJ conceived and designed the experiments and wrote the manuscript. All authors read and approved the final manuscript.

Reviewer's report

Title: Black tea extract prevents lipopolysaccharide-induced NF-kappaB signaling and attenuates dextran sulfate sodium-induced experimental colitis

Version: 1 Date: 31 May 2011

Reviewer: Rachel Marion-Letellier

Reviewer's report:
The objectives are well defined.

1. The supplier and the composition of the black tea extract are not mentioned. I think the lack of the BTE composition is an issue because it will help understand the potential compound responsible of the observed effect.

Reply: We agree with the reviewer. The BTE composition is as follows: oxidized polyphenols 20%, catechins 3-5%, protein 5-10%, carbohydrate 10-15%, caffeine 2.2-5.2%, potassium content <10%, moisture content <6%, mineral/ash content <20%. We added this information in the method section of manuscript.

2. I think that the use of colon length as inflammatory marker is not appropriate. It will be
better to determine colon length/weight as we generally found in the literature.

**Reply:** We did not check the colon weight at that time. So, we cannot use the data of colon length/weight as inflammatory marker.

3. The number of rats or experiments should be mentioned in the figures.

**Reply:** We added the number of mice or experiments in figure legends as recommended.

4. There is a significant void of analysis from the in-vivo study.

**Reply:** We added the densitometry data in figure 6. Please refer to figure 6B.

5. Some data from in-vivo study should be perhaps presented in a more limited number of figures with panels.

**Reply:** We added the densitometry data in figure 6. Please refer to figure 6B.

6. To assess inflammation, measure of colon production of cytokines will be more relevant.

**Reply:** The rationale for these experiments was to verify whether BTE prevent colon inflammation via blockage of NF-κB signaling pathway in *in vitro* and *in vivo* studies. The goal was not to make a comparative analysis of IL-12p40, IL-23p19, IL-6 and IL-1β expressions between BMM and colon tissues following TLE exposure. We have clarified this point in the text.

7. For histological analysis, representative slices in addition of the histological scores will be
appreciated. Moreover, it will be more relevant to represent histological scores in scatter plots (instead of bars) because it allows seeing individual scoring results.

Reply: In this experiment, the each group included just 6 samples number. So, we think that bar graph is better than scatter plots graph for presentation of histologic scores.

8. Figure 1 and 2 are good to provide representative gels, however it will improve the manuscript to also present this data in graph form as a measure of arbitrary density units with statistical analysis.

Reply: We added the densitometry data in figure 1 and 2C as recommended.

9. Figure 1: Number of experiments? Legends of the figures require more explanation. Did the first lane of the gel represent a control without LPS?

Reply: We described the number of experiments in figure 1 legend as recommended. The first lane represents a control without LPS stimulation.

10. Figure 2: Number of experiments?

Reply: We described the number of experiments in figure 2 legend as recommended.

11. Figure 3: y axis should be a disrupted axis or started at 0%.

Reply: We changed the weight reduction curve as recommended. Y axis was disrupted and started at 0% in new figure 3.
12. Figure 4: see above concerning the studied parameter.

**Reply:** We did not check the colon weight at that time. So, we cannot use the data of colon length/weight as inflammatory marker.

13. Figure 5: In the legend, the sentence “DSS-exposed mice demonstrated significantly better colitis” is not appropriate.

**Reply:** We corrected that sentence as follows; Histological evaluation of the colon of BTE-fed, DSS-exposed mice indicated a significant decrease in colon inflammation compared to control diet-fed, DSS-exposed mice. Please refer to results section and figure 5 legend.

14. Figure 6: There may be benefit from presenting this as measure arbitrary density units of replicate western blots?

**Reply:** We added the densitometry data in figure 6 as recommended.

**DISCUSSION:**

15. Discussion is poor: repetition of results, no explanation of potential mechanisms. No review of the literature. The discussion required to be extensively modified.

**Reply:** We modified the discussion section as recommended. Please refer to red color sentence in discussion section.

16. How BTE can interfere with NF-kappaB?

**Reply:** Our study showed that BTE inhibited LPS and DSS-induced NF-kB signaling via the
the blockage of IκBα phosphorylation/degradation and phosphorylation of NF-κB/p65 in vitro and in vivo studies.

17. The sentence concerning BTE composition is not appropriate.

**Reply:** We revised the sentence concerning BTE composition. Please refer to red color sentence in discussion section.

18. Extrapolation from experimental studies to IBD concerning the effects of BTE has to be modified.

**Reply:** We modified extrapolation from experimental studies to IBD concerning the effects of BTE as recommended. Please refer to red color sentence in discussion section.

19. Addition of a Model diagram of effects will be useful.

**Reply:** In this study, we showed the anti-inflammatory effect of BTE in chemical induced colitis model. We already a model diagram for anti-inflammatory effect of BTE as below inserted. However, we did not find the exact mechanism of anti-inflammatory effect of BTE in this study. So, we think that a model diagram of effects is not needed.
Reviewer's report

Title: Black tea extract prevents lipopolysaccharide-induced NF-kappaB signaling and attenuates dextran sulfate sodium-induced experimental colitis

Version: 1 Date: 4 June 2011

Reviewer: Julio Galvez

Reviewer's report:

This study describes the preventative intestinal anti-inflammatory effect of black tea extract in the DSS model of mouse colitis, as well as reports the effects of this extract on NF-kB signaling in bone narrow derived macrophages. This is the first study evaluating the effects of this extracts and confirms previous studies performed with some of its active components.
The aim of the study is clear, and the experiments seem to be well conducted, being the data analyzed properly. Some minor revision should be done to clearly improve the quality of the study:

- How was BTE obtained and/or provided? Is it possible to show the general chemical composition of BTE? This would be essential to characterize the extract, for instance by showing its polyphenolic content, or the content of specific compounds.

Reply: We added this information in the method section of manuscript. See reviewer’s 1 comment.

- In the in vivo studies, how was the proportion of BTE to be incorporated in the diet selected? Is there any plausible reason for this? It is interesting to note that both concentrations showed a similar beneficial effect. Maybe, lower doses should have been used to establish a dose-response effect.

Reply: The reviewer raises another excellent point. Previous studies showed that ingestion of BTE 200-1000 mg/kg/day could be reached in a regular diet since many commonly consumed black teas contain an appreciable amount of this phytochemical. BTE was mixed with a control chow to a final concentration of 0.2% and 1%. A mouse typically eats about 2g of chow/day, which will translate to about 20mg day of BTE (1% diet). Obviously, this simple determination does not take into account the metabolic aspect of the compound (absorption/secretion). Nevertheless, from the standpoint of BTE ingestion from an oral diet, our approach has definite physiological relevance.

- In Figure 1, only the dose of 100 ug of BTE is shown. Representative bands from the other different concentrations assayed should be included in this figure.
Reply: As described in method section, BMM were pretreated with various concentrations of BTE (0-200 µg/ml) after which they were stimulated with LPS (0.5-1 µg/ml) for times indicated (0-1 h). We evaluated the effect of BTE on LPS-induced pro-inflammatory gene expression by RT-PCR. The effect of BTE on LPS-induced pro-inflammatory gene expression in BMM was most clear in the dose of 100 µg/ml of BTE, although also showed the inhibitory effect in lower and higher concentrations of BTE. So, we showed the data about the dose of 100 µg/ml of BTE.

- In the present study a preventative dosing protocol was used, but it would have been more interesting a curative approach. maybe, this will be the aim of future studies. However, the authors should explain why they have used this model, by comparing it with other experimental models in which some of the components of the extracts have been assayed.

Reply: The acute DSS model is useful for the study of innate immune mechanisms of colitis and tissue repair. DSS, a sulfated polysaccharide administered to mice in drinking water, is directly cytotoxic to enterocytes of the basal crypts and leads to barrier damage, microbial translocation, and induction of a colitis phenotype that is independent of the adaptive immune system. In contrast to DSS, colitis induced with the haptens TNBS, DNBS, or oxazolone instilled into the colon depends both on an acute oxidative injury with innate activation and on CD4+ T cell stimulation by autologous or microbial antigens). The rationale for our experiments was to verify the impact of BTE on blockage of innate signaling pathway such as NF-κB in *in vitro* and *in vivo* studies. So, we chose the DSS-induced colitis model. We have clarified this point in the text. Please refer to red color sentence in discussion section.

- The histologic evaluation of the colonic samples from colitic animals should be described in
the text according to the different parameters evaluated and representative pictures included in the manuscript.

Reply: Briefly, we evaluated each slides for following parameters: inflammation, depth of injury and crypt damage. The inflammation was graded on a scale of four grades: 0, none; 1, slight; 2, moderate; 3, severe. The depth of injury was graded on a scale with four grades: 0, none; 1, mucosal; 2, submucosal; 3, transmural. The crypt damage was graded on a scale with five grades: 0, none; 1, basal 1/3; 2, basal 2/3; 3, only surface epithelium intact; 4, entire crypt and epithelium lost. The percentage of involvement was also graded on a scale with four grades: 1, 0-25%; 2, 26–50%; 3, 51-75%; 4, 76-100%. An overall score was calculated as the product of each parameter severity and involvement area. Theoretically, the overall scores could range from 0 to 40. We already described the references including these informations in the method section of the manuscript.

- Is there a probable mechanism that could justify the effects of the BTE? Can it be ascribed exclusively to its antioxidant properties?

Reply: Our study showed that the inhibition of NF-κB activation and apoptosis is at least partially responsible for anti-inflammatory effects of BTE in chemical induced colitis model. Further studies are needed to clarify the exact mechanism of anti-inflammatory effect of BTE.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests