Author's response to reviews

Title: Mitochondria-targeted antioxidant MitoQ ameliorates experimental mouse colitis by suppressing NLRP3 inflammasome-mediated inflammatory cytokines

Authors:

Amarjargal Dashdorj (amka0206@gmail.com)
Jyothi K.R. (jyothibiosci@gmail.com)
Sangbin Lim (dark2lsb@nate.com)
Ara Jo (ahahyeah@naver.com)
Nam Minh Nguyen (minhnam1984@gmail.com)
Joohan Ha (hajh@khu.ac.kr)
Kyung-Sik Yoon (sky9999@khu.ac.kr)
Hyo Jong Kim (hjkim@khmc.or.kr)
Jae-Hoon Park (jhpark@khu.ac.kr)
Michael P Murphy (mpm@mrc-mbu.cam.ac.uk)
Sung Soo Kim (sgskim@khu.ac.kr)

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Author's response to reviews: see over
Dear Dr. Sabina Alam

RE: Manuscript MS: 3967196349611999

Manuscript title: Mitochondria-targeted antioxidant MitoQ ameliorates experimental mouse colitis by suppressing NLRP3 inflammasome-mediated inflammatory cytokines.

We would like to thank you for giving us the opportunity to revise our manuscript. As the reviewers suggested in your letter on May 10th, we have revised the manuscript again. We hope that the present manuscript will be deemed acceptable for publication in the BMC-Medicine.

The following provides our point-by-point responses to the reviewer’s comments. In addition, the figure 7 was made exclusively for this study to support our hypothesis. We believe the statements presented in response to the reviewer’s comments have allowed us to further improve our manuscript. **We would appreciate your reviewing our revised manuscript and look forward to hearing from you soon.**
Thank you very much.
Sincerely,

Sung Soo Kim, M.D., Ph.D.

Department of Biochemistry and Molecular Biology,
School of Medicine,
Kyung Hee University,
#1, Hoegi-dong, Dongdaemoon-gu,
Seoul 130-701, Korea
Tel: 822-961-0524
Fax: 822-959-8168
E-mail: sgskim@khu.ac.kr

Reviewer 1
Reviewer: Holger K Eltzschig
Reviewer's report:
The present studies show that ROS originated from mitochondria play a causative role in the pathogenesis of IBD. ROS levels, oxidative injury and inflammatory cytokines IL-1beta and IL-18 were increased in colon tissue of DSS-induced colitis mice and significantly decreased by treating with MitoQ. Also, excessive activation of NLRP3 inflammasome which is responsible for those cytokines was suppressed by MitoQ. Based on these findings the authors conclude that mtROS play a critical role in the pathogenesis of IBD and MitoQ is a possible therapeutic molecule for the
treatment of IBD. These are novel and important findings. The conclusions are well supported by
the data presented.

Major comments:

1. The authors should expand their discussion on the interdependent role of hypoxia and
inflammation (see for example Eltzschig et al. Hypoxia and Inflammation; NEJM 2011 and
Eltzschig et al. Purinergic signaling during inflammation; NEJM 2012). How does the
hypoxic environment of the inflamed mucosa impact their findings?

Response: In accordance with reviewer’s suggestion, the discussion in the manuscript is expanded with
hypoxia, inflammation and ROS. (Please read page 14, line 390-401).

2. What role do regulatory T cells play in this context (see for example Clambey et al.
Hypoxia-inducible factor-1 alpha-dependent induction of FoxP3 drives regulatory T-cell
abundance and function during inflammatory hypoxia of the mucosa; PNAS 2012)?

Response: Possible role of regulatory T cells in our context is described in the manuscript. (Please read
page 4, line 103-107 and page 15, line 398).

Reviewer 2

Reviewer: Atsushi Mizoguchi

Reviewer’s report:

This manuscript proposes the potential therapeutic effect of MitoQ (an orally available
mitochondria-targeted derivative of the antioxidant ubiquinone) on acute colonic injury induced
by oral administration of dextran sulfate sodium (DSS). Overall, this is a well written manuscript
that contains attractive and translational information. However, there is a terminological, but
important, issue to be corrected for avoiding mislead the readers.

1. Minor Essential revision: The authors emphasize DSS colitis as a model of inflammatory
bowel disease (IBD). Although DSS colitis is useful to study the mechanism of acute
intestinal damage and following wound healing process, it is still under very controversy
whether this injury model can be used as a model of IBD. In addition, the authors used an unusual protocol for DSS administration, which should cause continuous epithelial damages by the chemical. Therefore, the authors need to change the term “IBD” to “acute colonic damage or injury”. If the authors eager to keep it, additional studies to test the therapeutic effect of MitoQ on another well accepted mouse IBD model (e.g. IL-10 knockout mice) would be necessary.

Response: According to reviewer’s suggestion, we modified the statement. But, in some conditions we used the term “acute phases of IBD” in reference to other publications (J Immunol. 2012, 188(12):6309-18; Am J Physiol Gastrointest Liver Physiol 2010, 298(6):G878-83). (Please read page 3, line 77; page 5, line134 and 135; page 13, line 347; page 15, line 413; page 16, line 442-444).