December 21, 2012

Dear Editor

Please find enclosed the edited manuscript in Word format (file name: 1281-review.doc).

Title: Inhibition of Pacemaker Activity in Interstitial Cells of Cajal by LPS via

NF-κB and MAP Kinase

Author: Dong Chuan Zuo, Seok Choi, Pawan Kumar Shahi, Man Yoo Kim, Chan Guk Park, Young Dae Kim, Jun Lee, In Yeoup Chang, Insuk So, Jae Yeoul Jun

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We carefully rechecked the reviewers comments and have done or changed the format. We hope this revised version of manuscript and our response satisfies both reviewers.

Answers to the Reviewer A’s comments

Comment 1. The title is not a good title and does not give information about ICCs and their rhythmic activity. Please change the title.

Ans 🡪As per you suggestion, we have changed the title of the manuscript which also gives the information that the experiment is carried out in ICC. We have changed the title as “Inhibition of Pacemaker Activity in Interstitial Cells of Cajal by LPS via NF-κB and MAP Kinase”

Comment 2. It is not clear which NF-κB inhibitor has been used in this study. Please clearly state the name of chemical which was used for NF-κB inhibition with a reference for the concentration which was applied.

Ans 🡪 NF-κB inhibitor used in this experiment is SN-50 supplied by Calbiochem. We have replaced “NF-κB inhibitor” with SN-50 in manuscripts, Figure 4 and also included the drug name in Solution and drugs section under material and methods. The reference for the concentration applied is “[Mitsiades CS](http://www.ncbi.nlm.nih.gov/pubmed?term=Mitsiades%20CS%5BAuthor%5D&cauthor=true&cauthor_uid=12173037), [Mitsiades N](http://www.ncbi.nlm.nih.gov/pubmed?term=Mitsiades%20N%5BAuthor%5D&cauthor=true&cauthor_uid=12173037), [Poulaki V](http://www.ncbi.nlm.nih.gov/pubmed?term=Poulaki%20V%5BAuthor%5D&cauthor=true&cauthor_uid=12173037), [Schlossman R](http://www.ncbi.nlm.nih.gov/pubmed?term=Schlossman%20R%5BAuthor%5D&cauthor=true&cauthor_uid=12173037), [Akiyama M](http://www.ncbi.nlm.nih.gov/pubmed?term=Akiyama%20M%5BAuthor%5D&cauthor=true&cauthor_uid=12173037), [Chauhan D](http://www.ncbi.nlm.nih.gov/pubmed?term=Chauhan%20D%5BAuthor%5D&cauthor=true&cauthor_uid=12173037), [Hideshima T](http://www.ncbi.nlm.nih.gov/pubmed?term=Hideshima%20T%5BAuthor%5D&cauthor=true&cauthor_uid=12173037), [Treon SP](http://www.ncbi.nlm.nih.gov/pubmed?term=Treon%20SP%5BAuthor%5D&cauthor=true&cauthor_uid=12173037), [Munshi NC](http://www.ncbi.nlm.nih.gov/pubmed?term=Munshi%20NC%5BAuthor%5D&cauthor=true&cauthor_uid=12173037), [Richardson PG](http://www.ncbi.nlm.nih.gov/pubmed?term=Richardson%20PG%5BAuthor%5D&cauthor=true&cauthor_uid=12173037), [Anderson KC](http://www.ncbi.nlm.nih.gov/pubmed?term=Anderson%20KC%5BAuthor%5D&cauthor=true&cauthor_uid=12173037).Activation of NF-kappaB and upregulation of intracellular anti-apoptotic proteins via the IGF-1/Akt signaling in human multiple myeloma cells: therapeutic implications. [*Oncogene.*](http://www.ncbi.nlm.nih.gov/pubmed?term=Activation%20of%20NF-kB%20and%20upregulation%20of%20intracellular%20anti-apoptotic%20proteins%20via%20the%20IGF-1%2FAkt%20signaling%20in%20human%20multiple%20myeloma%20cells%3A%20therapeutic%20implications) 2002; 21(37): 5673-83”

Comment 3. It is not clear which JNK inhibitor has been used in this study. Please clearly state the name of chemical which was used for JNK inhibition with a reference for the concentration which was applied.

Ans🡪 In the current experiment, JNK inhibitor II has been applied supplied from Calbiochem with the same name. We have replaced “JNK inhibitor with JNK inhibitor II” in the manuscript. Reference for the concentration applied is “[Qanungo S](http://www.ncbi.nlm.nih.gov/pubmed?term=Qanungo%20S%5BAuthor%5D&cauthor=true&cauthor_uid=15705601), [Das M](http://www.ncbi.nlm.nih.gov/pubmed?term=Das%20M%5BAuthor%5D&cauthor=true&cauthor_uid=15705601), [Haldar S](http://www.ncbi.nlm.nih.gov/pubmed?term=Haldar%20S%5BAuthor%5D&cauthor=true&cauthor_uid=15705601), [Basu A](http://www.ncbi.nlm.nih.gov/pubmed?term=Basu%20A%5BAuthor%5D&cauthor=true&cauthor_uid=15705601). Epigallocatechin-3-gallate induces mitochondrial membrane depolarization and caspase-dependent apoptosis in pancreatic cancer cells. [*Carcinogenesis*.](http://www.ncbi.nlm.nih.gov/pubmed?term=Epigallocatechin-3-gallate%20induces%20mitochondrial%20membrane%20depolarization%20and%20caspase-dependent%20apoptosis%20in%20pancreatic%20cancer%20cells) 2005; 26(5): 958-67”.

Comment 4. Fig 2 shows double labeling for c-kit and either TLR4, iNOS or COX2. Have these cells been treated with LPS. If yes, please provide pictures of the expression of these proteins before incubation with LPS.

Ans🡪 We performed immunocychemistry in this study for confirming LPS action is mediated by TLR4 and also ICCs can produce prostaglandins and nitric oxide. So, Figure 2. showed the expression of TLR4, iNOS or COX2 in ICCs by double labeling with c-kit and other proteins. For this experiment, we did not pretreat the cells with LPS so these pictures are the expression of the listed proteins before the incubation with LPS.

Comment 5. Discussion, first paragraph: “…. PGE2 and NO are inhibitory mediators on intestinal motility and produced by COX-2 and iNOS, respectively”. PGE2 and NO can be produced by COX-I and nNOS/eNOS as well. Please revise this sentence or provide evidence that ICCs do not express COX-I or nNOS/eNOS.

Ans🡪 We have revised the sentence as “PGE2 and NO are inhibitory mediators on intestinal motility and produced by COX (COX -1 or 2) and NOS (nNOS/eNOS/iNOS), respectively.”

Comment 6. Discussion, first paragraph: “…We have shown that LPS inhibited pacemaker currents of intestinal ICCs by activating ATP-sensitive K+ channels through the release of PGE2 and NO”. Please provide a reference for this sentence.

Ans🡪 Reference for the sentence has been mentioned on the discussion section.

“We have shown that LPS inhibited pacemaker currents of intestinal ICCs by activating ATP-sensitive K+ channels through the release of PGE2 and NO[17].”

( Reference : Zuo DC, Choi S, Shahi PK, Kim MY, Park CG, Kim YD, Lee J, Chang IY, Lee HS, Yeom SC, Moon HJ, Seong SY, So I, Jun JY. Action of lipopolysaccharide on interstitial cells of Cajal from mouse small intestine. *Pharmacology* 2012; 90: 151-159).

Answers to Reviewer B’s comments

Comment 1. The title needs revision and it doesn’t match the content of the manuscript, at least should include “interstitial cells of Cajal”.

Ans🡪we have changes the title of the manuscript and included “interstitial cells of Cajal” in it. The changes title is “Inhibition of Pacemaker Activity in Interstitial Cells of Cajal by LPS via NF-κB and MAP Kinase”

Comment 2. The major outcome of this study is to measure the pacemaker currents of cultured ICCs, suppressed by LPS and attenuated by various inhibitors. However, this is not consistently mentioned over the whole manuscript. For example, in the abstract the aim is “to investigate LPS related signal transduction in interstitial cells of Cajal (ICCs) from mouse small intestine” and the conclusion is “LPS can activate ICCs to release nitric oxide and prostaglandin E2 through toll-like receptor 4 and these are mediated by mitogen-activated protein kinases and nuclear factor κB”. In the introduction section, it was mentioned as “to clarify the involvement of NF-κB, ROS and MAPKs that contribute to PGs or iNOS induction in ICCs and changing of pacemaker activity in LPS-treated ICCs”. Actually, there was no data in the current manuscript that support the conclusion “the involvement of NF- κB, ROS and MAPKs that contribute to PGs or iNOS induction in ICCs”. Therefore, the authors should modify the test to be consistent and not to be overstated.

Ans🡪 As per your suggestions, we changed sentence conclusion in abstract from “LPS can activate ICCs to release nitric oxide and prostaglandin E2 through toll-like receptor 4 and these are mediated by mitogen-activated protein kinases and nuclear factor κB” to “LPS inhibit the pacemaker currents in ICCs via prostaglandin E2- and nitric oxide–dependent mechanism through toll-like receptor 4 and suggest that mitogen-activated protein kinases and nuclear factor κB are implicated in these actions”.

And also we changed sentence in discussion from “In summary, the current study provides evidence in cultured ICCs that LPS activates ICCs to release NO and PGE2 by deriving iNOS and COX-2 gene expression by activating proteins of the NF-κB family through ligation with TLR4. Moreover, p42/44 MAPK and p38 MAPK are involved in this event” to “In summary, the current study provides evidence that LPS inhibited pacemaker currents in cultured ICCs via PGE2 and NO-dependent pathways. NF-κB and MAP kinases are involved in this inhibitory action.”

Comment 3. More detailed description on their previous study (reference #17) in the introduction section, in particular, production of PGs and iNOS on the pacemaker currents of LPS-treated ICCs, will strengthen the rationale for the current study.

Ans🡪 As per your suggestions, we changed sentence in introduction from “our recent report that LPS has direct action on pacemaker activity in ICCs from mouse small intestine[17], can verify this” to “Recently we reported that LPS inhibited the pacemaker currents in cultured ICCs from mouse small intestine. LPS-action was blocked by cyclooxygenase (COX)-2 inhibitor or nitric oxide synthase inhibitor, suggesting prostaglandins (PGs) and nitric oxide (NO) are involve in these actions[17], can verify this.”

References and typesetting were corrected and attaching the figure files as PPT.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology.*

Sincerely yours,



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