Author’s response to reviews

Title: Ticagrelor and clopidogrel suppress NF-kB signaling pathway to alleviate LPS-induced dysfunction in vein endothelial cells

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Author’s response to reviews:

Dear Editor in Chief:

Thank you very much for your careful review and constructive suggestions with regard to our manuscript (BCAR-D-19-00665).

We appreciate editor’s comments and thoughtful suggestions that greatly improve the quality of our manuscript. We have carefully evaluated these comments and suggestions, and responded to them point-by-point and revised the manuscript accordingly. In addition, all the authors agreed and approved the contents of the manuscript. Please feel free to contact us with any further questions and we are looking forward to your positive consideration.

Sincerely,

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Answers to critiques point by point:

Editor Comments:

1. Please be precise and state clearly that this is an in vitro study; I would suggest to add this specification in the title in order to inform readers. As well, the topic of acute coronary syndrome is not the focus of the paper, that is rather focused on the molecular biology of ticagrelor and clopidogrel. For this reason, the title is misleading and needs to be changed.

Answers: We have changed the title to "Ticagrelor and clopidogrel suppress NF-κB signaling pathway to alleviate LPS-induced dysfunction in vein endothelial cells", which clearly states that the study is in vitro.

2. In addition to what above reported, please include limitations to the study and include the translational perspective of this study: which is the importance of the results of the paper from a clinical standpoint?

Answers: We didn’t have a relevant disease model and cannot directly explain the effects of ticagrelor and clopidogrel inhibiting the NF-κB signaling pathway on the disease. Whether ticagrelor and clopidogrel inhibiting the NF-κB signaling pathway has a therapeutic effect on the disease requires further study. However, the study provided a direction for future research. The above has been explained in the manuscript.

3. Authors’ contributions: Please use initials to refer to each author's contribution in this section, for example: "FC analyzed and interpreted the patient data regarding the hematological disease and the transplant. RH performed the histological examination of the kidney, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript."

Answers: we has corrected it.

4. Please could you remove duplicate figures in your manuscript file and upload the final version.

Answers: we has corrected it.


Answers: we has corrected it.
Reviewer reports:

Mette Bjerre (Reviewer 1):

1. Please mention the number of setups used for the different methods (n=3)?
   
   Answers: All experiments were repeated 3 times or more, which was mentioned in the statistical analyses of the manuscript.

2. Figure 2B: legend should be changed to clopidogrel.
   
   Answers: We are sorry for this error, we corrected this error.

3. Error bars are missing in Fig 2B (4B) and 2D (4D) - please mention number of experimental setup repeats (n=) - looking at the figures one may get the impression that the experiment is only performed once - if that is the case I recommend that the experiments are repeated 3 times as described in Figure 1 and Figure 3.
   
   Answers: These experiments were repeated more than 3 times. Previously, only one set of data was used for the mapping. Now that we have corrected it, we are very sorry about this error.

4. What is relative intensity refereeing to? protein/GAPDH. I suggest that figure B and D are shown relative to control (DMSO) - which will improve the readability and show fold changes instead.
   
   Answers: The relative intensity refereed to protein/GAPDH, and we have modified Fig 2B (4B) and 2D (4D) according to this suggestion.

5. Please include your thoughts of study limitations and how the results may translate into treatment.
   
   Answers: The limitation of this study is that no disease model was used. Therefore, this study does not directly explain the effects of ticagrelor and clopidogrel inhibiting NF-κB signaling pathways on the treatment of disease. The role of ticagrelor and clopidogrel inhibiting the NF-κB signaling pathway in the treatment of disease requires further research. However, this study provides direction for future research.
Rita Pavasini (Reviewer 2):

1. There are several typos in abstract. Please revise.

   Answers: We are sorry for the error, we corrected the error.

2. The sentence "The results showed that ticagrelor and clopidogrel can inhibit the degradation of IKBα and phosphorylation of p65, prevent p65 from entering the nucleus, reduce the production of TNFα, IL-1, IL-8, IL-6 and IL-2, and reduce the changes in life activities caused by LPS, such as cell viability, apoptosis, cell cycle, cell migration ability, and vascular formation. These results provide a new theoretical basis for ticagrelor and clopidogrel to cure ACS" has no sense in the context of introduction. It could be useful in conclusion.

   Answers: We are very grateful to the judges for making this suggestion, which has been removed from the background.

3. It is not clear why authors talk about a possible mechanism of ticagrelor and clopidogrel on NF-κB in the context of acute coronary syndromes. In a paper of Campo et al. (Thromb Haemost. 2017 Mar 23;117(6):1208-1216). The effect of ticagrelor and clopidogrel was analyzed and compared in patients with stable coronary artery disease and COPD. HUVEC were incubated with patients’ serum and this justify the fact to say that the two drugs act in endothelial dysfunction in a specific clinical context. So here it is not clear why talking about acute coronary syndromes and not of stable patients. Is the model studied a model of acute coronary syndromes?

   Answers: The model of acute coronary syndrome was not used in the study, so it is inappropriate to talk about a possible mechanism of ticagrelor and clopidogrel on NF-κB in the context of acute coronary syndromes. We have corrected it in the manuscript. Campo et al. demonstrated that HUVEC were incubated with patients’ serum and this justify the fact to say that ticagrelor and clopidogrel act in endothelial dysfunction in a specific clinical context, but did not clarify its mechanism. Our study demonstrated that the two drugs alleviate LPS- induced endothelial dysfunction by inhibiting NF-κB pathway.
4. Interestingly in the already mentioned study (Thromb Haemost. 2017 Mar 23;117(6):1208-1216) the level of 29 tested cytokines and chemokines (EGF, Eotaxin, G-CSF, GM-CSF, IFN-α2, IFN-γ, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-17α, IL-1 receptor antagonist (ra), IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IP-10 (CXCL10), MCP-1, MIP-1α, MIP-1β, TNF-α, TNF-β, and VEGF) and their level did not change between ticagrelor and clopidogrel, neither over time. So how can authors justify the differences found?

Answers: Campo et al. demonstrated that the level of TNFα, IL-1, IL-8, and IL-6 did not change between ticagrelor and clopidogrel[1], while our results showed the mRNA level of TNFα, IL-1, IL-8, and IL-6 changed between ticagrelor and clopidogrel. The difference may be caused by differences in research methods. Campo et al. used immunoassay to detect the level of TNFα, IL-1, IL-8, and IL-6 in serum of patients after treatment with ticagrelor and clopidogrel[1]. We used qPCR to detect the mRNA level of TNFα, IL-1, IL-8, and IL-6 in LPS-stimulated human umbilical vein endothelial cells (HUVECs) after treatment with ticagrelor and clopidogrel. In addition, the mechanisms which regulate TNFα, IL-1, IL-8, and IL-6 are more complicated in patients than in LPS-stimulated cells.

5. Were there any differences between ticagrelor and clopidogrel in results obtained? Considering the well-known pleiotropic effect of ticagrelor, it is surprising that the effect of the two drugs is similar.

Answers: There are differences about the mRNA level of TNFα, IL-1, IL-8, and IL-6 between ticagrelor and clopidogrel. Our results just showed that it is similar to alleviate LPS-induced dysfunction in vein endothelial cells, which does not conflict with the pleiotropic effect of ticagrelor.

References