Author’s response to reviews

Title: Hemopexin promotes angiogenesis via up-regulating HO-1 in rats after cerebral ischemia-reperfusion injury

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Author’s response to reviews:

Dear Dr. Wenbo Zhu:

Thank you very much for your consideration and positive comment to our manuscript entitled “Hemopexin promotes angiogenesis via up-regulating HO-1 in rats after cerebral ischemia-reperfusion injury” (MS: BANE-D-17-00261). The suggestions and criticisms are very helpful for the improvement of our manuscript. We also responded point by point to reviewers’ comments as listed below.

Point 1 Authors need provide more accurate responses for some concerns of reviews, including Point 3 from Reviewer 1, Point 5, 6, 8, 10 from Reviewer 2. Especially, authors should fully address the concern about the “penumbra”.

Point 3 from Reviewer 1 Please explain the link between eNOS to HPX, HO-1 and ZnppIX

Re: It was reported that eNOS played an important role in regulation of vascular permeability (reference 26, 27). eNOS expression mediated by HO-1 during ischemia reperfusion process was confirmed in the present research through the use of ZnppIX, inhibitor of HO-1. eNOS promoted repairment of vascular endothelial function and was related to neovascularization and neurologic functional improvement of HPX during ischemia reperfusion. More explanation has been added in the third paragraph of discussion.
Point 5 from Reviewer 2 Authors described that the model was deemed successful when the score was between 1 and 3 using the method of Longa. However, no data were presented in the text.

Re: After the rats were awake, the behavior of rats was evaluated, the score between 1 to 3 was seemed to be successfully molding and participated in the following experiments. This is the behavior criterion for judging the success of modeling but not the main result in the present research so we did not reveal the data. We have added “Rats failed to reach this standard were ruled out in the following experiments.” in the section of “Cerebral Ischemia Reperfusion Model” in Materials and Method.

Point 6 from Reviewer 2 How was the "penumbra" defined in this study? Cerebral infarction would be completed at the timing of 7 days after ischemic insult. Because authors evaluated blood vessel density in the ischemic penumbra, assessment method should be clarified.

Re: We have added the relevant reference about the definition of “penumbra” in part of Real Time PCR in Materials and Method, the position the first occurrence of it and briefly described the method we got penumbra. The blood vessel density was represented by the mean blood vessel count at each 200 higher magnification field of view. We have added the assessment method in part of Immunohistochemical Staining (IHC) in Materials and Method. Firstly, search for three ischemic penumbra zones at low magnification, then the number of new vessels in 3 regions was calculated under high magnification(200 higher magnification), and the density was measured by the mean number of three regions.

Point 8 from Reviewer 2 The method used for PCR should be clarified. How were the penumbra and ischemic core detected? How was the penumbra tissue obtained? How large were the obtained samples? Did they include cortex, striatum, and white matter? Please specify.

Re: According to Ashwal S et al. study(Ashwal S, Tone B, Tian HR, Cole DJ and Pearce WJ. Core and penumbral nitric oxide synthase activity during cerebral ischemia and reperfusion. Stroke 1998; 29: 1037-1046; discussion 1047.), the middle coronal brain section was used for determining the ischemic penumbra. We initially made a longitudinal cut (from top to bottom) approximately 2 mm from the lateral of sagittal suture through right hemisphere, then made a transverse diagonal cut at approximately the “2 o’clock” position to separate the core from the penumbra. We have added the relevant reference and briefly described the method we got penumbra.in part of Real Time PCR in Materials and Method. They include part of the cortex, striatum, and white matter.

Point 10 from Reviewer 2 In neurobehavioral evaluation, authors defined that score 3-7 showed severe neurological dysfunction, 8-11 moderate, and 12-18 were divided into mild neurological dysfunction. However, those assessments did not appear in results.
Re: The neurobehavioral evaluation was done through Garcia score method and the neurobehavioral scores (NBS) were got in each group. The higher the score, the better the neurobehavioral function was. The results are presented in Figure 1. Comparison of NBS scores in the five groups of rats at 24h and 7d after I/R. The results were listed at the beginning of the results.

Point 2 The language of this manuscript need to get revised by a native speaker or get editing service. The past tense and the present tense used in this manuscript is confusing. And it’s notable that “cytotoxicity” in Line 4, Paragraph 2, Section Background should be “cytotoxic”. More such errors would be found out and get corrected.

Re: The manuscript has been revised by a native speaker and we have carefully checked the errors and have corrected them.

Point 3 Authors don’t need to mention both of abbreviations and their full names for more than one time in the same section. Only if the abbreviation shows in Section Abstract or Section Result for the first time, authors have to provide the corresponding full name.

Re: We have revised accordingly.

Point 4 Authors should add the full information of all reagents and instrumentations. Ones in the articles of BMC series journals would be suggested for reference.

Re: The full information of all reagents and instrumentations has been added in the in the part of Immunohistochemical Staining (IHC) in Materials and Method.

Point 5 “Each group” in Line 1, Paragraph 1, Result Section should be specified.

Re: The groups have been marked following the corresponding value of NBS respectively.

Thank you again for your suggestion. We hope that the revised version of the manuscript could meet your requirement. We look forward to hearing from you soon.

With best wishes,

Sincerely,
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