Reviewer’s report

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

Version: 0 Date: 30 Jul 2017

Reviewer: Martin Schlaepfer

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Review BANE-D-17-00261

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

Relevance: 8/10

Originality: 6/10

Validity (methods and conclusion): 8/10

Clarity of writing, data and tables: 3/10

Summary: The authors expose rats to middle cerebral artery occlusion (MCAO) or sham operation. The MCAO groups receive several treatments 1. no additional treatment, 2. Vehicle injection, 3. Hemopexin (HPX) injection, 4. HPX and Zinc-protoporphyrin-IX (ZnppIX) injection.

Hemopexin helps to induce neovascularization in the stroke penumbra, while this could not be seen when ZnppIX was injected at the same time. ZnppIX serves as a heme-oxygenase (HO) inhibitor and the authors observe that HO-1 is induced by HPX and abrogated by HPX and ZnppIX injection at the same time.

Functional assessment of the animals shows, that the neurological score in the HPX group is also better than in HPX+ZnppIX.

Strengths and limitations:

The authors have made a very good experimental design, nicely conducted experiments that support their hypothesis.

That being said, the manuscript is at times extremely difficult to follow: the manuscript will need thorough language editing before publication and - in order to walk a reader through the manuscript - some explanations.
Major comments:

1. The abstract does not really support your conclusion the way it is written currently: for readers, less familiar with the field, the following things have to be explained: hemopexin-1 serves (together with heme) as an HO-1 inducer, and ZnppIX is an HO-1 inhibitor. This needs to be explained in the abstract as well as in the introduction. Currently the important explanations follow in the discussion and only then the whole experimental setup begins to make full sense.

2. Page 5, line 40: please indicate the animal use and care committee approval number, as well as the date of the approval.

3. Please explain the link between eNOS to HPX, HO-1 and ZnppIX.

4. Discuss that better functional neurobehavioral tests might not just be a result of neovascularization but might be also a result of attenuated cell death (J Cereb Blood Flow Metab. 2009 May;29(5):953-64)

5. If possible the following experiments should be conducted: Assessment of neuronal cell death would be extremely interesting.

6. The authors claim that HO-1 plays the central role in neovascularization therefore it is extremely sad that HO-1 was actually never determined on a protein level but only on an mRNA-level. Protein levels should be measured too.

Minor comments:

There are innumerous typos and language errors so that the manuscript is extremely hard to read. Please have the manuscript carefully edited by a native speaker or by someone fluent with English!

1. Abstract page 3, line 13: ischemia stroke -> ischemic stroke

2. line 19: weighting -> weighing

3. line 23: abbreviations are not introduced in the abstract (MCOA, HPX, ZnppIX)

4. line 35: Carcia -> Gracia

5. Background: line 38: Reperfusion injury occurred on stroke patients - reperfusion injury occurs in stroke patients

6. The commas in the very same sentence are partly in front and partly after the spacing (line 42)
7. Line 50: I don't understand this sentence

8. line 56. Excessive free heme was highly cytotoxic, and as such, caused endothelial injury (either use the past tense OR the present tense but be consistent).

etc. etc. etc.

9. Page 6, line 56 The term: the rat neck was broken to death cannot be written as it is. One option would be: Rats were sacrificed by cervical dislocation (I hope after prior anesthesia or CO2 application).

10. Page 7: immunohistochemical staining: please indicate the full information of the antibodies used (host, if possible article number)

11. Page 7, line 27: in what was goat serum dissolved (I assume in PBS)

12. Page 8, line 34: please indicate SD instead of SEM (SEM's are usually used for estimates and not for describing results).

13. Page 8, line 37: please introduce the term LSD-T test (I assume that's the "least significant difference test").

14. Page 10, line 40: there's no unit for the vessel density - please indicate

References:

Li RC et al: J Cereb Blood Flow Metab. 2009 May;29(5):953-64

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

**Quality of written English**
Please indicate the quality of language in the manuscript:

Not suitable for publication unless extensively edited

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