Author’s response to reviews

Title: The attenuation of neurological injury from the use of simvastatin after spinal cord ischemia-reperfusion injury in rats

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Author’s response to reviews:

Dear Pf Zhu

We would like to thank you and reviewers for the careful and thoughtful suggestions and correction for revision, most of which we have incorporated in the text of the revised manuscript. We described in detail all changes referring to each reviewer’s critique in the order mentioned in the review. As a result, we believe that our manuscript is greatly improved. Sincerely

Dr Han

Reviewer reports:

Wei Yin (Reviewer 1): The authors only used a single HE stain to count the normal neurons in Fig 1. It was not enough to convince the neuroprotection effects of the drug. At least more experiments like Nissl or TUNEL stain should be supplement in MS. : The authors appreciate this comment. A single HE stain was used in this study since HE stain with counting the number of normal motor neuron is one of the most commonly adopted methods for the evaluation of the degree of spinal cord injury in a spinal cord IR injury model (Protective effect of delta opioid agonist [d-Ala2, d-Leu5] enkephalin on spinal cord ischemia reperfusion injury by regional perfusion into abdominal aorta in rabbits. Neuroscience Letters. Volume 584, 2015, Pages 1–6; Reduction of spinal cord ischemia/reperfusion injury with simvastatin in rats. Anesth Analg. 2011 Sep;113(3):565-71; Acute Normovolemic Hemodilution Can Aggravate Neurological Injury After Spinal Cord Ischemia in Rats. Anesthesia & Analgesia: 2012,1,1 p 1285–1291).

Additionally, either thionin, crysyl violet (Nissle stain) or HE stain are suggested to be equally effective for the preparation of motor neuron counts from spinal cord section. (Treat-NeuroMuscular Disease_NeuroMuscular Network. SOP. SMA-M.1.2.004. http://www.treatnmd.eu/downloads/file/sops/sma/SMA_M.1.2.004.pdf) (Cardiotrophin-1, a Muscle-Derived Cytokine, Is Required for the Survival of Subpopulations of Developing Motoneurons. Journal of Neuroscience 15 February 2001, 21 (4) 1283-1291)

Cheng Ni (Reviewer 2): 1. As the authors
indicated that trauma or injury is unpredictable, post-treatment studies provide greater clinical implications than the pre-treatment study since. This study designed to evaluate the preventive effect of simvastatin after IR injury on neurologic damage (Simvastatin has been proved to be valuable in prevention of neurologic damage prior to IR injury), the results have their clinical significance, and could be accepted after necessary revision. Thank you for your comment. This study was performed to explore if simvastatin has therapeutic effect on spinal cord ischemia-reperfusion injury. The result of the current study suggested that simvastatin has therapeutic effect on spinal cord ischemia-reperfusion injury. We are currently conducting the follow-up study to find out the possible mechanism of the therapeutic effect of simvastatin including anti-apoptotic, anti-inflammatory, or anti-oxidative action. 2. A blank control group without ischemia-reperfusion should be added to provide baseline levels of MDS and motor neuron numbers in SD rats. According to the comment, the result of a blank control group without IR injury (sham group) was added in the method (Page 7, Group Assignment) and result section (Page 11, line 5 & 18). The MDS of a sham group (n = 10) was 0 (0) at each time point and the number of normal motor neuron of a sham group was 35 (3.8). As 10 mg/kg simvastatin group is the only group that has significant difference compared with IR group, groups with larger doses (such as 20 or 50 mg/kg) could be necessary to reveal the optimal doses. The safe dose range was explored by administrating various doses to a healthy sham animal before the study. In a pilot study, 50 mg/kg was administered to the sham animal but some non-specific negative effects such as weight loss were found without histological change in spinal cord. Thus, simvastatin within the dosage range from the previous pilot study was tried in the current study. However, we absolutely agree with you in that we need to explore higher doses for further study. The following sentence was described in the second limitation of the discussion session (Page 15, line 1-7). “Second, larger doses could be necessary to reveal the optimal doses. The safe dose range was explored by administrating various doses to a healthy sham animal before the study. In a pilot study, 50 mg/kg was administered to the sham animal but some non-specific negative effects such as weight loss were found without histological change in spinal cord. Thus, simvastatin within the dosage range from the previous pilot study was tried in the current study. Further study is needed to determine the optimal dosage for the application of human spinal cord IR injury.” 4. The study should provide possible mechanism related to simvastatin treatment. It has mentioned anti-inflammatory and antioxidant effects, thus, supplementary experiments including ROS and inflammatory cytokine tests could be acceptable. The authors absolutely agree with you and this was described in the limitation (Page 14, 1st limitation). This study was performed to explore if simvastatin has therapeutic effect on spinal cord ischemia-reperfusion injury and we could not perform ROS or inflammatory cytokine tests in this study. In this study, we found out that simvastatin has therapeutic effect on spinal cord IR injury. And we are currently performing the follow-up study to find out the possible mechanism of the therapeutic effect of simvastatin including anti-apoptotic, anti-inflammatory, or anti-oxidative action. If improvements to the English language within your manuscript have been requested, you should have your manuscript reviewed by someone who is fluent in English. If you would like professional help in revising this manuscript, you can use any reputable English language editing service. We can recommend our affiliates Nature Research Editing Service (http://bit.ly/NRES_BS) and American Journal Experts (http://bit.ly/AJE_BS) for help with English usage. Please note that use of an editing service is neither a requirement nor a guarantee of publication. Free assistance is available from our English language tutorial (https://www.springer.com/gb/authors-editors/authorandreviewertutorials/writinginenglish) and our Writing resources (http://www.biomedcentral.com/getpublished/writing-resources). These cover com-