OPEN PEER REVIEW REPORT 1

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Title: Shuxuetong injection protects cerebral microvascular endothelial cells against oxygen-glucose deprivation reperfusion
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COMMENTS TO AUTHORS

In the present paper, the authors investigated whether SXT protects cerebral microvascular endothelial cells (bEnd.3) from damages induced by oxygen and glucose deprivation (OGD/R).
Although the paper is potentially interesting as Chinese Medicine has becoming of increasing interest, it needs major revisions.
Introduction
In the introduction, the authors point to the importance of ischemic cerebral injury in severe brain dysfunctions and to the fact that t-PA is the only effective treatment for rescuing ischemic brain tissue although its narrow therapeutic window and risks of cerebral hemorrhage restrict its application. Then, the authors revise some papers in which brain microvascular endothelial cells (BMEC) are used as a model to investigate the effects of ischemia. After that, the authors introduce SXT and describe its manufacturing technology. In the present work, the authors used a mouse brain endothelial cell line (bEnd.3) to investigate the effect of SXT after cells are exposed to oxygen and glucose deprivation. In respect to the many brain endothelial cell lines that have been established and employed as in vitro BBB models, the authors need to introduce the advantage to use bEND.3 cells as a convenient and useful model for evaluating SXT effects on BBB function.
The aims of the work should be better defined and clearly summarized at the end of the introduction.
Results
The results show that SXT increases cell viability, decreases LDH leakage and cleaved-caspase-3, while increases bcl-2 expression. SXT also attenuates the cell morphology injury, a finding that is difficult to evidentiate with the methodology used in the present study. In addition, SXT reduces the intracellular ROS level and the mitochondrial superoxide level as determined by fluorescent probes. Moreover, SXT inhibits both mRNA levels of TNF-α, IL-1β, IL-6 and iNOS as well as protein levels of ICAM-1 and VCAM-1. In this respect, mRNA determination of inflammatory factors should be correlated with their protein expression. In addition, Western blots for ICAM-1 and VCAM-1 need replicates to be shown. Finally, SXT increases cell TEER, decreases FITC-Dextran values, while increases Claudin-5, Occludin and ZO-1, a finding that is difficult to extrapolate from the blots presented in Figure 6. Then, the authors investigated the effects of STX on the NF-κB signal activation, but the interpretation of their results is complicated by the poor quality of the blots. Finally, the authors determined whether SXT acts on VEGF and p-ERK1/2. The inhibitory effect of SXT on VEGF cannot be extrapolated from the blots of Figure 8A. In addition, the best effect of SXT on p-ERK1/2 was

determined at 1/32 and 1/128, but not at 1/64 concentration, a finding difficult to understand. Together, Western blots are in general of very poor quality and the experiments must be repeated in order to improve their quality. Actin is the loading control and must be run on each gel. Please, also include the replicates.

Discussion

The discussion is mainly a repetition of the results without a real interpretation of the present data. Most of the discussion is dedicated to revise key characteristics of the ischemic stroke, an aspect that is amply described in the literature. The authors also need to discuss their data in light of developing strategies to counteract human diseases in light of clinical application of the compound. For instance, according to a recent paper of Jiang et al. (2016), the clinical medication characteristics of the herbal extract Shuxuening are basically consistent with combined medicine including antiplatelet drugs. What about SXT clinical application? The conclusion of the authors that the potential mechanism of SXT regulation of ischemic damage is likely to be related to changes of VEGF, ERK1/2 and NF-κBp65 signal pathway is merely descriptive since no mechanicistic relationship among these events has been tested.