Reviewer's report

Title: Neuroprotective effect of the hairy root extract of Angelica gigas NAKAI on transient focal cerebral ischemia in rats through the regulation of angiogenesis

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Reviewer: Yinye Wang

Reviewer's report:

In the manuscript, the authors investigate the neuroprotective effect of AG water extract in MCAO-induced ischemic rats and the working mechanism linked in the BBB destruction. In a rat ischemia/reperfusion (I/R) model, post-ischemic treatment with AG extract resulted in reduced infarct volume, decreased brain edema, diminished activation astrocytes and microglia.

Novel aspects of this study are the effect of AG extract on the BBB permeability and angiogenesis.

There are major points and minor points as follow:

Major points:
1. Rats subjected to I/R were treated with AG extract (single dose, ip or po? time point? no clear, n=5), the statistical data showed very little deviation, it is unbelievable.
2. It is rational that the exudation experiment should be described after I/R observation.
3. Authors are unable to make a conclusion that “our result indicates that AG extract induces angiogenesis after ischemic damage in the brain by increasing VEGF and Tie-2 expression”, since there is no angiogenesis data support.
4. Though it is true that the up-regulation of VEGF expression after MCAO promote angiogenesis, however it increases the BBB permeability and aggravates ischemic brain edema at the same time (Mandriota et al. J Biol Chem. 1995, Zhang et al. J Cereb Blood Flow Metab. 2002). The author showed that the administration of AG significantly increased the expression of Ang-1, VEGF, and tight junction proteins ZO-1 and Occludin. How do the authors explain the influence of elevated VEGF?

Minor points:
1. (Method) there isn’t the description for Nissl staining.
2. 25.6±3.8%, 81.7%±1.2%, p < 0.05, p<0.05. The format should be consistent.
3. The relationship between the PI3K/Akt signaling pathway and VEGF and Ang-1/Tie-2, or Occludin and ZO-1 should be explained.
4. References format should be consistent.
5. Figure 1(B): The histogram of brain infarction volumes (n=5 per group). Values
are expressed as mean ± SD of three separate experiments. It is not clear, N=?.

6. Figure 2B: there should be statistical data of surviving neurons by Nissl staining. Only one sample?

7. Figure 4D: The authors state that they observed a "significant" increasing expression of Tie-2 by using 10 mg/kg of AG, and it is hard to understand when its mean value and deviation were quite close to the Vehicle group. Similar question between Sham and Vehicle group.

8. “The treatment of AG extract at doses of 50 and 100 mg/kg in MCAO rats significantly increased the expression of Ang-1, and Tie2 (Fig. 5A) and VEGF (Fig. 5B) compared with the vehicle group. In addition, AG extract significantly induced phosphorylation of Akt (Fig. 5A), and expression of PI3K (Fig. 5B).” This sentence is kind of confusing to me. There are error.

9. Evans blue exudation detection with Fluorescence method or spectrophotometry is not clear, it is inconsistent in ‘methods’ and in ‘caption’

10. Sentence “AG extract at doses of 10, 25, 50 and 100 mg/kg inhibited(?) infarction volume at 25.6±3.8%, 21.2±7.8%, 18.5±2.8%, and 14.2±4.1%, respectively” deliver incorrect fact

11. The writing should be thoroughly improved.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests