OPEN PEER REVIEW REPORT 2

Name of journal: Neural Regeneration Research
NRR-D-19-00556
Title: Improved prognosis of subarachnoid hemorrhage by L-Cysteine depends on H2S-attenuated neuro-inflammation, complement deposition, oxidative stress and endoplasmic reticulum stress
Reviewer's Name: Guilherme R. França
Reviewer's country: Brazil
Date sent for review: 2019-10-10

COMMENTS TO AUTHORS

In this work Lingxiao Wang and collaborators show a continuity of previous group work. The anti-inflammatory and neuroprotective effects of L-Cys were clearly demonstrated here. Since L-Cys is a well-known and low-cost molecule, this work ultimately suggests the possibility of a therapeutic application of L-Cys as a possible treatment for brain hemorrhagic lesions, aiming at reducing neurological damage.

Abstract:
Suggestions to diminish English misunderstandings in the abstract:
#page 15, line 16: "… mechanisms remain unearthed…", substitute the world unearthed by unknown or other word more adequate.
#page 15, line 24: "The results exhibited an expected…", please substitute the world exhibited by shown.
#page 15, line 31: "All of the four mechanisms investigated additively contributed to the EBI which were soothed by L-Cys treatment". This phrase is confusing. I suggest rewriting this sentence by "All of the four mechanisms investigated attentively contributed to decrease EBI after L-Cys treatment", or something similar.

Introduction:
I suggest replacing the last paragraph of the introduction (page 17, line 9: "Complement system, including various complement protein contributes…") in place of the second to last (page 16, line 48: "Since the polymorphism of CBS cannot be altered in a certain SAH patient,…").

Results:
Authors should write the respective drug concentrations in the legends, images or in the text body to facilitate data interpretation. This recommendation is for all results.

Figure 1
Figure 1 a) Observing brain images it is possible to see some hemorrhagic differences between SAH, L-Cys and L-Cys + AOAA groups. Why the authors decide not to describe these hemorrhagic patterns in the results? Besides, the hemorrhagic characteristics between SAH, L-Cys and L-Cys + AOAA groups do not affect the final inflammatory responses?
Figure 1b) The authors should point images with arrows the morphological alterations described in the text.

Figure 2:
The effect of L-Cys is reducing activation of microglia after SAH is clear and well defined. However, the sentence (relative to Figure 2a, page 21, lines 36 and 37) that describes microglial morphology is confusing. Above follow the sentence:

#line 36 and 37: "SAH insult significantly activated microglial cells with increased numbers ($p < 0.01$) and more ramified, amoeboid-like appearance…". Ramified microglia morphology is an indicative of resting microglial cells. On the other hand, amoeboid appearance is an indicative of activated microglial cells. I recommend authors check the sentence and describe the correctly microglial morphology. Besides, it would be interesting to add a magnified image, emphasizing morphological alteration on microglia.

Figure 5: Please, check on the representative image of the PCR bands 3a). Animals that submitted to SAH followed treatment with L-Cys + AOAA drugs are missing. Apparently just a typo.

The representative image of western blotting for Chop protein (Figure 5a) in SAH animals treated with L-Cys does not appear to represent the graph. There is no visual difference between bands. I strongly recommend choosing a more representative image.

Discussion:
I strongly recommend improve discussion about figure 6.

I suggest taking off this paragraph (page 27, lines 4-17)
However, we acknowledge it that some limitations exist in this study. Firstly, microglia activation was observed to be suppressed after L-Cys treatment but we didn't investigate whether there's any M1/M2 shift for the microglia subtype. Secondly, we didn't specify the certain cell type which responded most to L-Cys or H2S. Thirdly, the molecular basis underlying complement system activation following SAH is far from complete. Last but not least, the differently altered ATF6 pathway required further investigation.