

Reviewer's report

Title: A Randomized Trial of the Effects of the Noble Gases Helium and Argon on Neuroprotection in a Rodent Cardiac Arrest Model

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Reviewer: James Sleigh

Reviewer's report:

Synopsis

Zuercher et al. present a new study using their rat cardiac arrest (CA) model that aims to test whether the noble gases helium and argon are able to reduce CA-associated neurodegeneration. This work is similar in experimental design and analysis to their previous publication in BMC Anesthesiology (doi: 10.1186/1471-2253-15-2.) that assessed the effects of different anaesthesia regimes on neuroprotection in the CA model. In their latest work currently under review, the authors show that the noble gases tested provide no obvious clinical or histological benefits. Their results and conclusions are counter to previously published work from other groups, and possible reasons for these discrepancies are provided in the discussion.

Overall, the manuscript is relatively well written, the statistics are nicely performed and the study contains some provocative data given previous findings. However, I have a number suggestions detailed below, which I believe will improve the flow and understanding of the manuscript, and the interpretation of the data. The majority, if not all, of my concerns can be remedied by improved explanations and further discussion. The points are largely described in sequential order.

Abstract

1. In addition to the NDS, TRT, and OFT, the vertical pole test is also used to analyse recovery from CA - this could be included in the Methods and Results section of the abstract.

Background

2. I appreciate that references are provided later, but a citation or two (perhaps a review paper) to back up this first statement would be useful - "Although many substances affecting neuronal inflammation have neuroprotective properties in in-vitro and animal experiments..."
3. The second part of the final sentence in the first paragraph, should be amended to say, "the only neuroprotective measure known to have a profound effect on survival and functional outcome after cardiac arrest in humans is temperature management."
4. Typo: change lessens to lessen.
5. This whole section is a bit short and should be expanded to provide a better introduction to the field for the uninitiated reader - the neuronal pathology elicited by cardiac arrest should be elaborated upon; further information on the benefits of xenon and how it was first identified could be given in paragraph two; the results from references 21-24 could be expanded to highlight to the reader the past results showing positive effects of argon in rat and pig CA models.

Materials and Methods

6. It is stated throughout the manuscript that eight non-ischemic sham animals were included in the study, yet not incorporated in the randomisation process. In the Methods it is mentioned that this is for "internal laboratory purposes (quality of histology, natural history after anaesthesia only)." I think this reasoning should be expanded to at least a full sentence to increase understanding. It seems to me that it would have been very easy to include those extra eight rats in the randomisation process, and it seems odd to have excluded them. Nevertheless, I do appreciate that these animals were not used for the main statistical analyses, and that they are not central to the conclusions of the manuscript.

7. Please provide a reference for this sentence, "All animals underwent the same anaesthesia and instrumentation procedures, and, except the nonischemic sham animals, experienced cardiac arrest/resuscitation with a standardized protocol." I know that there is more information later, but a reference would help here.
8. Were any quality controls in place to confirm the presence of helium or argon in the oxygen mixtures? i.e. what assurances/positive control can the authors provide that helium and argon were indeed present in the respective treatment groups?
9. The sources of the equipment and reagents should be provided - this may help to clarify point 8.
10. Typo: surgically to surgical
11. How were the manual chest compressions delivered? By hand? How was the 220 bpm maintained if done by hand?
12. "sc" should be expanded.
13. Typo: wounds closured to wounds closed
14. Is there any evidence (a reference) that the sevofluorane anaesthesia and fentanyl would have altered apoptosis in the sham-treated animals?
15. Water and food consumption is likely to significantly affect recovery post CA; was it confirmed in any way (levels of remaining food/water) that the treatment groups were eating and drinking appropriately and at similar levels?

16. Methods are best described in order that the results are presented, e.g. Fluor-Jade B staining before cresyl violet.
17. A sentence elaboration on each test - NDS, VPT, and TRT- would be very useful here.

Results, Figures, and Tables

18. More details should be provided on the baseline characteristics at this point - e.g. what haemodynamic analyses were performed, what respiratory variables are being alluded to?
19. I think that the data in tables 1 and 2 should be presented and described before the histologic results, as the latter are presented in Table 3. Please rearrange order to reflect presentation sequence.
20. For clarity, I would add, ", as assessed by pyknotic cell counts." At the end of this sentence, "In the cresyl violet stained slides, no cells with signs of ischemic damage could be seen in the non-randomized nonischemic sham animals."
21. Typo: cresyl to cresyl
22. Rationale for looking at the CA1 region of the hippocampus should be provided either in the introduction or here in the results, rather than just in the discussion.
23. Typo: lactat to lactate.
24. Similar to the introduction, I feel that the results section can be expanded to aid the reader's understanding. For example, a single sentence covers the TRT, NDS and VPT analyses. This is nice and concise; nonetheless, the tables provided are very detailed, and

a better description of the analyses in the results, would really ease reader digestion of the data.

25. Including the words "degenerating neurons" in Figure 2 title would be useful.
26. In my opinion, the ordering of information within figures 2 and 3 is a bit clumsy, i.e. Fig. 2B and D are mentioned before A and C. I think a rearrangement would be useful so that the overview panels are provided before the zoomed in panels.
27. Figure 2: arrows pointing to regions of staining would be helpful (similar to Figure 3), i.e. in the CA1 and hilus regions.
28. Figure 2 typo: FJ to FJB?
29. I would make sure that there is uniformity in scale size and lettering in Figures 2 and 3.
30. To improve immediate understanding of a figure, I often think that having labels such as, "Sham" and "Cardiac Arrest" somewhere on the figure is useful. I appreciate that this is preference, so will leave this to the authors.
31. Table 1: MAP is not defined
32. I think emboldening or in some other way highlighting the linear regression P value would make table interpretation easier, i.e. by identifying the headline result of the table section.
33. It is stated in the methods, but a re-statement in table legends of what the Friedman and Wilcoxon tests are comparing would be useful.

34. My preference, again, but I really think that presenting the data included in tables 1-3 in graphical form would drastically improve visualisation and interpretation of the information for the majority of readers. This could perhaps be incorporated into supplementary figures.
35. FJB staining is observed in the CA1 region of the hippocampus and the cortex, while pyknotic cells are only alluded to in the hippocampus; did the authors look for these cells in the cortex also?

Discussion

36. Please reference the figures/tables within the text so that the reader can easily see to which data you are referring.
37. Can the authors speculate on discrepancy between FJB and cresyl violet staining results?
38. This sentence should be clarified: "The pigs used in the Ristagno's experiment had a combined no-flow and low-flow time of 817 seconds."
39. This sentence is clumsy and should be amended: "The duration of administration also differed significantly between our model and the above cited ones, we had a longer administration time (24 hours), compared to 1, respective 4 hours."
40. Reference to "her" air and argon groups should be amended to be more scientific.
41. Rationale for not looking at the CA3/4 regions, which were shown to be improved in a previous model, should be provided. If this was performed in details, allusion to this should be made in the results.

42. MCAO should be defined.

43. Please change CV to cresyl violet, or alternatively define and use the acronym throughout.

44. Beginning sentences with, "But" and "And" is a bit clumsy.

Are the methods appropriate and well described?

If not, please specify what is required in your comments to the authors.

No

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 am able to assess the statistics

Quality of written English

Please indicate the quality of language in the manuscript:

Acceptable

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