OPEN PEER REVIEW REPORT 2

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-21-00457
Title: Functional recovery and muscle atrophy in pre-clinical models of peripheral nerve transection and gap-grafting in mice: Effects of 4-aminopyridine
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COMMENTS TO AUTHORS

In this study, the authors examined whether 4-AP, a potassium channel blocker, could aid functional recovery after peripheral nerve transection injuries, with the rationale that this drug has previously been shown to aid recovery after a sciatic nerve crush injury. The authors studied the effects of this drug in three peripheral nerve injury models. Overall, the data are solid, but the results are not described in enough detail for a reader to fully appreciate the study and its outcomes. More care should be taken to define acronyms, state the rationale for the measurements and assessments made in each section of the results, and to make summary statements in each of the results sections. Some of the important information to understand the study are buried in the methods section and should be brought up to the front of the results section. The methods section should be reserved for technical information, and not rationale and experimental set up statements.

Specific comments on Results section:

(1) The authors should provide more context at the start of the results section. The results section starts abruptly and assumes that the reader has gone through the methods section in detail (which is not always the case). The authors should add one sentence to the start of the results section to set the stage for the study. For example:

Here we set out to ask whether 4-AP can improve functional recovery in three peripheral nerve injury models:(1) stepwise nerve transection with gluing (STG); (2) 7 mm irreparable nerve gap (G-7/0), and (3) 7 mm isografting in 5 mm gap (G-5/7) (Figure 1A-C).

The following information from the Methods should then be moved to the Results section so that readers of the article do not have to go through the methods (unless they are interested in experimental details) to understand the story.

"As described recently for Stepwise Transection and Glue (STG) method (Lee et al., 2020) and shown in Figure 1A, the nerve was first transected to 80% of its width to prevent gap formation between the cut ends, then 10 µl of fibrin glue was applied around the transection site and the nerve was completely transected before complete clotting of fibrin glue. This method was highly effective to minimize and standardize the gap formation to 1-2 mm between the cut nerve ends. In graded nerve gap and grafting model (Figures 1B-C), pairs of mice were used in series for cascade syngeneic nerve grafting in 1 set of experiments. In mouse # 1, a large gap was created by dissecting out 7 mm (G-7/0) of right sciatic nerve section. The proximal stump of the sciatic nerve was buried underneath the muscle with 10 µl of fibrin glue. Then, in mouse # 2, a medium gap was created by dissecting out 5 mm of right sciatic nerve section and the 7 mm dissected nerve section from mouse # 1 was grafted and kept in good alignment with fibrin glue at both ends (G-5/7)."

(2) It would be easier to follow if the next part of the results was to define outcomes for these models with the recovery measures, as stated : "Figure 2A shows that post-injury SFI recovery in the saline STG model was significantly better than G-7/0 and it was ~40-50% of the baseline"

However, the authors need to define the acronym and state how they measured SFI recovery.

They also do not conclude on the G-5/7 model. It looks similar to the STG data - is that the case?

(3) After defining the models, then the authors can comment on the effect of 4-AP. As stated "We had 10 mice in each injury model, only one mouse in G-7/0 4-AP group died after 8 weeks of surgery. In STG model, 2 mice in both saline and 4-AP groups failed to produce measurable footprints after 4 weeks of surgery. In G-5/7 model, >40-50% of mice in saline and 4-AP groups failed to walk properly after 5 and 9 weeks of surgery, respectively. However, all mice in G-7/0 model were able to walk until the end of experimental protocol."

The authors have packed a lot of information in this short paragraph, and they need to break it down more with a final conclusion (and refer to the figure).

The authors are talking about footprints out of the blue. Are the footprints part of the SFI measurement? If so, does this mean that the animals that could not walk were not included in the data analysis in Figure 2A-D? SFI needs to be defined and rationale given.

Most importantly, the authors make no conclusion about the 4-AP treatment. It looks like it was not beneficial in any situation. If so, they need to make that point.

Finally, if 4-AP is not effective, how do they know the drug was working at all? There is no positive control. I know that the authors have previously published a crush injury model and shown that 4-AP was effective in that scenario, but having that as a control group here would have been helpful.

(4) In the section describing Figures 3-5, the authors should focus on the question and not on the figure number.

For example, they state "Figure 3 shows representative whole mount immunofluorescence images of left (uninjured) and injured right sciatic nerves in saline and 4-AP groups stained with NF-H, CD31, MP0, and DAPI 12 weeks after STG."

What is the question? Is it : To examine the histology of the nerve 12 weeks after injury, we performed immunostaining with NF-H, to label peripheral nerve axons, CD31, to label blood vessels, MP0 to label myelin, and DAPI to label nuclei.

The authors should then make specific statements about the data in each figure. For example " We first compared the STG model treated with saline or 4-AP. We found xxx…

They should then make a conclusion that relates to whether 4-AP had a noticeable effect or not..

They should then move onto, " We next examined the G-5/7 model….(Figure 4)

My question here is also what about the G-7/0 model? If there is a rationale for not adding this data, please give it.

(5) The authors start the next section with "Absolute values for muscle mass (mg), CSA (μm2) and MFD (μm) 12 weeks after nerve transection and gap-grafting are shown in Table 1. Figure 6 shows quantitative and normalized RTA muscle mass, CSA and MFD (all nerve injuries were on the right side). "

What is CSA? What is MFD? What is RTA? These acronyms should all be defined and the rationale for each functional test given.

The data in Table 1 should be graphed instead of in table form to make it easier to compare groups. Or is this the data in Figure 6? It is confusing.

For the bar graphs in Figure 6, all data points should be shown to know how many animals per group were analysed.

A conclusion should be made on each measure - muscle mass, CSA and MFD - which measures were significantly improved by 4-AP in the G-7/0 group and what does that mean about rescue of muscle function?

(6) The authors state: "Further analysis of muscle fiber size distribution in G-7/0 model (Figure 7) revealed that while permanent nerve gap increased the propensity of smaller muscle fibers in the injured hindlimb muscle of both saline (Figure 7A) and 4-AP (Figure 7B) groups, 4-AP treatment was able to preserve most muscle fibers within 35-50 μm ranges (Figure 7C) compared with the saline group at 25-40 μm ranges."

This section does not explain fully figure 7. In figure 7, the authors have graphed LTA and RTA but do not define in the results what they are.

In Figure 7A, it seems they are compared injured and uninjured sides, and so I presume this graph just shows that there was sustained injury? How long post-injury were these measurements made?

In Figure 7B, it seems that the difference in fiber frequency between left and right is gone after treatment of 4-AP, so I presume this means that fiber frequency back to normal levels.

In Figure 7C, fiber frequency comparing saline and 4-AP to show improvement.

Some sort of conclusion for each graph should be given and all of the data should be fully explained.