Reviewer's report

Title: Fasudil improves survival and promotes skeletal muscle development in a mouse model of spinal muscular atrophy

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Reviewer: Umrao Monani

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Spinal muscular atrophy is a common neuromuscular disorder for which there is currently no effective treatment. In the article by Bowerman and colleagues, the authors extend a previous finding in which they showed that Smn depletion perturbed the ROCK pathway and increased RhoA GTP levels. RhoA GTP is an important regulator of the actin cytoskeleton. Here the authors provide additional evidence of the importance of the ROCK pathway in SMA biology by using an FDA-approved inhibitor of the pathway, Fasudil, to partially rescue the SMA phenotype in a mouse model of the human disease. Fasudil significantly enhanced survival without affecting other phenotypic characteristics such as body size and motor performance. At the cellular level, pre-synaptic pathology persisted whereas the post-synapse exhibited marked improvement. The improvement in post-synaptic morphology correlated with enhanced muscle fiber size and restoration of normal myogenin regulation, suggesting a primary effect of Fasudil on the muscle rather than the motor neurons. Consistent with previous studies by the authors involving the use of ROCK inhibitors, Fasudil did not alter Smn levels, highlighting the feasibility of therapeutic strategies that do not increase Smn protein. The article by Bowerman adds to the importance of the ROCK pathway in SMA pathogenesis and serves as further rationale for contemplating modulators of the pathway in the treatment of the human disease. The study is well-conceived, thorough and, apart from the minor concerns indicated below, merits publication in the journal. My specific criticisms are described below:

• On page 4 the authors reference the differences between the two SMN genes citing an article by Lorson et al, 1999. It would be appropriate to also reference an article from the Burghes group (Monani et al, 1999) that documented precisely how many differences there are between the two genes. It would also be appropriate to cite the article by Coovert et al, 1997 later in the paragraph when referencing SMN2 copy number and disease severity.

• Figure 1 - The legend suggests 5 cohorts of mice whereas only three curves are apparent on the graph. It appears that the remaining two curves do not show up on the graph simply because they are masked by the Smn2B/+ (vehicle) curve. If so, it would be useful to indicate this in the legend.

• Figure 1 - It is indicated that a treated animal perished because of dystocia. Does this mean that treated Smn2B/- mice are able to breed despite their severely compromised body size?
• The authors reference an article by Boyer in the text but missed including it in the bibliography section.

• Figure 3 - The myofibres of treated animals are more than twice as large as those of untreated animals. Not surprisingly, the corresponding endplates are also larger. Considering that muscle mass constitutes the bulk of an animal's weight, it is puzzling that the treated animals did not differ in size or weight from their untreated littermates. Can the authors comment?

• On page 17, Bowerman and colleagues state that, “…given sufficient time, Fasudil administration allows for the improved development of NMJs in Smn2B/- mice.” What might be the mechanism underlying this improved development? Could the authors comment or speculate?

• On page 30, bibliographic information for reference #21 is not complete.

Quality of written English: Acceptable

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

Declaration of competing interests:

No competing interests