Author's response to reviews

Title: Selected statins produce rapid motor neuron loss in vitro

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Author's response to reviews: see over
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Dr Homa Tajsharghi, Editor
BMC Musculoskeletal Disorders

Dear Dr Tajsharghi,

The authors are indebted to the referees for their careful review of this manuscript and their appreciation for this work. The comments of the referees have been addressed in detail as described on the pages that follow this one. The authors gratefully acknowledge that the manuscript has been substantially improved by the process of peer-review.

The manuscript has been revised to conform to the formatting requirements as specified in your office’s correspondence and website. We have cropped the images to remove excess white space.

It is hoped that the manuscript will be acceptable for publication in your journal and again we thank the reviewers and the editorial staff for their helpful feedback and kind assistance.

Please find a point-by-point response to the referees’ comments below.

Kind regards,

Beth Murinson
Referee 1: Minor essential revisions:

(1) simvastatin is used clinically as an inactive prodrug lactone which is converted in vivo to active acid form. It is essential to state in which form (lactone or open acid) simvastatin was used in this study.

Simvastatin lactone was used in these studies. This is now specified in the manuscript.

(2) Fig. 1C: concentrations of statins should be in micromoles rather than milimoles

The concentrations of statins used are now specified in micromoles.

Discretionary revisions:

(1) Discussion section seems too short. For example, authors mention the possible role of LXR but this issue should be explained in more detail because in the current form is unclear for a non-specialist.

The discussion has been expanded to address the concerns of the referee. A more extensive discussion of potential mechanisms is provided with additional references to contextualize the impact of statins in ALS in counterpoise with the ongoing controversy about statins in peripheral neuropathy. A copy of the manuscript with track changes in place is provided so that the revisions may be readily identified.

(2) What mechanism(s) authors propose to explain specific toxicity of statins to motorneurons vs other neurons or glial cells?

The mechanisms of statin toxicity for motoneurons is a topic of great interest. We have expanded the discussion to address this point. Clearly further experiments are necessary to elucidate the potential mechanisms.

(3) It would be interesting to test if and which mevalonate derivatives prevent the toxic effect of statins on motor neurons. Such experiments could explain or at least suggest the mechanism of toxicity.

In experiments which were not replicated, we did observe that mevalonate was able to partially reverse the effects of the statins. However, because these experiments were not repeated and the protocol remained un-optimized, we do not report these results here.

Referee 2:

Minor Essential Revisions:

1 - study demonstrated statin effect in 'spinal' motor neurons. What about effect in 'cortical' motor neurons? Were cortical 'motor' neurons included in paradigm testing cortical neurons that
demonstrated no effect of statins? Authors need to clarify and if not, must restrict their conclusion to 'spinal' motor neurons only and make this distinction clear in manuscript.

The cortical neurons were derived from E18 rats for which no particular effort was made to separate out the motor cortex. Thus we have revised the manuscript to more specifically address spinal motoneurons.

2 - The rat model used in study was not an ALS animal model. While this is a good place to start, it would be of interest to determine effect of statins on rate of motor neuron degeneration in an ALS animal model. ALS experts generally do not think statins cause ALS, the controversy revolves around the effect of statins on disease progression. It would be most informative to determine the effect of statins on damaged ALS motor neuron. The authors should mention this distinction and I encourage them to perform this study in the future on an ALS mice/rats if not already planned.

These studies were intended as a 'proof of principle' studies to determine if there were any direct effects of statins on cultured spinal motoneurons. It is likely that the cultured spinal motoneuron are vulnerable to insults that are not problematic for motoneurons in vivo. While we do not consider the system to be a 'model of ALS', we do consider that it is a useful system for better understanding the neurobiology of spinal motor neurons. We do plan to investigate the effects of these treatments in an ALS model once funding is secured. We have revised the discussion to indicate that other experiments are needed to elucidate ALS-specific mechanisms.

3- typo pg 3 para 2 - change "except for" to "except for"

Done.

4- page 10 para 1 - last sentence - inference to fluvostatin difficult to understand here. best to remove this sentence or clarify.

This sentence has been revised to better convey the intended meaning. It serves as a transition to the subsequent paragraph which reviews the pharmacokinetics of fluvastatin in s