Reviewer's report

Title: Proteasome Inhibition Alleviates Prolonged Moderate Compression-induced Muscle Pathology

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Reviewer: Anders Nedergaard

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Siu and coworkers
The concept of deep pressure ulcer was a new one to me prior to this manuscript, but having read quite a lot of the sparse literature on the topic i must fully agree that this is indeed a topic of clinical relevance and one that has hitherto been underappreciated in the scientific community. The model used appears to be a solid and relevant model for the human condition and thus very useful to the community, but the manuscript has got some language issues, and

Major compulsory revisions

1 - The article as such, but especially the background section is quite flawed in terms of language and grammar. Almost all grammatical errors are related to faulty use of plural forms and definite/indefinite articles.

2 - the background section presents a somewhat simplistic view on the function of the ubiquitin proteasome system (UPS) in muscle. The UPS is important for a score of conserved cellular processes, including regulating cell cycle, protein quality control as well as specific regulation of specific signaling pathways by modifying singling through ubiquitination itself or degradation of components in signaling cascades. Merely describing it as a degrader of bulk protein in muscle provides a very incomplete picture of the relevant biology.

The reason i make this comment is that it is unlikely that deep pressure ulcers are initiated by increased proteolysis. A number of upstream processes, of which the most important are likely hypoxia and hypoperfusion, most likely create a cascade of events that at very bottom results in net degradation of muscle protein. This leads to the next comment

3 - given that the pressure stimulus creates a cascade of events, and that it is unlikely that UPS mediated proteolysis is at the top of this chain of events, I'd like to see some kind of reflection on the effects of the used proteasome inhibitor. In the discussion section of the article it is presented as if the effects of MG132 is solely on the net proteolysis of bulk muscle protein. MG132 has been shown to inhibit NFkappAB activation and induce HSP's, of which the latter increases protein stability and possibly even protects against DNA damage. This could very well be related to the prevention of pressure induced appearance of DNA strand breaks.

4 - in the light of the previous comments I would like to see some supporting data
if tissue form the experiment is still available. Specifically, I would to see data of expression of some markers of hypoxia (especially HIF's, but more can be found here http://www.antibodybeyond.com/reviews/tumor-markers/hypoxia-marker.htm ), to see hat level MG132 affects the pressure induced damage at.

Also, i would like to see data of HSP expression at the protein or mRNA levels, as the MG132-induced expression increases of HSP's could explain some of the protection conferred by MG132 treatment.

5 - also, if tissue is still available, I would like to see western blots corresponding to the immunostainings performed

Minor essential revisions

1 - the use of "ubiquitin proteasome" various places in the manuscript should be replaced by (the)"ubiquitin proteasome system" (and/or a fitting abbreviation). Using the form suggested in the manuscript is grammatically questionable and impairs readability

2 - PCR primer sequences should be supplied

3 - was amplification effeciency for the PCR reactions assessed?

4 - line 197-204. Considering that the data were negative and not shown, these lines could be rephrased and cut down. E.g. "no signficant differences were observed with pressure treatment or MG132 administration. We did however observe a net increase of MAFbx/Atrogin-1 expression with the pressure treatment alone, but this did not reach significance due to high variability in the response."

Discretionary revisions

1 - line 41 - destruction should be degradation

2 - line 47 - cdc53 is normally known as cullin. perhaps this should be added in parantheses?

3 - line 59 after "revealing the" should have inserted "etiology of"

4 - line 82 - how frequntly was the maintenance doses of anesthetics given?

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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

Declaration of competing interests:

I declare that I have no competing interests