Reviewer's report

Title: Characterization of GLPG0492, a selective androgen receptor modulator, in a mouse model of hind limb immobilization

Version: 1 Date: 21 May 2014

Reviewer: Paula Tavares

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Major Compulsory Revisions

General evaluation

My overall opinion is that there are too much information (without correlation between them) that authors can use separately. Thus, there is a lack of correlation between the two sets of experimental work. There are also a poor explanation of the work aims, as well as a very confusing explanation of the obtained results.

Some of the inconsistent of the results is mainly due to a misinterpretation of the data or a poor choice of methodology.

The results are confusing since the information on the text is, in several cases, in disagreement with graphics and figures. Moreover, some figures had the statistical significances at the wrong column.

Moreover, the conclusion is not in agreement with the obtained results.

My advice is that authors can revise the article according to the suggestions pointed below.

Abstract:

The paper abstract is ambiguous namely in the aims of the work and the conclusions are not in agreement with the obtained results (more details in the observations about the conclusions).

Background

The state of the art is reasonable accept but fail in the clarifications of the aims. The last sentence of the second paragraph is in disagreement with the conclusions of the authors.

Methodology:

Muscle fibres that are homogeneous for a myosin heavy chain isoform may be heterogeneous in regard to myosin light chain isoforms, although, in general, fast myosin heavy chain isoforms associated with fast light chain and the same for slow isoforms. Nevertheless, the atrophy studies need more accuracy in fibres type analysis and a histochemical staining of myosin ATPase should be considered. Moreover, in the background section (first paragraph, line 16) the authors referred the myosin heavy chain as been involved “...in the degradation of both regulatory and structural protein...”. However, the authors used the
analysis of myosin light chain in their methodology, as previously report.

It is difficult to understand the use of two different muscles to perform different experiments and correlate the obtained results in order to obtain a work conclusion. The authors used the gastrocnemius muscle for fiber type and atrophy analysis and tibialis anterior (?) for gene and metabolic studies. In my opinion these are a very dangerous correlations since tibialis anterior is a highly glycolitic muscle and gastrocnemius, a mixed muscle (containing both glycolitic and oxidative fibres (in Balb/cj mice). Due to the characteristics of this strain there are also a great variability in marked variation of fiber size, hypertrophic fibers, fiber splitting, fat replacement, perivascular inflammation, endomysial fibrosis and inflammation. These facts may question the working design of the study.

There’s some question that I like to be answer by authors. For instance for the sacrifice of the animals what method was used? How it was made the blood collection (the methodology may interfere with the obtained results)? How it was established the dose of GLP0492 for animals administration?

For the determination of FCSA how many fibers were count and how the normalizations of the results was made?

Results:

In this section there are serous mistakes that I strongly advise authors to correct. Authors should maintain the same designation between legends and tables/figures: ex: casted (in the legend) and immobilized (in the table -3) or 0 mg/Kg GLPG0492 and vehicle.

In figure 1 the significance symbols are at the wrong position (### in vehicle).

Authors referred that there is no changes in prostate weight in contrast to TP (last sentence of the first paragraph of Results & Discussion). However, in the figure 1C there is a significant reduction in prostate weight with GLPG0492. How authors explain this discrepancy? There’s also another discrepancy in figure 2B concerning the FCSA. The significances in the figure or the text are wrong. Which results are the real results?

Continuing in figure 2C there is missing information in the graphics. What is the meaning of the numbers in the X-axis? Once more the text and the figure 2B and 2C are not in agreement. In figures 3 and 4 the vehicle or 0 concentration of the drug is replaced by CTL. The authors should maintain the same nomenclature overall the figures, tables and text.

In the 5th paragraph, line 9-11 the results are not in agreement with the body of the text. Once more the significance symbols are placed wrong or the results are incorrect.

When the authors denote an increase in ketone bodies they strongly suggested that those increase is due to fatty acid oxidation. These imply a question: did the authors analyse the liver and the body composition of the animals? What about the body mass of the several groups of the animals? The treated group lose weight?
Another question is that the presented work as no sufficient data to take conclusions about oxidative stress (6th paragraph, line 2).

I consider important to show the data about IGF-1 as well as the data concerning the lack of LC3 gene regulation after immobilization.

I also think that is very important to include a discussion that relates MurF1 with the protein analysis indicated in the table 3.

In table 2 the legend is incomplete (as well as the others) and should be maintained the same presentation of table 1. It may be include in the legend “…the results of GLPG0492 (ng/ml) are expressed as mean ± SEM.” And present results as 8.62 ± 2.33.

In table 3, I think that is was important to compare the presented results also with the results from the animals treated with TP (testosterone propionate).

Conclusions:
In this paper author extrapolate the results obtained from an immobilization model (atrophy by disuse) to sarcopenia and cachexia. Spite the fact that this muscle conditions have linking points, different cellular mechanisms are associated to these pathologies or medical conditions. Moreover, sarcopenia has no defined models or specific markers, until now. In addition, the type of rats used have a genetic profile that is more related to dystrophies what is complete different from sarcopenia and cachexia. It is also important to consider that in each of these conditions the changes in fibers type and size are different.

Thus, it seems difficult to take those conclusions with the data provide in the present paper.

Minor Essential Revisions
The designation of muscle fibers instead of myofibers is preferable in order to avoid confusion with myofibril.

Some references are at the wrong place at the text.

**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