Reviewer’s report

Title: Cellular pharmacodynamic effects of Pycnogenol® in patients with severe osteoarthritis: a randomized controlled pilot study

Version: 0 Date: 27 Mar 2017

Reviewer: Stefan Toegel

Reviewer’s report:

This study investigates the effect of a nutritional extract (Pycnogenol) on molecular marker levels in articular chondrocytes, synovial fluid and serum obtained from arthroplasty patients. The authors argue that this setting allows studying the activity of a dietary supplement (including its bioactive metabolites!) in an in vivo setting that also takes bioavailability into account. This is a good point and makes the manuscript interesting.

However, there are a number of limitations and weaknesses of the manuscript which should be addressed before publication can be considered.

*) OA patients - who received neither Pycnogenol nor placebo - served as control. Although the authors indicate this limitation in their work, the scientific value is compromised by this limitation.

*) The impact of the study is largely limited by the fact that only one statistically significant result (that is relevant for the study) is presented (Figure 3). Due to the lack of other significant results, large parts of the discussion are rather speculative.

*) line 46: "…investigated in serum…”

*) Official gene symbols should be used throughout the manuscript to indicate mRNA levels and avoid confusion with protein levels (e.g. IL1B instead of IL-1ß which would indicate the protein).

*) The conclusion of the abstract should be revised to be more specific and related to the study.

*) The manuscript should unambiguously state why endstage OA patients were used in this study. Is there any benefit expected from clinical application of the extract in endstage patients?

*) The first phrase of the introduction (line 61) should be revised to improve wording.

*) The statement in line 82 ("Subsequent investigations….") should indicate the biological background of the previous studies. Which cells, which disease context…?

*) Methods: Regarding synovial fluid, please provide details on how samples were collected and processed (centrifugation, pre-treatment,…)
*) line 157 ("All samples were shock-frozen..."). This statement is misleading as it obviously does not include "knee fragments" (preceding sentence).

*) Methods, line 174 ("Residual articular cartilage..."). Which anatomical tissue regions were selected for cell isolation (loaded/unloaded, un/affected)?

*) The authors cite the MIQE guidelines. Did they check for RNA integrity of the preparations using qualified methods as outlined in these guidelines?

*) The citation and general adherence to the MIQE guidelines is appreciated. However, there is one issue regarding the use of reference genes. The authors state that SDHA was found to be unstable under the experimental conditions. So why was it still used for normalization to produce the data shown in figures, but then omitted to allow re-calculation which sometimes even improved significance? If omission of SDHA is justified, then the presentation of the data (that were generated using the 2 remaining reference genes) would be more straightforward and meaningful.

*) Line 275ff: Please indicate in which group the "tendency towards down-regulation" was observed.

*) The result "Correlation analysis of biomarkers" (line 330ff) is rather off-topic and not related to the study aims. It should be shifted to the supplementary data.

*) The relation of the results presented in the final paragraph of the results section (line 337ff) to the study is unclear. It appears that these data are obtained from a different study submitted elsewhere (line 338f). Please report full data and the respective methods or delete the entire section if reported elsewhere.

*) Discussion (line 349). In fact, this study does not investigate the "molecular effects of the …extract…on … markers". Please revise wording.

*) The legend to Figure 2 should indicate what the data are referred to. What is "1"? Levels in control groups should be given in the figure.

*) Figure 3: the legend should provide more details on data processing. The figure should indicate the statistical difference.

*) Figure 3 and 4 can be combined and presented as 2 panels of the same figure.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?  
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?  
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English  
Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests  
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests
I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal