Reviewer’s report

Title: Calcitonin Substitution in Calcitonin Deficiency reduces particle-induced Osteolysis

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Reviewer: Christophe Nich

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Reviewer’s comments – BMC Musculoskeletal Disorders- MS 1941545385526339 -

Title: Calcitonin Substitution in Calcitonin Deficiency Reduces Particle-Induced Osteolysis"

Since my first review of the manuscript, an important effort has been made to reorganize it and to clarify the message. Authors provided appropriate modifications to focus on the objectives of the work. It might be suitable for publication in the BMC Musculoskeletal Disorders journal, provided authors address following issues.

Major comments

The authors did not employ a control group in the calca -/- group. Specifically, it would have been helpful to supply calca deficient mice with a vehicle or placebo only. Hence, it is well known that such an injection might consistently influence tissue reaction to an inflammatory stimulus in mice. This limitation should be thoroughly discussed in the appropriate section.

Minor comments

1. Introduction, line 9: Delete « in-vivo » in the sentence « ….in in-vivo particle-induced osteolysis ».

2. Introduction, line 23: please, do not begin a sentence with an abbreviation. Replace “CT” by “Calcitonin”.

3. Introduction, lines 24-25: The primary reason why CT is considered a valuable therapeutic agent in Paget’s disease is its ability to slow down bone resorption by inhibiting osteoclasts activity. Hypocalcemia should not be viewed as a desirable therapeutic effect in Paget’s disease or in osteoporosis. Please re-phrase this all sentence or delete it.

4. Introduction, page 4, lines 2-3: “In this manuscript we are following our previous study on alpha-CGRP deficiency to analyze the influence of CT on particle-induced osteolysis”. Provide reference here.

5. Introduction, page 4, lines 8-9: “The increase of bone formation accompanied
by an increase in bone resorption raises the question as to how this would react on UHMWPE particles”. I am not sure the formulation of this sentence is correct. I would say”…. raises the question of the consequences on particle-induced osteolysis.”

6. Introduction, page 4, line 9: Replace “We believed…” by “We hypothesized….”

7. Introduction, page 4, line 9: Replace “…..that Calca deficiency might show greater osteolysis compared to the WT.” by “ …. that Calca deficient mice might…..”

8. Introduction, page 4, lines 13-14: The sentence “It is still of great interest to gain a better understanding of the effect of CT and its interaction with alpha-CGRP in particle-induced osteolysis.” does not bring much information. Delete it.

9. Introduction, page 4, lines 15-16: “This in-vivo study investigates the impact of CT deficiency and CT substitution in a murine particle-induced osteolysis model in Calca -/- mice.” Obviously, this study is in vivo, so you do not need to mention it. Similarly, you do not need to mention that the murine model was employed, as you say the experimental procedures were applied to (calca-/-) mice. Please rewrite and simplify this sentence.

10. Methods, page 5, line 7: There are 6 experimental groups (not “5”). Table 1 does not demonstrate groups, but rather “presents” or “shows” them. Employ appropriate terms in the sentence. Table 1 needs to be presented with a legend. Include number of animals per group (n=?) and also the sum at the end of each row and column.

11. Methods, page 5: the paragraph “Particles” should come before the paragraph “Surgical procedure” in text.

12. Results, page 6, lines 6-8: “Significantly higher serum levels of OCN (preoperative p<0.01; postoperative p<0.001), DPD (p<0.01; p<0.01), OPG (p=0.0000002; p<0.01), and phosphate (p<0.001; p=0.08) were found in the sham groups compared to the wild-type (Figure 1).” I don’t see in the context what are the « sham groups », since, for instance, group 1 is both « sham » and « WT » (cf Table 1). Please use the same nomenclature along the manuscript when you refer to the experimental groups. The post op serum level of phosphate is not significantly different between groups (p=0.08), therefore the sentence is not correctly formulated.

13. Results, page 6, lines 11-12: “DPD/creatinine levels revealed significant differences in osteolysis between Groups 1 and 2 (p<0.05), though…” the term should be accurately defined in the Methods section.

14. Results, page 6, lines 16: “….but serum parameters did not correlate with the extent of osteolysis in any of the groups.” How was defined the “extent of osteolysis”?
15. Results, page 8, “Bone Histomorphometry”: the sentence “Formation of fibrous and granulomatous scar tissue surrounding the osteolytic lesions around the midline suture was found analog to previous publications [5, 22].” does not refer to a morphometric parameter, so this description does not belong to this paragraph.

16. Results, page 9, lines 1-2: “Calca -/- mice with and without CT substitution had a greater quantity of fibrous tissue compared to the wild-type.” What group do the authors refer to? How was the fibrous tissue quantified?

17. Results, page 9, lines 7-8: Bone resorption in Group 5 does not seem to be significantly different from Group 6 (p value?). So, why do the authors state that “Bone resorption in the groups with UHMWPE particles was more pronounced compared to that in the corresponding control groups.” (lines 2-4)?

18. Results, page 9, lines 9-11: “The eroded surface was significantly reduced by 20.6 % after CT substitution of Calca deficient mice with particle implantation (p<0.05).” what experimental groups do the authors compare? Describe with more accuracy your results.

19. Results, page 9: the results reported in the paragraph “Bone Thickness” are histomorphometric parameters, and should be presented in the corresponding paragraph (“Bone histomorphometry”).

20. Results, page 9, lines 18-19: “Particles in the calcitonin substituted Groups 5 and 6 did not lead to a significant change of bone thickness (201µm ± 6µm vs. 197µm ± 8µm)”. Although the difference is not significant, authors should indicate the p value.

21. Results, page 9, lines 25-25: “…significant, prompted us to performed comparisons of groups as before”. Data have to be described with more accuracy: what kind of test did authors employ following ANOVA?

22. Results, page 10, line 1: “9.65 ± 5.26 (range 0 – 22) compared to 16.17 ± 8.26 (range 6 – 43) in Group 2”. Indicate the p value.

23. Discussion, page 10: “Particle-induced osteolysis has been extensively studied in the past…” Avoid vague terms. Rewrite and replace with explicit study variables from your citations, or delete.

24. Conclusion: in my opinion, the statement that “CT can reduce particle-induced osteolysis,.............” is partially true, since it is only observed only in calca -/- mice. Once again, conclusions must be drawn on data obtained in the Results section.

25. References 5, 7, 20, 38: citations should include all named authors, up to the first 30 authors. Make correction.

26. Figure 1 Legend: not correct. The term “Differences” does not belong to a figure title but rather to its comment. Re phrase it.
27. Figures 2, 4, Legends: Replace “….via histomorphometry” by “….by histomorphometry”.

28. Figure 2 does not appear on my copy!

**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