Author's response to reviews

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

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Author's response to reviews: see over
Submission of "Calcitonin Substitution in Calcitonin Deficiency Reduces Particle-Induced Osteolysis" – MS 1941545385526339

Dear Professor Patel,

Thank you very much for the review of our manuscript entitled "Calcitonin Substitution in Calcitonin Deficiency Reduces Particle-Induced Osteolysis" (MS 1941545385526339).

Enclosed please find the reviewers’ comments and the answers to their questions. The manuscript was revised by a native speaker. This original work has not been previously published and is not under consideration for publication elsewhere. All authors have seen and agree with the content of this manuscript. All authors have no conflicts of interest. The corresponding author takes full responsibility for the submission. We hope that you will find this paper suitable for publication in BMC Musculoskeletal Disorders.

Sincerely,
Dr. med. Max Daniel Kauther
Reviewer 1: Christophe Nich
Reviewer's report:

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.

This comment is thoroughly discussed in the Discussion. The daily injection of calcitonin in Groups 5 and 6 might have had an influence on local tissue reaction or systemic inflammation. As we did not employ a control group the reaction of a vehicle or placebo is unknown. Nevertheless, calcitonin led to decreased osteolysis, whereas a local or systemic inflammation due to injections would possibly have lead to increased osteolysis. It is known that endotoxins lead to increased osteolysis in the calvarial model [39].

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

   “in-vivo” has been deleted.

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

   This has been corrected.
“CT” has been replaced 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 sentence or delete it.

The sentence has been changed. “Salmon CT is preferred as a therapeutic agent against Paget´s disease and osteoporosis because of its ability to slow down bone resorption by inhibiting osteoclasts activity, even though calcitonin is not widely used to treat osteoporosis [15-17].”

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.

Reference [5] has been included.

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.”

The sentence was changed. “The increase in bone formation accompanied by an increase in bone resorption raises the question of the consequences on UHMWPE particles.”

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

“We believed” has been replaced 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…..”

“…..that Calca deficiency might show greater osteolysis compared to the WT.” has been replaced 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.

The sentence has been deleted.

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.

The sentence has been re-phrased. “This study investigates the impact of CT deficiency and CT substitution on particle-induced osteolysis.”

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.

The sentence and Table 1 have been changed. “Table 1 shows the 6 different groups.” The Table caption has been changed. “Table 1: Study design of the 6 different groups. Each group consisted of 5 mice.”

As every group consisted of 5 animals we did not include the number of animals and the sum of the 6 different groups, as this does not provide any additional information for the reader.
11. Methods, page 5: the paragraph “Particles” should come before the paragraph “Surgical procedure” in text.

The order of the paragraphs has been changed.

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.

The sentence has been rewritten. “Preoperatively, significantly higher levels of OCN (p<0.01), DPD/creatinine (p<0.01), OPG (p=0.0000002), and phosphate (p<0.001) were found in the Calca -/- mice compared to the wild-type (Figure 1).”

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.

The term DPD/creatinine is now defined in the Methods section (page 6, line 19). “DPD values were normalized to the urinary creatinine concentration and expressed as nmol DPD / mmol creatinine.”

14. Results, page 6, lines 16: “....but serum parameters did not correlate with the extent of osteolysis in any of the groups.” How was the “extent of osteolysis” defined?

The sentence has been rewritten. “Analysis of the postoperative serum parameters demonstrated significant inter-group differences, but the change in
the serum and urine parameters could not be correlated with the change in BV/TV or the eroded surface."

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.

The sentence has been deleted.

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?

The sentence has been deleted.

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)?

The sentence has been deleted.

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.

The sentence has been changed: “The eroded surface was significantly reduced by 20.6 % after CT substitution of Calca deficient mice with particle implantation (Group 4 vs. Group 6) (p<0.05). “
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").

"Bone Thickness" is now presented in the "Bone Histomorphometry" paragraph.

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

The p-value has been added. "Particles in the calcitonin substituted Groups 5 and 6 did not lead to a significant change in bone thickness (201µm ± 6µm vs. 197µm ± 8µm; (p=0.39))."

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

The t-test was employed. "...though not significant, prompted us to perform t-test comparisons of the groups as before."

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.

"The number of osteoclasts per bone perimeter in Group 1 was found to be 9.65 ± 5.26 (range 0 – 22) compared to 16.17 ± 8.26 (range 6 – 43) in Group 2 (p=0.17)."

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.

The sentence has been deleted.
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.

The Conclusion has been partly rewritten. The unexpected finding of increased osteolysis in the Calca -/- mice and the expected reduction in osteolysis in Calca -/- mice due to CT substitution suggest that CT has an osteoprotective impact on particle-induced osteolysis. Calcitonin appears to have a stronger effect on osteoclasts than on osteoblasts in particle-induced osteolysis in Calca -/- mice. These findings might be helpful for the prevention of aseptic loosening and could offer further treatment options.

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.

Figure 1 Legend has been rephrased. Figure 1: Preoperative serum and urine levels of WT and *Calca* -/- mice. Differences were found in OCN, DPD/creatinine, OPG, and phosphate. The data are expressed as mean ± SD. (a: p<0.01, b: p<0.001)

27. Figures 2, 4, Legends: Replace “….via histomorphometry” by “….by histomorphometry”.

“Via” has been replaced by “by”.

28. Figure 2 does not appear on my copy!

Figure 2 will be uploaded again.

Reviewer 2: Gerald Atkins

Reviewer’s report:
The paper is somewhat improved following attention to the reviewers’ comments. However, I still have several concerns:

**Major compulsory revisions:**
1. Expression is still lacking throughout and the paper requires further thorough editing by a native speaker of English.

   The manuscript has been thoroughly edited by a native speaker of English.

2. Page 8: With reference to Table 1 there are 6 groups not 5.

   The sentence has been changed. “Table 1 shows the 6 different groups.”

**Minor essential revisions:**
3. Page 12: Reword the sentence ‘Neither in the alpha-CGRP deficient mice, nor in the Calca -/- mice with additional CT substitution the effect of particle-induced osteolysis was significant’ to read ‘Neither in the alpha-CGRP deficient mice, nor in the Calca -/- mice with additional CT substitution was the effect of particle-induced osteolysis significant.’

   The sentence has been re-worded as suggested by the reviewer.


   The word has been replaced.

5. Page 12: By ‘retraction of osteoclast formation’ do the authors mean ‘decrease in osteoclast formation’?

   The sentence has been changed. “decrease in osteoclast formation” has been included.
6. Page 12: The finding that CT could inhibit PE-induced osteoclast resorption in Calca-/- mice despite PE inducing osteoclast formation 2-fold is very interesting. However, the authors’ explanation that CT induces bone formation and subsequently reduces osteolysis is difficult to understand. Can they elaborate on this paradoxical finding?

The sentence “These data directly implicate calcitonin as a negative regulator of bone formation through a previously unsuspected mechanism.” has been deleted.

7. Conclusions: (expression) re-write the sentence ‘Although some pathways of this impact remain unclear…’

The sentence has been shortened. “Calcitonin appears to have a stronger effect on osteoclasts than on osteoblasts in particle-induced osteolysis in Calca-/- mice.”

Discretionary revision:
8. Page 12: As noted in my initial review, the study by Gooi et al (now included as reference 12) is further evidence that CTR is expressed on osteoblast-lineage cells, namely osteocytes.

Page 12: “Despite the known influence of CT on osteoclasts, there is only little evidence to support the theory that there is a CT receptor on the surface of osteoblasts [34, 35]. Hoff et al. discussed unknown pathways of CT which could also induce an indirect activation of osteoblasts [20]. Gooi et al. recently described the CT-receptor on osteocytes with a negative regulation on bone formation [12].”