Author's response to reviews

Title: Combination therapy with alfacalcidol and risedronate improves the mechanical property by ameliorating the biological apatite c-axis orientation of bone in ovariectomized rat model of osteoporosis.

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Author's response to reviews:

January 13, 2009
Dr. Joseph Dunckley
Associate Editor, BMC-series journals
Re: MS: 1620472204221632 Revision

Dear Dr. Dunckley,

We thank you very much for your letter of December 10, 2008. Please find a revised version of our manuscript entitled “The combination therapy with alfacalcidol and risedronate additively improves the mechanical property in lumbar spine by affecting the material properties in an ovariectomized rat model of osteoporosis.” We found the comments of the reviewers very helpful in improving our paper. According to their suggestions, we performed additional statistical analysis, and several data have been shown in this article. Some figures and tables are added and revised. In the present study, we addressed the advantage of the combined therapy with alfacalcidol and risedronate at their sub-therapeutic dose of each drug over each monotherapy.

On separate pages, we provide point-by-point responses to the referee’s comments. We hope that the revised manuscript has improved significantly and is now acceptable for publication in your journal. If further information should be necessary, please do not hesitate contact me.

Thank you very much for your attention.

Sincerely yours,

Ayako Shiraishi, Ph.D.
Response to Referee 1

We appreciate the reviewer’s constructive and valuable suggestions, which we have found very helpful in improving our manuscript.

Reviewer 1 Comments:

Additive treatment effects is defined by the authors as “the sum of interaction between "of "additive effect is the absence of interaction (independent of each other, not the sum of the interaction), but a sum effect of the two treatments."

These are valuable suggestions. At first, we had referred to Dr. Ego Seeman Paper (JCEM 87(3):985–992) and used p value at 0.10. According to the reviewer’s suggestion, we rewrote some parts of this manuscript, especially in statistical methods, results and discussion, to make the definition of additive treatment effects clear. We show the result with a one-way ANOVA and a two-way ANOVA models for each parameter in Table 1 and 2 to demonstrate no interaction of the two agents. The significant difference among all groups was assessed using Tukey-Kramer test. The effects of ALF and RIS were considered to be independent and additive if the interaction effect was not statistically significant (P>0.05) in a two-way ANOVA model and the effects by the combination treatment are significantly higher than the OVX control group or the each drug alone group (P<0.05).

Since the title of the paper and major issue of the study are evaluating the additive improvement of the combined treatment in each parameter measured, ........and demonstrate no interaction but a sum improvement effect of the two agents.

Table 2: ALF and RIS interaction should be ALF x RIS (not ALF+RIS). It should also examine any sum improvement effect to conclude an additive effect or not. “Table 2 should include the actual values, one-way ANOVA significance, 2-way ANOVA ALF factor, ....... and BAp c-axis orientation data, et al in order to show if there is any improvement by the combine intervention and in Discussion.”

We appreciate the reviewer’s comments. As the reviewer suggested, we revised Table 1 and Table 2 including a one-way ANOVA significance as well as a two-way ANOVA significance.

Figure 3: It is fine to use figures to illustrate the additive effect, but it is not necessary unless list most of the important parameters instead choose two to
This is a very important point. Because we showed the P value in Table 1 and 2, original Figure 3 was deleted.

Experimental Design: The rats were divided into 7 groups based on the BMD of lumbar vertebrae (L2-L4). The method of in vivo scanning should be described in the Methods.

We thank you for this advice. We showed the method of in vivo scanning in p.6.

Second paragraph of the Discussion: “These results …….. bone formation (6, 24)”. The current findings measured only serum OC as bone formation marker and the result do not show any change. You may discuss and compare the dose used in the current study with previous reports.

This reviewer’s comment is very important. Because serum OC level did not change by ALF treatment in this experiment and this was not essential for the purpose of this article, we deleted the data of serum OC level from the result.

Response to Reviewer 2

We appreciate the reviewer's constructive and valuable suggestions, which we have found very helpful in improving our manuscript.

Major Compulsory Revisions

1) Please strongly consider revising the title of the study.

We thank you for this advice. According to the reviewer’s suggestion, we revised the title. The title reflects the results of this study, suggesting that, in respect of the improvement in the mechanical strength of lumbar spine, the effects of ALF and RIS were considered to be independent and additive, and the material properties such as the BAP c-axis orientation and the microstructural parameters, not the BMD, contributed to the reinforcement of spinal strength.

2) The introduction paragraph on BAP c-axis is confusing as it fails to define what Bap c-axis orientation is and does not sufficiently explain how …….. mechanical properties. This information is essential as ……. with such a measure.

Based on the reviewer’s comment, we improved the paragraph in background of our manuscript (p. 4-5).

3) The final sentence of the introduction necessitates clarification. Statements such as “focused on differences ……..(the second sentence of this paragraph is much better). Please state ……..ameliorating changes in mechanical properties”.

This is a very important point. We rewrote the background and clearly showed the purpose and the hypothesis of this study (p.5).
It is not clear why only ultimate load was reported? At a minimum, the other extrinsic... At an absolute minimum, this needs to be addressed in the discussion.

This is also valuable suggestion. When we discuss the biomechanical properties of bones, the extrinsic properties should be considered. We added the values of the stiffness and the energy in the result and revised Figure 2 and Figure 3.

5) The use of 0.10 as a significance value ........, or use a more traditional value of 0.05.

According to the reviewer’s suggestion, we use a more traditional value of 0.05 and revised the article.

6) The results/discussion repeatedly refers to dose-dependent changes. While in most of these cases, the two doses of either ALF or RIS appear different, ........ it is not valid to say there is a dose-dependent effect.

The reviewer’s comment is very important. We rewrote the result and the discussion to a mention that the effect of ALF or RIS is dose-dependent when the two doses of compounds are significantly different from each other.

7) The first paragraph of the discussion necessitates major revision. There is no data presented to show the amelioration of Bap c-axis was ........ ; in the femur the combination was better than ALF but not RIS). One cannot make such broad statements without a broad effect.

We thank you for this advice. According to the reviewer’s suggestion, we revised the discussion. The bone volume and thickness did not alter significantly in the combination group, however, the bone surface and trabecular separation were decreased compared with each drug monotherapy. The BAp c-axis orientation was significantly improved in the combination group compared to the OVX control group. Therefore, we revised the result and the discussion to a mention that the combination effect regarding the microstructural parameters was the inhibition in becoming thinner of trabecular bone, and the Bap c-axis orientation was regarded as one of the causes that contributed to the reinforcement of bone strength.

Minor Essential Revision

1) The paper would benefit if it was edited for proper English as there are several areas that are missing words.

This is also very important. We revised English of the manuscript.

2) The introduction suggests bisphosphonates reduce fractures by increasing mineralization rather than maintaining bone mass/architecture. This is not a majority view and the evidence far more favors the preservation of
mass/architecture although there clearly is some role for mineralization. Please consider revising this text.

According to the reviewer’s suggestion, we cited the paper describing the effect of risedronate on bone volume and matrix mineralization (Ref. 8: Fratzl P et al. Calcif. Tissue Int. 2007, 81(2), 73-80) and rewrote the introduction (p.3-4) to a mention that although RIS had important action for calcification, it did not markedly change the bone mass and bone structure. This was also observed in this study. High dose of risedronate failed to ameliorate the changes in bone microstructure.

3) Please state the total number of animals, as well as the number in each group.

We thank you for this advice. The number of animals is shown in the method and Table 1.

4) Please provide additional information on dosing. For example, the dose of RIS (0.3 mg/kg) is……. It would be useful to explain how the doses were chosen, with references where appropriate.

This reviewer’s comment is very important. The aim of this study was to determine the additive effect of ALF with RIS at their sub-therapeutic doses on bone metabolism in an osteoporosis model of rats. We referred to Dr Erben paper (Ref. 35) and our previous studies (Shiraishi A et al. Calcif Tissue Int (2006) 78:152-161, Ref. 6), and therefore the dose of RIS and ALF used in this study were chosen.

5) Can the authors please clarify where the BMD measures were made (cortical or trabecular bone) ?

According to the reviewer’s suggestion, we show clearly in the method and the result that BMD was measured at the cortical bone part of the lumbar vertebrae.

6) What does the statement that Bap c-axis is a useful parameter for evaluating ‘history of bone formation’ mean ?

As the reviewer pointed out, “history of bone formation” is unclear. We wanted to mean it as “process of bone formation and growth”. In a skull bone with elliptical BAp c-axis distribution along the skull surface, for example, the ellipticity varies as a function of the distance from a sagittal suture. This change is closely related to “process of bone formation and growth” such as direction and speed of bone growth etc.. Therefore, we can estimate the process (history) of bone formation and growth by analyzing the BAp c-axis orientation. This was discussed in details in ref. 14. To avoid misunderstanding to the readers, we changed “history of bone formation” to “process of bone formation and growth”.

7) The methods concerning Bap assessment are unclear. It seems as if measures were made
on a cross-section in the ventral cortical bone, yet later is says the bones were cut and polished to the center? Please clarify.

We thank you for this suggestion. Measurements were performed at the ventral cortical position on the cross section which is a middle part and perpendicular to the cranio-caudal direction in L4. In this study, thus, analyzed L4 specimens were cut perpendicular to cranio-caudal axis. Since our explanation was unclear as the reviewer pointed out, we improved the description in the methods (p. 10).

8#Please only report the data as SE or SD.

We thank you for this advice. We revised and reported the data only as SE.

9) Please explain in more detail the way additive effects were assessed. What does the ‘sum of the interactions……. by the effects of ALF and RIS were considered additive when given in combination if the interaction was not significant?

This is valuable suggestion. We had referred to Dr. Ego Seeman Paper (JCEM 87(3):985–992) at first. However, according to the reviewer’s suggestion, we clearly rewrote some parts of this manuscript, especially in statistical methods, results and discussion, to make the definition of additive treatment effects. We show the result with one-way ANOVA and 2-way ANOVA for each parameter in Table 3 and 4, and demonstrate no interaction of the two agents. The significant difference among all groups was assessed using Tukey-Kramer test. If the interaction effect was not statistically significant (P>0.05) in a two-way ANOVA model and the effects of the combination group is significantly higher than the OVX control group or each drug alone group (P<0.05), the effects of ALF and RIS were considered to be independent and additive. When the effect in combination group is higher than that in the monotherapy group of each drug and the interaction is significant, the effect of each drug is synergistic, not an addition effect.

10) The text on interactions suggests that lines on figure 3 need to intersect for there to be an interaction. This is not true and thus the text (and possibly the method for assessing interaction) should be revised.

This is also an important point. We deleted original Figure 3 because it was not essential and necessary. The P values from a one-way ANOVA as well as a two-way ANOVA are listed in Table 3 and 4.

11#The value of Figure 5 is unclear. Please either emphasize why this is important or remove. The text regarding the ‘optimal state of bone mineral parameters’ is not supported by the data.

We appreciate the reviewer’s comments. The BMD and the BAp orientation are independent parameters for describing bone microstructure because they
correspond to density of BAp and orientation of the BAp c-axis, respectively. So, we have to consider both parameters. Moreover, the normal and most optimal balance between BMD and BAp orientation corresponds to that in the Sham group. As a result, we improved and emphasized the paragraph concerning with Figure 5 (p. 15-16).

12#The statement about ‘restoration of damaged trabeculae’ is not supported by the data.

This is also valuable suggestion. For the reason same as Major Point 7, we revised the discussion (p.17).

13) The text on a ‘unique mechanism’ with respect to changes in bone formation and……uncoupling of bone formation and resorption do not unambiguously show it to occur.

We thank you for this advice. We deleted the data of serum OC, because serum OC level did not change by ALF in this experiment and this was not essential for the purpose of this article.

14) The discussion suggests a goal of the study was to find ways to minimize adverse reactions to treatment but it’s not clear how this study really addresses such question.

This is also very important. The purpose of this experiment is not to find the method to reduce the side effect by treatment with ALF or RIS. We expected that the effect on bone and Ca metabolism were improved by the combination therapy at sub-therapeutic dose of each drug more than those of the monotherapy. As the result, the bone mechanical property was improved by combined treatment with ALF and RIS. Moreover, urinary Ca excretion in the high dose of ALF or RIS monotherapy group was increased and reduced significantly in comparison with those in OVX control group, respectively. However, in the combination therapy group, urinary Ca was equivalent to those in OVX control group. Therefore, we rewrote the discussion to mention that both the bone effect and the Ca metabolism were improved by the combination therapy with ALF and RIS. These results have addressed that the advantage of the combination therapy at sub-therapeutic dose of each drug over each monotherapy to treat Ca metabolism in osteoporosis.