Author's response to reviews

Title: Effects of JSOG-6 on protection against bone loss in ovariectomized mice through regulation of osteoblast differentiation and osteoclast formation

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Author's response to reviews: see over
Title: Effects of JSOG-6 on protection against bone loss in ovariectomized mice through regulation of osteoblast differentiation and osteoclast formation

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Dear Dr. Editor of BMC Complement Altern Med

Thank you for your letter of 24 January 2014, and the comments of manuscript 1059507997115854. According to reviewer’s comments, the manuscript was properly revised as followings.

Reviewer: Anne Blais

Reviewer's report:
This manuscript presents an interesting in vivo study showing that a traditional medicine can reduce bone loss. The in vitro study support that the effect is mediated by a stimulation of osteoblasts and an inhibition of osteoclasts. The in vitro studies showed a dose dependent effect. However the in vivo study the authors was not able to report a dose dependent effect for the concentration used.
There are too many tables and figures in the manuscript

Major Revisions

- The authors indicate that JSOG-6 treatment improve dose dependently the trabecular bone structure however figure 1B and 1C do not present any significant difference between the 3 different concentrations of JSOG-6 for the different parameters evaluated.

Response: As demonstrated in Fig. 1B, OVX destroyed the bone architecture of mice, but the treatment of E2 prevented the deterioration of bone destruction. In a similar way, JSOG-6 also alleviated the destruction of bone, and even the protective effect did not show dramatic activity in the bone histology the analysis of bone parameters was shown in a dose dependent manner depicted in Fig. 1C.

- The time at which the blood was collected for the measurement of the metabolic biomarkers should be indicated. The values for those parameters are drastically different 1 or 3 months after the OVX procedure.
Response: The time for blood collection was already mentioned in the Materials and Methods (Page 5): After 12 weeks of treatment, the animals were sacrificed, and blood samples were collected for serum isolation.

In the discussion the authors should suppress the sentence that indicates that JSOG-6 rescue the deterioration of bone micro architecture because the design does not allow such an affirmation.

Response: As suggested, the sentence was properly changed for the findings.

Minor Revisions

Table 1 should be discarded values are given in the text.

Response: As suggested, Table 1 was deleted and the composition was mentioned in the Materials and Methods (Preparation of test samples).

Figure 1 should be modified. Gain weight as function on time as compare to T0 and line should replace the histogram.

Response: As suggested, the gain weight was changed with line graph to be easily depicted the change of body weight in a time point.

Values of figure2 should be presented in a table.

Response: As suggested, Fig. 2 was presented in Table 2..

Reviewer: Yin Xiao

Reviewer's report:

Major revision in the methodology:
Reverse transcriptase-polymerase chain reaction (RT-PCR)

MC3T3-E1 cells were stimulated with 1 μg/mL LPS in the presence or absence of JSOG-6 for 48 h.

Why LPS is used? There is no mention of the LPS in the result and discussion. The rationale is not mentioned and the experimental design in this section is not fully understood.
Response: The use of LPS in the experiment was described on Page 8 Line 6~7.

LPS, a cell component of gram-negative bacteria, is an important mediator of pathological bone destruction associated with inflammation.

In addition, following sentences were mentioned in the Discussion part to described the use of LPS in the relation with the bone metabolism.

LPS leads to the intracellular induction of p38, JNK phosphorylation, and NFκB inmacrophages and monocytes, and promotes the differentiation and survival of osteoclasts through the production of several factors such as PGE2, interleukin 1, RANKL, and TNF.

Reviewer: Jean Langlois
Reviewer's report:

Major compulsory revisions
1. The structures of Methods and Results paragraphs from the abstract should be deeply revised in order follow conventional rules: methods should develop precisely the technics involved (microCT, serum biomarkers assessment, in vitro cultures etc...) and results should show the very objective resulting data. What we find here in this abstract is too fuzzy

Response: As suggested, the abstract part was changed and also results were described with the sentence for implying objective resulting data.

2. There are too many subjective comments in the Results part. We don't need introduction sentences like "To assess whether JSOG-6 exhibits anti-osteoporotic activity, an in vivo experiment was performed employing an OVX-induced bone loss mouse model" or "To determine the effects of JSOG-6 on the bone metabolic biomarkers, blood samples were collected, and biochemical analysis of the serum was performed in OVX mice" (same for the others sub parts). Second, we don't want to see subjective comments in the Results part (see "These data suggest that JSOG-6 treatment prevents ..." or "These findings suggest that JSOG-6 might modulate the process of osteoclastogenesis via ..."). These kind of subjective analyses are to be discussed in the last part of the manuscript (Discussion).

Response: As suggested, several sentences were properly changed without subjective comments.

3. Please revise introduction sentences of both Introduction and Discussion Parts. Starting from a wide overview (bone metabolism... osteoporosis ...treatment of osteoporosis ... JSOG6) to focus on the subject, for the introduction; reverse the process for the first paragraph of the discussion.
Response: The sentences were properly changed following the comments.

4. It seems awkward to finish the introduction (last sentence) with the results ("The findings demonstrate that JSOG-6 protects against...")

Response: As suggested, the sentence was deleted.

Minor Essential Revisions

5. Please be more convincing or precise than "These reported data suggest that JSOG-6 might have the potential to alleviate the symptoms of bone loss-associated diseases". It's probably more than just "to alleviate the symptoms", as you have spent hours and days to perform these experiments

Response: The sentence was properly changed and addressed the findings in the meaning of clinical value.

6. Please revise the sentence "In the present study, we investigated the activities of JSOG-6 in vitro and in vivo bone-remodeling models and examined its underlying molecular mechanism" (or add a second "in" between JSOG-6 and in vitro)

Response: As suggested, the part was corrected.

7. Whenever a "significant result" announced, we would like to see a "p-value" (between brackets at the end of the sentence for example)

Response: As suggested, p value was inserted in the indicated positions.

8. We regret there was no limitation stated (at least one limitation of each technic: why microCT and no histology? why these biomarkers and not different ones?)

Response: As suggested, each technique for the evaluation of biologically activity was described in the Methods and Results.

9. We regret no clinical or experimental perspective was stated.

Response: As suggested, clinical perspective with the findings was described in the Discussion parts.

10. It might be more logical to present experiments from in vitro to animal in vivo studies, in order to follow a progression in terms of possible clinical application.
Response: As suggested, clinical perspective with the findings was described in the Discussion parts.

As a result of these changes, the manuscript might be substantially improved and hopefully acceptable for *BMC Complementary & Alternative Medicine*.

With many thanks for your help and consideration.

With my best regards,

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