Author's response to reviews

Title: Protective effects of biochanin A on articular cartilage: in vitro and in vivo studies

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Author's response to reviews: see over
Dear Editor,

We are extremely grateful to the reviewers’ excellent comments and valuable advices about our paper. We have revised the manuscript, according to the comments and suggestions of the reviewer, and responded, point by point to, the comments as listed below.

**Replies to Reviewers**

First of all, we thank the reviewers for their positive and constructive comments and suggestions.

**To Reviewer: 1**

**Comment 1**

1. All experiments of Figure 2-5 indicated that rabbit chondrocytes were pretreated with various concentrations biochanin A for 2 h followed by co-treatment with IL-1beta for 24 h. Anyway, authors did not provide data of MMPs, TIMP-1, IκB-alpha and NFkB expression of chondrocytes treated by single biochanin A incubation (2 h). This data is related with ACLT animal model. Because authors did not discuss the inflammatory effect on ACLT model.

**Answer:**

Thanks very much for your comments and suggestions. We completely agreed with the advice of the reviewer. However, MMPs, TIMP-1, IκB-alpha and NFkB expression of chondrocytes treated by single biochanin A incubation (2 h) were not obviously changed, so the results were not shown in our manuscript. Only if the chondrocytes treated with biochanin A and inflammatory mediator (IL-1β), the
expressions were obviously changed. On the other hand, information about inflammatory effect on ACLT model has been discussed in our revised manuscript.

**Comment 2**

Histological assessment (page 8, L9-14) and explanation of Table 1 (page 30) is too simple to understand. Authors should also add remark of Table 1 to explain all histological scores including structural changes, cellular changes, Safranin staining and total score. Table 1 lacks data of normal group which is same as (a) of Figure 6. The description of sarfranin-O stain of result 3.5 and Figure 6 are too simple to understand. Moreover, the description on legend of Figure 7 is also too simple to read. If possible, Figure 6 and 7 can be merged together.

**Answer:**

Thank you very much for your carefulness and suggestions. The effects of ACLT on cartilage degradation were confirmed by many studies, so the comparison between normal group and other groups was not shown. We aimed to demonstrate the effects of biochanin A in OA models. We described the effects of biochanin A on osteoarthritic cartilage by using these two figures (Fig.8 and Fig.9) to make it easily understood.

**Comment 3**

The title of 3.1 (page 9, L4) should focus on cell viability.

**Answer:**

We have modified this title in our revised manuscript.

**Comment 4**
The title of 3.4 (page 10, L4-5) should mean “IL-1beta”-treated chondrocytes.

**Answer:**

We have modified this title in our revised manuscript.

**Comment 5**

Lacking statistical analysis between mock cells and IL-1beta-treated cells of Figure 2 and 3. Moreover, there are no P<0.05 (*) of Figure 2 and 3. If possible, authors should provide the dose-dependent results among various biochanin A after statistical analysis.

**Answer:**

Thank you for your suggestions. The statistical analysis between mock cells and IL-1beta-treated cells of Figure 2 and 3 have been added in our revised manuscript. P<0.05 (*) of Figure 2 and 3 was mentioned in figure legends.

**Comment 6**

There are no ratios obtained by normalizing the signal of MMPs, TIMP-1, IkBa and NFkB to that of beta-actin bands of Figure 4 and 5.

**Answer:**

Thanks for your carefulness and suggestions. We have added the ratios figures in our revised manuscript.

**Comment 7**

Units of biochanin A of Figure 6, 7 and Table 1, 5 and 50 uM, are not units of pharmacological dosages.

**Answer:**
The unit of “uM” is short for “umol/L”. We checked and referred many other references.

**Comment 8.(1)**

Authors should talk about the clinical anti-catabolic drugs on OA and comparing the efficacy between these drugs and biochanin A.

**Answer:**

Thanks for your suggestions. We have added relative information in our revised manuscript.

**Comment 8.(2)**

Authors mention that little is known about the effects of biochanin A on osteoarthritic models or chondrocytes (page 11, L20-21). Anyway, the balanced effects of biochanin A on adipogenesis and osteogenesis was published by ECAM (2013, article ID 846039).

**Answer:**

Thanks for your suggestions. We have added relative information and references in our revised manuscript.

**Comment 9.(1),(2)**

(1) Page 6, L1: 37 # : 30 #

(2) Page 6, L12: 95 #, 60 # : 95 #, 60 #

**Answer:**

Thanks for your carefulness. We have corrected the typo errors in our revised manuscript.
To Reviewer: 2

Comment 1

The effect of biochanin A on the production of secreted or intracellular MMPs proteins seems marginal (Fig. 3 and 4). In contrast, its inhibition on MMPs mRNA is much pronounced (Fig. 2). What is the reason for this contradiction?

Answer:

As to the values for Fig2, Fig3 and Fig4, we believe that biochanin A possesses the effects on the inhibition of MMPs at the mRNA and protein levels, however, their impact are not completely consistent. The effects of biochanin A on MMPs mRNA is much pronounced. While the effect of biochanin A on the production of secreted or intracellular MMPs proteins seems marginal. We speculate that the effect of biochanin A on the production of secreted or intracellular MMPs proteins is impacted by many other multifactors, which will be further studied.

Comment 2

In Fig. 6, please indicate the staining of cartilage (calcified cartilage, uncalcified cartilage; subchondral bone) with arrows. Description about the pathological changes for Fig. 6 and Table 1 needs to be provided in details.

Answer:

Thanks for your carefulness. We have indicated the staining of cartilage with arrows in Fig.6. Also we have added the description about the pathological changes for Fig. 6 and Table 1.

Once again, thank you for your patience and kindness. Looking forward to
hearing from you soon.

With kindest regards.

Yours Sincerely