Reviewer's report

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

Version: 1 Date: 27 July 2014

Reviewer: Cicero LT Chang

Reviewer's report:

Points are listed below.

Minor Essential Revisions

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, Ikbalpha 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.

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.

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

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

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.

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

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

8. Discussion section:

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

   (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).
9. Typo errors:
(1) Page 6, L1: 37 # : 30 #
(2) Page 6, L12: 95 #, 60 # : 95 #, 60 #
Please check manuscript thoroughly and carefully.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests.