Reviewer's report

Title: Effects of BIS076 in a model of osteoarthritis induced by anterior cruciate ligament transection in ovariectomised rats

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Reviewer: Nicole Walsh

Reviewer's report:

In this study, the investigators assess the effects of 2 formulations of porcine cartilage extract, hydroxyapatite and vitamin D3 (BIS076) to prevent OA onset and severity of joint damage following ACL transection in ovariectomised rats. The authors claim that BIS076 treatment in these animals reduced articular cartilage damage at the histologic level and lowered expression/release of markers of cartilage damage and inflammation, and protected bone micro-architecture. Two formulations of BIS076 are used which differ in the concentration of porcine cartilage extract and hydroxyapatite (a low and high dose of each), with a constant dose of Vit D3.

New agents that have a disease modifying effect on joint structure in the treatment of OA are required. However there are some concerns regarding the strength of the results in this study and the conclusions drawn from them.

Major Compulsory Revisions:

1) Fig. 1 Histologic analyses. The examples of histologic sections shown in Figure 1 are of poor quality. If these are representative of the whole group, then reliable histologic scoring would be challenging. Furthermore using HE stained sections only would prohibit scoring of aggrecan loss which is a component of the OARSI scoring scheme. Toluidine blue or Safranin O-fast green stained sections would be more suited for this purpose.

2) P6 last sentence: the composition of the porcine cartilage extract is listed as 40-65% type II collagen and 15-25% glycosaminoglycans, mainly chondroitin sulphate). How variable is the content of the porcine cartilage extract composition and was only one batch used in this preparation.

3) P7, 1st paragraph: clarification of the experimental groups is required. It is assumed that all rats underwent ovariectomy and then the groups were split into sham surgery and ACLT surgery groups and these then allocated to treatment groups. A sham surgery group + treatment are not included, which would have allowed assessment of BIS076 on the tissue and cytokine expression independent of OA.

4) P10, 2nd paragraph, Fig. 3 and Table 2. MicroCT studies.

- It is not clear, what the area for bone structure analysis was for the epiphysis? Did this include the subchondral bone (below cartilage but above the growth plate) and the primary spongiosa (immediately below the growth plate as shown
in Fig3. If this is the case, then an analysis of just the subchondral bone above the growth plate would be more informative.

- The authors claim that these is a similar tendency for bone loss in the epiphysis as observed for the metaphysis upon treatment. This is not correct. There was no difference (or trend) in any of the parameters in the epiphysis.

5) It is a little surprising that the changes in the metaphyseal bone are much greater than those observed in the epiphyseal bone both in terms of bone lost between ACLT and sham surgery groups and the ACLT control and treatment groups. Could the authors speculate why this may be the case. Furthermore, treatment had a stronger affect on the metaphyseal bone compared to the epiphyseal bone. Can the authors explain the differences in the different compartments of the bone?

6) p11 first paragraph and Figure 5.

- Analysis of serum markers of cartilage degradation and bone cell activity. The authors state in the text that there is an enhanced level of CTX-II in the sera between sham and control, however in Fig 5 this is not evident. Furthermore, although the authors are showing SD, many of the statistical significance is not convincing. Perhaps the authors could plot their data as a box and whisker plot or with individual points within the groups to show the spread of data.

- The apparent reduction in TRAP5B levels in the ACLT+control group compared to the Sham group is a little surprising given that there is a significant bone loss within the metaphysis. Can the investigators explain why this is. Have they done osteoclast counts within the histologic sections?

- Serum CTX-1 levels would have provided a better measure of bone resorption in addition to TRAP5b activity and RANKL/OPG.

7) The authors do not provide any discussion on how BIS076 may be eliciting its effects. This should be included in the discussion.

Minor Essential Revisions

1) P8 microCT methods paragraph. A clear description of how the region of interest for each anatomical location where bone microarchitecture was assessed should be provided. Details of the software used for MicroCT analyses (including versions) and thresholds used for analysis should also be listed.

2) In the background section of the abstract it is stated that “some of the available treatments are dietary supplements providing natural components that can help to preserve structural integrity of the joint”. To this reviewer’s knowledge, this statement is largely incorrect, at least in the treatment of human OA.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:

I declare that I have no competing interests