Author’s response to reviews

Title: Intra-articular injection of mono-iodoacetate induces osteoarthritis of the hip in rats

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Author’s response to reviews:

Dear reviewers,

Thank you very much for your kind letter. Based on your comments and requests, we have made the revision and all the changes were highlighted in green in the revised paper.

Thank you very much for giving me an opportunity to revise the above manuscript. I hope you are satisfied with the revised version, however, if there is more question, we are willing to revise it again.

Here below is our description on revision according to the reviewers' comments.
Reviewer #1:

The authors describe a rat model of hip osteoarthritis based on intra-articular injection of moniodoacetate (MIA). Saline injections are performed as controls. Rats were evaluated at 1, 2, 4, 6, and 8 weeks by X-ray, histopathology, and scored using the Kellgren-Lawrence (Mankin) system. Findings confirmed that intra-articular injection of MIA induces arthritis in the hip. Arthritic changes included joint-space narrowing, deformity of the femoral head, bone atrophy, surface irregularity of the articular cartilage, and a gradual increase in the Mankin score.

1) A stated purpose of the study is to "...establish an intra-articular injection technique to the rat hip..." (pg3 line 1). This technique was already published by the same group in April of 2015, (Mod Rheumatol. 2015 Nov;25(6):931-6. doi: 10.3109/14397595.2015.1023977. Epub 2015 Apr)

2. A novel rat model of hip pain by intra-articular injection of nerve growth factor-characteristics of sensory innervation and inflammatory arthritis.). However, the earlier manuscript did not include detailed descriptions of the intra-articular hip injection procedure. In the current manuscript the authors describe IA injections of the hip as technically challenging, yet only provide a brief description without accompanying figures or diagrams. If the purpose of the current manuscript is truly to provide this detail, then sufficient detail should be provided to enable readers to perform the technique, and in this respect the current manuscript falls short. A figure or diagram clearly depicting the various steps in sufficient detail should be included, as should experimental evidence that the protocol works to keep the injected material in the joint capsule without leakage as stated.

Author's reply

Thank you very much for your comments and suggestions. It is true that the intra-articular injection technique to the rat hip has applied in our recent article. This technique itself had established earlier in this study, but it takes much longer time to be accepted. As a result, the latter article has published without detail explanation about the technique. We had to omit the figures at the time of submission for want of space, but now we add them with reviewer's helpful suggestion.

2) The extent of analysis is very limited. Additional analyses might include pain scores (certainly within the expertise of this group), quantitative assessment of bone remodeling using micro-CT, OA biomarkers, synovial inflammation, cell death, etc.
Author's reply

It is hard task to evaluate pain scores in animal model, but a gait analysis using CatWalk system (Noldus Information Technology, Wageningen, The Netherlands) is going on as a behavioral testing. Moreover, immunohistochemistry and enzyme-linked immunosorbent assays of pain related molecules have been analyzed. These studies are submitted to elsewhere. Quantitative assessment of bone remodeling using micro-CT, OA biomarkers, synovial inflammation, and cell death has not been investigated yet, but they should be clarified for characteristics of rat MIA hip model in the future.

We add these limitations in the end of Discussion.

3) A more detailed discussion comparing the timeline of MIA-induced hip OA versus MIA-induced knee OA in rats would also be helpful.

Author's reply

Orita et al. reported that OA change was appeared after day 7 and was gradually progressed until day 28 in rat MIA knee model (Orita S, et al. Pain-related sensory innervation in monoiodoacetate-induced osteoarthritis in rat knees that gradually develops neuronal injury in addition to inflammatory pain. BMC Musculoskelet Disord. 2011;12:134.). Guzman et al. had already observed extensive areas of chondrocyte degeneration at day 1, moderate collapse of the cartilaginous matrix with marked loss of chondrocyte by days 5 or 7, and the progressive collapse until days 56 (Guzman RE, et al. Mono-iodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. Toxicol Pathol. 2003;31:619-24.). In this study of the hip, chondrocyte degeneration was absent both in the acetabulum and the femoral head, and was noted by days 14. It should be investigated whether time to cartilage degeneration is different between the hip and the knee. We add this point in the Discussion.

4) Other details that should be addressed:

- Figure legends are missing.

Author's reply

We are sorry of our careless mistake and add the legends.
5) Because there is also mild OA in saline-injected joints, these joints should also be shown. Importantly, since the goal of the manuscript is to establish IA hip injection model of OA, the saline-injected joints should be compared to age-matched naive joints without injection. Only then can the effect of saline injection be evaluated.

Author's reply

It is quite reasonable to show the figures of control group. We had to omit them at the time of submission, but now we add with reviewer's helpful suggestion.

6) Methods reference Kellgren-Lawrence scale, yet results describe Mankin Score.

Author's reply

Kellgren-Lawrence scale is radiological classification and Mankin score is pathological classification. Both scores reported in the Results, but we add more explanation.

Reviewer #2: Comments to the Author

Major Revision

1) The authors present a paper assessing cartilage morphology in hip OA. Although the concept of the paper is reasonable, the data presented in the paper is not particularly novel and does not provide in my opinion any new knowledge on cartilage morphology in the animals described.

Author's reply

It is true that cartilage morphology after MIA injection has already been reported in some knee models. However, to our best of knowledge, it is not documented in a hip model. We believe that this article has great impact on pain research of the hip and the readers of BMC Musculoskeletal Disorders.
2) If the author is only interested in establishing an animal model for hip OA, they should have done it in Old rats not in young, since hip OA is prevalent in old patients - Please include the reason for using young rats instead of old in discussion.

Author's reply

We applied rats at 6 weeks of age for several reasons. First, animal age was different in previous literature and then was adjusted to Orita's study of knee MIA model using 6 weeks old rat (Orita S, et al. Pain-related sensory innervation in monooiodoacetate-induced osteoarthritis in rat knees that gradually develops neuronal injury in addition to inflammatory pain. BMC Musculoskelet Disord. 2011;12:134.). Second, because we planed relatively long-term study for rat until 56 days, we thought that young rat would be suitable in that time.

It is of great interest whether effect of MIA shows age dependent progression or not. We include the reason for using young rats instead of old in discussion.

3) In page 3, the introduction part, line 19, the author have spoken about x-ray imaging and a new device, but there is no name of the equipment or the reference to show that it is different from the available imaging units. How does this sentence help in their study? It will be better if they explain this properly.

Author's reply

We documented a name of equipment as in vivo imager (Xtreme, Bruker, WI) at "X-ray imaging of the hip joint" in the Materials and Methods section. We add the name in the Introduction. We also add an article in Reference (Jung YK, Shin E, Kim BS. Cell Nucleus-Targeting Zwitterionic Carbon Dots. Sci Rep. 2015;5:18807).

4) Since the author's knew that they have to sacrifice the rats for histology, if the author had planned properly, according to my opinion, they should have added some more rats and analyzed GAG and relevant genes. May be immunohistochemical staining for cartilage specific proteins can provide a better value to the manuscript.
Author's reply

It is quite reasonable to investigate quantitative assessment of OA biomarkers, gene, and immunohistochemical staining for cartilage specific proteins. Unfortunately, they have not been investigated yet, but they should be clarified for characteristics of rat MIA hip model in the future. We add these limitations in the end of Discussion.

5) The discussion part should be more specific to the objective of the manuscript rather than discussing the relevance of pain in OA, while they haven't done any test to prove it.

Author's reply

With the reviewer 2's helpful suggestion, we omit the redundant sentences about pain.

6) While MIA injection induces OA in 2 weeks, in knees, how does it take 56 days to induce OA in hip - Probably the author's should discuss the principle behind this.

Author's reply

It should be investigated whether time to cartilage degeneration is different between the hip and the knee. In this study of the hip, chondrocyte degeneration was absent both in the acetabulum and the femoral head, and was noted by days 14. Orita et al. reported that OA change was appeared after day 7 and was gradually progressed until day 28 in rat MIA knee model (Orita S, et al. Pain-related sensory innervation in monoiodoacetate-induced osteoarthritis in rat knees that gradually develops neuronal injury in addition to inflammatory pain. BMC Musculoskelet Disord. 2011;12:134.). Guzman et al. had already observed extensive areas of chondrocyte degeneration at day 1, moderate collapse of the cartilaginous matrix with marked loss of chondrocyte by days 5 or 7, and the progressive collapse until days 56 (Guzman RE, et al. Monoiodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. Toxicol Pathol. 2003;31:619-24.).

We add this point in the Discussion.

7) Please update the reference section, add recent references for ACLT, MIA comparison.
Author's reply

The references are updated and following articles are included.

For ACLT,

For MIA,