Reviewer's report

Title: (5R)-5-hydroxytriptolide Inhibits Osteoclastogenesis through RANKL/RANK/OPG Signaling Pathway

Version: 2 Date: 8 October 2014

Reviewer: Toru Yago

Reviewer's report:

Comments to the author

The authors aimed to clarify the mechanism of to assess the effects of LLDT-8 on RANK/RANKL/OPG signaling pathway and osteoclastogenesis. In the current report, they demonstrated that LLDT-8 up-regulated OPG expression in CD3+ T leukomonocyte, and increased the ratio of OPG to RANKL in peripheral blood of RA patients. They also showed that LLDT-8 increased the ratio of OPG/RANKL in synovial fluid of RA patients. Next, they also showed that LLDT-8 inhibited secretion of osteoclastogenic cytokines including IL-1#, IL-6, IL-21 and that LLDT-8 promoted secretion of anti-osteoclastogenic cytokine, IL-10, in the supernatants of PBMCs from peripheral blood or SFMCs from synovial fluid with RA patients. Furthermore, they demonstrated that LLDT-8 may reduce osteoclastogenesis by inhibiting NF-#B signaling. Finally, they concluded that LLDT-8 could have a therapeutic potential for RA. The author's studies are interesting; however, I have major and minor concerns on their study.

Major concern:

1. They estimated osteoclasts only by TRAP staining. They described that “Osteoclast formation was determined to be TRAP-positive staining multinuclear cells using light microscopy.” in Methods; however, we could not detect multinuclear osteoclasts in Figure 5. I could not understand how they made the graph of Figure 6 from Figure 5. I recommend that they evaluate osteoclasts also by immunostaining with anti-vitronectin receptor antibody. I also recommend that they evaluate the ability of bone resorption when they determine osteoclasts.

Minor concerns:

1. They used “osteoclasisia” two times in the current paper. We do not use this word usually. It is better to change to another word.

2. In Background, the first paragraph is too long.

3. In page 7, they used “didn’t” two times. Furthermore, in line 194, they described “To examine the effect of LLDT-8 on the expression on CD3+ T leukomonocyte in synovial fluid, we isolated SFMCs from synovial fluid of RA patients...”. There were two periods.

4. In Results, they did not describe the explanation about Figure 5C. I think it is
the most important point in the current paper.

5. Throughout the paper, English should be revised by native speaker.

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.