Reviewer's report

Title: Rapamycin reduces bone growth, decreases angiogenesis and lowers chondroclastic activity in young rats

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Reviewer: H Anderson

Reviewer's report:

This report contains valid and important new data regarding the apparent negative effect of rapamycin (RAPA) on endochondral bone growth in children and young adults. The study is clinically relevant because of the current usage of rapamycin as an immunosuppressant to prevent rejection of renal allografts in children. The results presented are in good agreement with the findings of an earlier study, also in young rats, showing that rapamycin slows chondrocyte proliferation and leads to a decreased rate of endochondral bone formation with shortened long bones (Alvarez-Garcia, 2007). The present manuscript not only confirms several observations by Alvarez-Garcia, 2007 (e.g. that growth plate chondrocyte proliferation is reduced by rapamin), but also goes further by documenting that the resorption rate at the base of the growth plate is reduced in RAPA-treated animals, and that the height of the hypertrophic zone in RAPA-1 treated animals is actually greater than in controls. Furthermore, the present manuscript makes a number of new observations to confirm and support the above conclusions, including in situ hybridization to document changes in expression of a number of factors regulating growth plate growth, including levels of Type II collagen, histone 4 and mTOR (a rapamycin receptor involved in signal transduction), to name just a few.

Specific comments:

Page 8, paragraph 2. Regarding the increase in creatinine levels observed, perhaps this point should be clarified by stating that the rise in creatinine was observed in both controls and RAPA-treated animals, at 4 weeks.

Page 8, paragraph 3, fig. 1. It will be difficult for the inexperienced reader to estimate hypertrophic zone (HZ) heights, looking at the images presented in Fig. 1A and 1B. Thus, it should be indicated in the text exactly how the top and the bottom of the hypertrophic zone were identified, in order to measure its height. It also might be possible to superimpose dashed lines at the top and bottom of the HZ in the images of growth plates presented here, to make the point that the HZ was higher with RAPA treatment. Also, the RAPA-treated HZ shown in fig. 1B doesn't look significantly higher than control. Was the difference in HZ height at 4 weeks not significant in control vs RAPA-treated growth plates? Are the magnifications of photomicrographs in Fig. 1A the same; (because the control growth plate looks slightly more magnified)?
Page 11, line 3, Fig. 4B. Although the text states that "IGFBP3 protein expression was localized to the proliferating chondrocytes", examination of Fig. 4B suggests that there was much IGFBP3 expression in Hypertrophic chondrocytes of both RAPA-treated and control growth plates, at both 2 weeks and 4 weeks.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**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.