Reviewer's report

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

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Reviewer: Anita Oberbauer

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The manuscript entitled “Rapamycin reduces bone growth, decreases angiogenesis and lowers chondroclastic activity” by Sanchez and He addresses an important topic in pediatric care. Rapamycin is used as an immunosuppressant in organ transplantation yet also impairs bone growth. Application to children could have significant impact on growth parameters. This study investigates some aspects of the effects of rapamycin on the cartilage growth plate.

Major concerns: The authors use rats given oral rapamycin for 2 or 4 weeks and evaluate numerous morphometric parameters and the expression of an array of proteins. While many of the results are intriguing, the authors extend their conclusions beyond the scope of their data. For example, the title states that rapamycin lowers chondroclastic activity yet the data shows no difference in OPG expression, RANKL is transiently depressed at 2 weeks but is restored at 4 weeks, and a decrease in the number of TRAP stained cells (this last is supportive of the statement). They do not actually measure anything that directly measures resorption activity and the OPG and RANKL data do not support the claim. This is just one example and there are several others throughout the manuscript [such as defining that the rapamycin treated animals need more calories to grow which can only be stated if in fact additional calories were provided and the animals grew--- unlikely since those animals had free choice access to food; this is stated in the results too]. The authors are encouraged to stay within the scope of their findings.

Though rapamycin has a clear effect on many of the parameters after 2 weeks of treatment (from 3-5 weeks of age), the rapamycin effect is gone for many of the measurements by 4 weeks of treatment. Though the study concluded after 4 weeks, one is left wondering whether the animals would exhibit catch up growth. That should be, at the very least, considered in the discussion that the effects of rapamycin may only be transient. If the growth retardation remained throughout development, that gives additional impact to the authors’ study. The authors do not give proper thought to the 4 week findings. The absence of data from 4 weeks in the abstract emphasizes this concern. The authors also equate provision of rapamycin for 4 weeks vs 2 weeks (starting at 3 weeks) to providing rapamycin at a later developmental age in the abstract and discussion. This is not accurate.
Both the tibia and femur were collected but it would appear (though the figure legends do not state that but the methods do) only the tibiae were used for the IHC and in situ hybridization. Why?

The images depicted in figure 3 do not support the data analysis presented.

Figure 4 does not support the statements the authors make in the results: IGFBP3 does not decrease 44% after 4 weeks of rapamycin as stated.

The data presented in Figure 4 of a smaller hypertrophic zone does not fit with the data in Figure 1 that reports 2 weeks of rapamycin increases the hypertrophic zone.

The lack of concordance between the rapamycin dose used and what is given to children is a concern especially since many of the measurements were back to control levels by 4 weeks of treatment. Is this really a good model for a human study?

The discussion lacks substance.

Minor Essential Points:

• Abstract Methods section: “anthropometric” refers to the measurement of humans. The authors are measuring rats.

• Abstract Methods section, last sentence the word “for” should be omitted: …“to evaluate for …”

• Background: should be BrdU

• Methods: should include whether both left and right bones were harvested, were both measured and average length used or?? What were the anatomical landmarks for measurement? What is the replicate number per animal or was a single section used per animal? Were all measurements and assays done using the proximal tibial growth plate?

• Methods: define what is meant by "50-60 cell profiles" were assessed. Is that 50 or 60 cells of a given region or ?? Also define the area used for the reporting of TRAP positive cells: total area or surface area at the chondro-osseous junction (the latter being more relevant).

• Methods: why was a one way ANOVA used rather than a multifactorial analysis used?

• Results: the authors note a positive correlation between PTH and IGF-I is serum (not expression as the authors state) which is difficult to reconcile given that at 4 weeks of age rapamycin decreases PTH and increases IGF-I. But the authors are only focusing on the 2 week treatment period and neglecting the 4 week.

• The tables need to state that the data are presented as means + 1 SD. The "anthropometric" in Table 1 title needs to be changed. The statistical significance designation is unclear.
• The figures are poorly presented. As in the tables, the statistical significance designation is unclear. The figures do not have the a, b, and c panel designations alluded to in the text. The presentation between table and figures differs with the ordering of the treatments (e.g., main category weeks in tables but rapamycin vs control in figures).

• The results section should contain only results; there are elements of discussion within the results.

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