Reviewer’s report

Title: Rivaroxaban does not impair fracture healing in a rat femur fracture model: an experimental study

Version: 2 Date: 22 October 2014

Reviewer: Hanna Schell

Reviewer’s report:

Minor Essential Revisions

I. BACKGROUND
Line 69: “…goes up to one third to one half …” sounds more logical to me.

II. METHODS
Line 118-20: How was the pain medication administered, how often and for how many days post surgery?
Line 128: Please explain the wide range of buprenorphine-dosage.
Please provide information how many animals were assorted to which group (biomechanical testing, histology) either here or in the following paragraphs. Otherwise it is difficult to follow your statistical and scientific conclusions.
Line 143 following: In this paragraph about the biomechanical testing no technical problems are mentioned, nevertheless it says in line 270 ff.: “Due to technical problems we could not reach minimal quantity to perform statistical analysis for the 49-days-old treatment group.” So please elaborate what the mineral quantity would be and what kind of technical problems occurred.
Line 174: “…embedded in methylmetacrylate for 5 days…” infiltrated would be more precise.

III. RESULTS
Line 205: “…cortical gap…” Do you mean the fracture gap?

FIGURE LEGENDS
Fig 1: Please mark the (former) fracture gap to make it visible.
Line 328: I assume you mean the fracture gap?

Major Compulsory Revisions

II. METHODS
Statistical Analysis
Please provide the exact p-values.
How was the data tested for normality?
Did you work with mean or medians and which tests were used for which analysis (Biomechanical testing, µCT, histology)?

Line 189ff.: “…CT scan were illustrated with Box and whisker plots…” please compare to Fig. 3 and adjust.

Line 190 ff.: “For histological analysis three longitudinal sections were examined for each fracture callus specimen with two callus specimens examined for each animal.” What kind of results were achieved. I cannot find any histological results in the manuscript, apart from the overview-pictures in Fig. 1. Please complete.

III. RESULTS

Line 202 ff.: “Therefore we could illustrate the cellular and mineralised components of the callus in the longitudinal axis, which provides vital information on bone turnover or bone formation and resorption.” Please describe your precise findings and illustrate them with high-magnification pictures.

Line 205: “…cortical gap…” Do you mean the fracture gap?

Unfortunately I cannot follow your conclusions (line 206-209) from the presented results.

Line 211 ff.: biomechanical analysis: To my opinion, the strength of the non-injured contralateral limb should have been taken into account as well. Since Rivaroxaban is administered systemically, a systemic effect might be possible and should be analyzed. Please comment on that.

Line 222: Callus volume of treated animals is 36.7 mm³ at 28 days. In line 225 a value of either 28.37 or 42.17 is given. Which values are the correct ones and were used for statistical analysis?

IV. DISCUSSION

Line 252f.: “Our results would not be meaningful if we could not differentiate between hypertrophic cartilage formations from bony bridging.” This is the first time, cartilage is ever mentioned in the manuscript. I don’t see the context and the expression is questionable. Please rephrase and generate context.

Line 257f.: “To quantify the callus mineralisation we performed micro CT scans, as a valid assessment for bone stiffness.” To my knowledge, there is more to bone stiffness than mineralization, for example bone diameter. Figure 1 shows a clearly higher bone diameter for the treated group. Could that account for the higher torsional stiffness? Please discuss. I assume the photographs in Fig. 1 are representative for the particular group?

V. CONCLUSIONS

Comparing the photographs in fig. 1 I see a fracture healing much more advanced in the untreated control group at both time points. The femoral bone has regained its original form, whereas the treated group shows abundant callus formation. Due to the low magnification, an analysis of cellular components and activities is not possible for the reader, but my macroscopic impression is described above. From the presented results I cannot follow the conclusion of the
authors that fracture healing is not downgraded by Rivaroxaban. Alternative interpretations of the results were not taken into account or discussed (as highlighted in my review of the discussion/biomechanics)

FIGURE LEGENDS

“3 weeks later femurs of rivaroxaban treated rat (A2 + B2) demonstrate increased bone tissue compared to control group (A3 + B3).” Is that a good or a bad sign after 7 weeks of healing in a rat? To my knowledge, fracture healing should be finished by then and remodeling processes cause the bone to regain its former form.

Line 334f.: “non-significant decrease of increase of callus…” ??

FIGURES

Fig. 1: Why do you only show overviews which does not allow any deeper insight in histology?

The orientation of the single photographs seems to be inconsistent (e.g. A3-B3). Please provide a scale-bar and describe orientation of the specimens (femoral head/condyle).

Level of interest: An article of limited interest

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

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

'I declare that I have no competing interests'