Reviewer's report

Title: Systemic zoledronate treatment both prevents resorption of graft bone and increases the retention of new formed bone during revascularization and remodelling. A bone chamber study in rats.

Version: 1 Date: 15 April 2006

Reviewer: Brigitte von Rechenberg

Reviewer's report:

General
The manuscript contains interesting material that is worth publishing. It reports about an experimental animal study in mice, where allogenic bone grafts were introduced in a titanium chamber and implanted in the tibia. There, graft resorption and/or new bone formation was followed over 6 weeks with or without the osteoclasts inhibiting drug zoledronate. Results demonstrated that grafts resorbed faster and new bone formation was less in untreated grafts. The authors conclude that treatment with zoledronate is an effective drug to prevent undue bone resorption in clinical cases of bone defects and osteonecrosis. Although the study shows potential, the manuscript shows weaknesses that need addressing before it can be published.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

General comments

Title:
The title should reflect that the authors were studying allografts and not autografts.

Abstract:
The description of the background does not reflect the content of the study. The authors did not prove that zoledronate reduces the resorption of necrotic bone, rather a frozen allogenic bone graft. In conclusions: The authors should limit their conclusions on bone grafts and not extend it to other orthopedic diseases, which were not part of the study. The abstract should be re-written.

Background:
Page 5, first paragraph: Why do the authors start with the topic of osteonecrosis. Osteonecrosis is different on a pathological level than a bone matrix, where living cells have been destroyed through freezing. The implantation of allogenic, pretreated allografts should not be compared with conditions such as corticosteroids treatment, sickle cell anemia, etc. Also the subchondral bone has other problems related to overlying cartilage degeneration. The authors should clearly stay with i) (allogenic) graft survival and remodeling and ii) effect of bisphosphonates in their introduction. Although the results of this study may once be applied in cases of osteonecrosis and other diseases mentioned in the first paragraph, they are not the focus of the current study. The reviewer recommends to start with the second paragraph where bisphosphonates are introduced and then in a second paragraph summarize their findings with the first study where the authors tested obviously the effect of alendronate.

Methods:
The authors rely on the reader to be familiar with their first study. Although this is partly legitimate, each manuscript has to stand on its own. Therefore, the authors need to be more specific about the surgical method of implantation and screw fixation. Also the method of anaesthesia and analgesia of the rats should be given to allow the reader to assess whether animal welfare was maintained during
the study.
The description of how the authors performed the statistical analysis is missing, although they report in the results sections their P-values. Please, give short description.

Conclusions:
Again, the authors should focus on their own study with bone grafts and discuss these results. A critical comparison of results of the current with their previous study is missing. Also missing is a critical view on graft resorption in controls in a chamber without mechanical load. Would another animal model, where grafts would have been subjected to mechanical load, have had another outcome? They mix their discussion and conclusions with other orthopedic diseases that they have not studied in this experiments and therefore should exclude.

Specific comments:
Page 3, first paragraph: Background:
Line 1: The first sentence about osteonecrosis is out of context. Focus on the grafts.
Line 4: …..osteonecrosis occurs …..
Line 6: Was it really necrotic bone or bone grafts?

Page 3, second paragraph: Methods
Line 3: The rats were killed, and their tibiae were harvested.

Page 3, third paragraph: Results
Line 1: …control specimens in the titanium chambers were almost totally resorbed …..

Page 4, first paragraph: Conclusions
Line 4: In our model an increased net formation of new bone was found in the grafts which…..

Page 5, first paragraph: Background
Delete entire paragraph and use second paragraph for introduction about the effect of bisphosphonates.

Page 5, second paragraph:
Line 7: Did they really show that bisphosphonates reduce the resorption of necrotic bone in the true pathological sense, or just mineralized bone? Tumor metastases may contain nectrotic bone, but usually there are also viable cells and living bone parts. Osteoporosis has also nothing to do with necrotic bone.

Page 6, paragraph 2:
Use this paragraph as introduction about bisphosphonates. Instead add a paragraph about the implantation of pretreated allografts including immunological aspects. Take reference to the first study with alendronate.

Page 7, paragraph 1:
Specify the surgical implantation enough that the reader can follow the technique. Give anaesthetic and analgetic regimen.

Page 7, paragraph 2: Grafts
Line 1: How were the donor animals killed?
Line 4: Were the bone grafts harvested under constant irrigation while using the cutter?

Page 7, paragraph 3: Surgical procedure
Line 3: What was the size and diameter of the hole?
Line 4: Give screw size
Line 5: Was the chamber now placed subcortically or subcutaneously?

Page 7, paragraph 4: Injections
Line 1: When were the injections started, directly after implantation or later? When was the last injection before sacrifice?
Page 8, paragraph 2:
Line 4: What was the magnification and how were the power fields chosen, such as parallel or at the
front of the original graft?
Line 6: Give number of official animal permission in brackets.

Page 9, paragraph 1:
Line 1: In all other specimens ......
Line 3: Were all grafts (controls and treated) replaced “halfway through the grafts”? Or were there
differences between groups? One would assume if the histologic measurements are considered.
Line 4: Did the bone deposit directly onto the mineralized matrix of the control grafts, or was there
fibrous or marrow tissue in between remodeled grafts and old matrix?

Page 10, paragraph 1:
Line 2: It is not correct that the authors showed in this study that the zoledronate is as effective in
preventing bone resorption as alendronate. They only demonstrated that in grafts treated with
zoledronate more bone was present compared to untreated controls. The authors can discuss their
previous results with alendronate in the same animal model, but not include the previous results in
the current study. Re-write the first paragraph.
Line 4: Discuss sc versus iv. Injection separately, if this point is really based on different results with
iv Injections in other studies. Otherwise leave it away.

Page 10, paragraph 2:
Line 9: “Osteoclasts are necessary for bone formation by the osteoblasts”. This statement is not
correct. The relationship between osteoclasts and osteoblasts is different and more complex.
Osteoclasts are dependent on cell signals released by osteoblasts to get recruited and activated
(IL-1, IL-6, PGE2 and possibly IL-11). Their activity is again inhibited through the mediator NO, and
through the soluble substances released by the bone matrix after osteoclasts resorption.

Page 11, paragraph 1:
Line 7: …be discussed, such as that in contrast to cortical bone....... 
Line 17: leave necrotic away.

Page 11, paragraph 2:
At the end the authors should add something about long-term remodeling of the grafts under the
influence of bisphophonates?

Figure & figure legend:
Fig.1: It would be desirable if it were indicated where and how the graft chamber is fixed in relation tc
the tibia.
Fig.2 and 3: Make clear where the old graft and the newly deposited bone is in relation to the picture
in both, the legend and the figures.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the
author can be trusted to correct)

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the
major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research
interests

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

The reviewer has no competing interests with this study.