Bilateral Distal Femoral Epiphyseal Defect Models for Safety Testing: A 5-Week Rat Bone Healing Study

Luis Fernando Negro Silva, Julius Haruna, Pritpal Malhi, Simon Authier, Yannick Trudel, Raluca Kubaszky, Michel Assad
INTRODUCTION
Low trauma fractures represent clinical complications for an aging population. Most fractures are known to occur in long bones with consequences such as pseudoarthrosis and relatively high non-union rates. In the last 20 years, substantial efforts in the field of biomechanical and biomaterials research have been devoted to the development of synthetic osteoconductive graft substitutes. The grafts may also contain drug-eluting therapeutic agents with osteoinductive and antimicrobial properties to improve bone healing and to prevent iatrogenic infections. The rat is the most frequently selected preclinical rodent model to investigate drug safety, however scarce data is available relative to the use of this murine species in orthopedic models.

MATERIALS AND METHODS
Following an IACUC-approved protocol from an AAALAC-accredited institution, nine (9) female Sprague-Dawley rats were used to evaluate whether 2-mm critical-size defects in distal medial lateral femoral condyles were adequate for use in drug safety evaluation focusing on potential impacts on bone remodeling. Under anesthesia, both hindlimbs were prepared and draped under sterile condition. Then, bilateral cylindrical defects of 2x5mm (DxL) were created in distal medial lateral femoral condyles (Figure 1). Digital radiography was performed on all femurs immediately after surgery and 5 weeks post-surgery (Figure 2). At 5 weeks post-operative, all femoral defect sites were harvested, decalcified, and embedded in paraffin. Central longitudinal histological sections were then taken from the bone defect site and prepared for microscopic examination by staining with hematoxylin and eosin (H&E) and Goldner’s Trichrome.

Histopathology scoring was performed on the quality of new bone formation, while the extent of new bone formation was quantified by histomorphometry. Regardless of treatment, all defect sections demonstrated clinically significant bone remodeling characterized by cortical bone repair and an ingrowth of bony trabeculae consisting of a mixture of woven and lamellar bone (Table 1, Figures 3 to 7). These results demonstrate that creating a 2-mm long-bone defect can be used to evaluate potential drug effects on bone healing in preclinical models. In contrast, a 2-mm femoral epiphyseal defect would not be considered a critical-size defect in the rat long bone, but larger bone defects (e.g. ≥3mm) may be explored for this purpose. When this is achieved, this murine femoral defect model may then represent a promising model to evaluate the superiority of new bone substitutes with osteoconductive and osteoinductive properties.

RESULTS AND CONCLUSION

Regardless of treatment, all defect sections demonstrated clinically significant bone remodeling characterized by cortical bone repair and an ingrowth of bony trabeculae consisting of a mixture of woven and lamellar bone (Table 1, Figures 3 to 7). These results demonstrate that creating a 2-mm long-bone defect can be used to evaluate potential drug effects on bone healing in preclinical models. In contrast, a 2-mm femoral epiphyseal defect would not be considered a critical-size defect in the rat long bone, but larger bone defects (e.g. ≥3mm) may be explored for this purpose. When this is achieved, this murine femoral defect model may then represent a promising model to evaluate the superiority of new bone substitutes with osteoconductive and osteoinductive properties.

Table 1 - Quantification of bone ingrowth

<table>
<thead>
<tr>
<th>New Bone Formation Sham Defect Right Femur (%)</th>
<th>New Bone Formation Saline/Defect Left Femur (%)</th>
<th>Soft Tissue Formation Sham Defect Right Femur (%)</th>
<th>Soft Tissue Formation Saline/Defect Left Femur (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 95.66</td>
<td>95.50</td>
<td>4.34</td>
<td>4.50</td>
</tr>
<tr>
<td>SD 3.65</td>
<td>4.37</td>
<td>3.65</td>
<td>4.37</td>
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<tr>
<td>N 9</td>
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No significant differences between sham and saline-treated groups.
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