EXTRACORPOREAL SHOCKWAVE THERAPY AS A COUNTER MEASURE FOR BONE LOSS ON EARTH AND IN SPACE

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INTRODUCTION

Human bone continually remolds itself as part of the normal repair and regeneration process. The fundamental mechanisms of remodeling are not well understood, but the process appears to be triggered through normal microdamage incurred during physiological loading. This microdamage may cause trauma to the cells, disrupt normal transport of cytokines, chemokines, growth factors and other signaling molecules [1]. Each of these factors may, in turn, trigger a cascade of cellular events that result in osteoclastic resorption and subsequent osteoblastic apposition. The ultimate goal of this research program is to apply extracorporeal shock waves in the intent to mimic naturally occurring microdamage that stimulates bone tissue to rebuild. Whereas a continued lack of physiological activity, including exposure to microgravity, will result in osteoporosis or osteopenia, our working hypothesis is that prophylactic application of extracorporeal shock waves to create microdamage in bone will stimulate the remodeling, repair and renewal cascade. A critical first step in testing our hypothesis was to test the feasibility of using lithotripsy to create microdamage in bone. In the current study, shock waves are applied to an *ex vivo* and *in vivo* rat femur model to assess acute damage induced by shock wave application.

MATERIALS AND METHODS

Since these studies are unprecedented, an *ex vivo* study was first carried out to define appropriate lithotripsy regimes for creation of microdamage in the middiaphysis of the rat femur. Thereafter, the protocols were applied in *vivo* to two cohorts of five rats and microdamage was assessed.

*Ex vivo study:* Ten skeletally mature rats (325 ± 25 g) were obtained in a frozen state from a previous study. Nine rats were subjected to lithotripsy (Modulith®, SLX lithotripter, Storz Medical AG, Kreuzlingen, Switzerland) at one of three energy levels (applied at 2 GHz) and numbers of waves (e.g. 0.46 mJ/mm² at 500, 1000, and 1500 waves, Table 1). One rat served as a baseline control. The treatment was administered to the middiaphysis of the right femur and comparisons were made with the contralateral control femur.

*Table 1. Experimental matrix for *ex vivo* study.*

<table>
<thead>
<tr>
<th>Sample Treatment</th>
<th>0.46 mJ/mm²</th>
<th>0.82 mJ/mm²</th>
<th>1.06 mJ/mm²</th>
</tr>
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<tbody>
<tr>
<td>500 waves</td>
<td>1000 waves</td>
<td>1500 waves</td>
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*In vivo study:* This study was IACUC approved. Twenty skeletally mature rats (325 ± 25 g, one at 255g) were anaesthetized via 3% Isoflurane (Abbott Laboratories, Chicago IL) inspiration. Four groups of five rats were subjected to either the 15 kV lithotripsy treatment or the 1.06 mJ/mm² lithotripsy treatment for 500 or 1500 shocks, resp. (Table 2), at a rate of 2 GHz, while under anesthesia. The shock waves were applied to the middiaphysis of the right femur, with the same focal volume as in the *ex vivo* experiments. The rats were euthanized immediately after surgery with CO₂ asphyxiation. The samples were analyzed versus the contralateral femur.

*Table 2. Experimental matrix for *in vivo* study.*

<table>
<thead>
<tr>
<th>Sample Treatment</th>
<th>0.46 mJ/mm²</th>
<th>1.06 mJ/mm²</th>
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<td>500 waves</td>
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<td>1500 waves</td>
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*Specimen preparation and analysis:* Post-lithotripsy procedures were the same for *in vivo* and *ex vivo* studies. Femora were excised, fixed in ethanol, and then bulk stained in 0.5mM calcein for 4 hours under vacuum. The samples were then embedded in PMMA and the mid-diaphysis of each bone was serially cut and polished into 20 transverse slices (100 ± 10 µm). From each animal, three sections within the focal volume of the lithotripter were analyzed; the three comparable contralateral specimens were analyzed as a baseline control. Specimen morphology was evaluated using an epifluorescent microscope with computer controlled stage (Leica, Exeter, PA). Microcracks were defined as oblique structures in the bony matrix that were stained by calcein. The number of cracks (Cr.Nu), mean crack length (MnCr.Le.), and total crack length (To.Cr.Le.) were measured using OpenLab imaging software (Improvision, Lexington MA).

RESULTS

Ex *vivo* study: Microcracks were observed in all bones subjected to shock wave treatment and were located within the volume of focus of the lithotripter. The average crack length was 100 ± 35 µm, which falls in the general range of sizes reported in the literature. All bones treated with shock waves showed significantly higher numbers of microcracks than did their contralateral controls (*p < 0.05*).

In *vivo* study: As in the *ex vivo* study, microcracks were observed in all treated bones (Fig. B) and were confined to the volume of focus of the lithotripter. For the cohort subjected to 1500 waves at 1.06 mJ/mm², the mean number of cracks within one plane of the focal volume was 18.56 ± 6.8 (Fig. A) and the mean crack length was 91.62 ± 6.7 (Fig. A). All bones treated with shock waves showed significantly higher numbers of microcracks than the contralateral controls (*p < 0.05*). The animals tolerated the procedure well and no outward signs of discomfiture were apparent. In a limited number of rats, a pin prick sized haematoma was visible subcutaneous to the application area.

![Figure A-1](image1.png)

**Figure A-1** Total Number of Cracks

**Figure A-2** Net Length of Cracks

![Figure B](image2.png)

**Figure B** Microdamage near periosteal surface of bone section take from within the focal volume.

DISCUSSION

These studies show, for the first time to our knowledge, that exogenous application of extracorporeal shock waves in living rats result in microdamage that is morphologically similar to damage produced through physiologic loading. The *ex vivo* study served to define an appropriate treatment regime for the rat femur. Application of this regime *in vivo* was a critical next step in testing the efficacy of this novel treatment as a countermeasure for bone loss on Earth (osteoporosis) and in space (osteopenia).

REFERENCES


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