ANABOLIC EFFECTS OF INTERMITTENT PTH THERAPY ON BONE TRAUMA HEALING UNDER SIMULATED WEIGHTLESSNESS CONDITIONS

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INTRODUCTION

In the absence of effective and practical countermeasures, astronauts could develop sufficient osteopenia to make the occurrence of a cortical bone fracture under normal loading highly likely during long duration space flight (e.g. Mars mission). Ongoing research studies in our lab, and that of previously published reports, indicate that bone trauma healing under simulated weightlessness is significantly impaired [1-5]. Thus, it is likely that bone fractures incurred during long duration space flight will be impaired and may potentially lead to a fracture non-union. The occurrence of fracture in an astronaut and particularly those fractures that fail to heal would present a deep space mission team with problems of decreased mission effectiveness. As well, severe secondary complications may occur, potentially leading to a life-threatening situation for the astronaut. Thus, effective countermeasures that normalize the repair response will be required.

One solution to the above dilemma would be to enhance callus remodeling by incorporating an adjunctive anabolic therapy, such as intermittent PTH therapy, to promote bone formation and remodeling. With respect to its potential use in deep space missions, intermittent PTH therapy has been shown to reduce hind limb bone loss in ovariactomized tail-suspended rats, and enhance the amount of bony callus tissue surrounding tibial fractures in rat [6-7]. In the current study intermittent PTH therapy was utilized in an attempt to normalize the impaired fracture healing that results from non-weight bearing conditions. We hypothesized that intermittent PTH therapy will augment and normalize the impaired cortical bone healing under non-weight bearing conditions.

METHODS

Model System – A state of weightlessness was modeled using a conventional hind limb unloading protocol in the rat species.[1]

Typically, cancellous bone volume loss of 3-5% after four weeks is expected with this model system, similar to the rate of bone loss experienced by astronauts. After confirming a state of osteopenia in the non-weight bearing groups at seven weeks, all rats were subjected to bilateral, mid-diaphyseal fibular osteotomies [8]. Bilateral 0.2-mm wide osteotomies were placed in the mid-diaphysis of both fibulae.

Experimental Groups – Six-month-old female Sprague-Dawley rats were used in these studies. They were either subjected to a hind limb unloading protocol for seven weeks (non-weight bearing, NWB; n = 5 per group), or allowed free cage activity (weight-bearing, WB; n = 5). These rats were subjected to bilateral, mid-diaphyseal fibular osteotomies at the end of the 7-week period. The NWB rats were divided into three groups (n=5 per group) and given different doses of PTH: 0 (control group), 80, or 120 µg/kg BW, 5x per week beginning immediately post surgery for a 5-week period.

Outcomes - Values for the following parameters were collected from the hard callus (or calcified reparative) tissue region: volume of hard callus, rate of callus formation, and cortical bone volume. Specifically, the hard callus repair response was monitored longitudinally using in vivo micro-computed tomography (micro-CT) imaging at 1-, 7-, 14-, 21-, and 28- days post-op. Rate of hard callus formation was calculated from the time-dependent change in segmented cancellous bone tissue within the healing callus region from day 1 to day 14. The extent of hard callus bridging was assessed from ex vivo images obtained at 35 days post-op (5-week time point).

RESULTS

The fibular osteotomies, for rats in the WB group, healed with a full bridging hard callus by five weeks post surgery (Figure 1A). Conversely, rats in the NWB group exhibited and incomplete bony union across the trauma site. (Figure 1B). For the PTH treated groups, mineralized callus formation was evident, however not uniform when compared to the WB group (Figure 1C and 1D).

Table 1 lists the parameter values for hard callus and cortical volumes and callus formation rate. The maximum extent of hard callus volume occurred at day 14 (WB, NWB), day 28 (NWB,120 PTH), and day 21 (NWB,0 PTH). The callus volume was only 20% for NWB, 35% for NWB,80 PTH and 32% for NWB,120 PTH that of the WB callus content (p < 0.001, ANOVA). The rate of hard callus formation was diminished to only 19% for NWB, 28% for NWB,0 PTH, and 31% for NWB,120 PTH groups as compared to that of the WB group. The cortical volume, representative of a periosteal reaction, was only 28% for NWB, 42% for NWB,0 PTH and 3% for NWB,120 PTH that of the WB cortical volume content (p < 0.001, ANOVA).

While callus volume increased relative to the PTH dosage in the NWB groups, this increase was not found to be significant. Further, an unforeseen drop in cortical bone formation rate was witnessed in the NWB,120 PTH group.

Table 1: Hard callus parameter values

<table>
<thead>
<tr>
<th>Group</th>
<th>Callus Volume</th>
<th>Formation rate</th>
<th>Cortical Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>6.99 ± 1.82</td>
<td>1.50</td>
<td>2.74 ± 0.44</td>
</tr>
<tr>
<td>NWB,0 PTH</td>
<td>1.39 ± 0.60</td>
<td>0.65</td>
<td>0.76 ± 1.21</td>
</tr>
<tr>
<td>NWB,80 PTH</td>
<td>2.44 ± 1.26</td>
<td>0.97</td>
<td>1.16 ± 1.55</td>
</tr>
<tr>
<td>NWB,120 PTH</td>
<td>2.21 ± 1.08</td>
<td>1.08</td>
<td>0.07 ± 0.59</td>
</tr>
</tbody>
</table>

* Determined from a 1-cm region of interest at the osteotomy site

DISCUSSION AND CONCLUSIONS

Bone trauma does not heal normally in rats during an extended period of simulated weightlessness. These initial findings support a potential effectiveness of intermittent PTH therapy to augment bone trauma healing under conditions of extended weightlessness. The 80 µg/kg group had the greater increases in cortical bone volume as well as in callus volume. However, in the case of the 120 µg/kg PTH group we witnessed a drop in cortical bone formation rate. The low range of data for the 120 µg/kg group could possibly be due to varied responses, or it could indicate a dose threshold after which treatment is rendered ineffective. While these data validate our hypothesis that the healing response has been improved, it has not been optimized. Specifically, further studies to evaluate whether (1) lower dosages of PTH peptide are reasonable or (2) if a combination therapy approach would be required to normalize the bone regeneration response and ameliorate the impaired fracture repair of NWB situations are warranted.

ACKNOWLEDGEMENTS

This work was supported by (1) the National Space Biomedical Research Institute through NASA NCC 9-58, grant BL00045, (2) Post-Doctoral Trainee Award, Cleveland Clinic Orthopaedic Research NIH Training Grant (T32 AR 50959), and (3) imaging in part by NIAMS Cleveland Clinic Musculoskeletal Core Center Grant #1P30 AR-050953. Technical support provided by Laura Burch and Matt Richardson.

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