LOW MOLECULAR WEIGHT HEPARINS CAUSE OSTEOBLAST APOPTOSIS - A PUTATIVE MECHANISM FOR IATROGENIC OSTEOPENIA

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Introduction
Thromboprophylaxis using a low molecular weight heparin (LMWH) results in reduced vascular disassembly and delayed bone formation in a rabbit model of fracture healing1. Prolonged LMWH administration decreases cancellous bone volume and circulating alkaline phosphatase levels in a rat model2, and pathological fractures have been found in otherwise healthy individuals receiving long-term LMWH therapy3. However the mechanism of LMWH induced osteopenia remains unclear. Osteoblast apoptosis, or programmed cell death, is a key regulator of osteoblast cell number, both in physiological skeletal turnover and in conditions which disrupt the dynamic balance between bone formation and resorption4. This current study tested the hypothesis that alterations in osteoblast activity and life span (i.e. apoptosis) could explain the reduced bone formation associated with LMWH therapy.

Materials and Methods
Experiment 1
12 Male New Zealand White rabbits underwent fracture of floating ribs and were randomized to receive either enoxaparin 2 mg or saline SC daily for 14 days. After 28 days the rib was excised and either tested in torsion for strength, stiffness and energy absorbed to failure, or examined for cell apoptosis using TUNEL immunostaining.

Experiment 2
Primary human osteoblasts, isolated from patients undergoing emergent trauma surgery, were treated with TNFα and anti-Fas IgM, or enoxaparin (0 – 3.2 ug/mL) for 24 hours, in the presence or absence of a peptide inhibitor of caspase 6 and 8 (IETD), and cell apoptosis was measured using annexin V and propidium iodide staining on flow cytometry. To determine the mechanism of LMWH induced apoptosis, osteoblasts were treated with enoxaparin in the presence or absence of a reactive oxygen species scavenger PDTC, and the percentage of cells with impaired mitochondrial membrane potential was determined using the DePsipher dye method. To examine the effect of LMWH on osteoblastic activity, cells were treated with enoxaparin (0-3.2 ug/mL) for 0 – 18 days in the presence or absence of VEGF 10ng/mL, and alkaline phosphatase activity (nitrophenol assay) and bone nodule formation (von Kossa staining counted at X40 magnification) were measured. The following data represents the mean +/- standard deviation. ANOVA was employed to determine statistical significance* at p < 0.05. N=6 patients for in vitro experiments; each measurement was performed in triplicate. N=6 rabbits/group for in vivo data.

Results
Daily enoxaparin resulted in increased callus cell apoptosis and reduced biomechanical integrity when compared to controls.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>APOPTOTIC INDEX</th>
<th>STRENGTH (Nm)</th>
<th>STIFFNESS (Nm)</th>
<th>ENERGY (Nm/deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>11 (5)</td>
<td>0.21 (0.03)</td>
<td>0.014 (0.001)</td>
<td>1.98 (0.5)</td>
</tr>
<tr>
<td>LMWH</td>
<td>37 (7)*</td>
<td>0.15 (0.002)*</td>
<td>0.01 (0.001)*</td>
<td>1.64 (0.4)</td>
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Conclusions
Thromboprophylaxis with enoxaparin increases programmed cell death in fracture callus and attenuates bone repair in a rabbit model. In vitro, LMWH significantly reduces bone formation and differentiation, while the life span of primary human osteoblasts is significantly reduced through primarily caspase independent mechanisms.

References