LOW MOLECULAR WEIGHT HEPARIN DELAYS ENDOCHONDRAL OSSIFICATION DURING FRACTURE HEALING

*Street, J; *O' Regan, K; *McGrath, M; *McGuinness, A; *Redmond, H Paul
+*Department of Academic Surgery, Cork University Hospital,, Cork, Ireland. Department of Surgery, Cork University Hospital, Cork, Ireland, 353 21 922371, Fax: 353 21 344230, hrb_street@yahoo.com

Without prophylaxis, deep-vein thrombosis (DVT) occurs in 50-70 percent of patients undergoing total hip arthroplasty, total knee arthroplasty, acute fixation of hip fractures and in patients with multiple injuries. In Europe at least, low-molecular-weight heparins (LMWH) are the gold-standard for thromboprophylaxis in orthopaedic surgery and their use reduces the risk of associated DVT by approx 50%. Like unfractionated heparin (UFH) LMWH exerts its anticoagulant effect by activating antithrombin, while its advantages over unfractionated heparin include more predictable anticoagulant effect, better bioavailability at lower dose and a longer half-life. LMWH causes less bleeding than UFH because of less platelet binding, reduced effect on microvascular permeability and lower affinity for endothelial cells. However, LMWH, in a rat model, decreases cancellous bone volume, decreases osteoblast and osteoid surface and decreases circulating alkaline phosphatase activity in a dose dependant manner, with no effect on osteoblast activity. Similarly, in the clinical setting, when given for more than 8 weeks, LMWH may cause osteoporosis (2.6 %). Endothelial cells and pericytes disassociate from native vasculature and transform into bone and cartilage progenitor cells in a process of disassembly. Brighton demonstrated that transformed pericytes from a disassembling microvessel retain basal lamina remnants which are also found in chondroblasts and polyomorphous mesenchymal cells. Indeed the pivotal role of vasculature to chondrogenesis and osteogenesis has very recently been illustrated by Gerber et al. These facts considered we tested the hypothesis that LMWH through its effect on the transforming vascular cells would delay endochondral ossification during fracture healing.

Thirty-six male New Zealand white rabbits, aged 16 weeks, each weighing ~ 2.1kg, were anaesthetised and two of the floating ribs on each side of every animal were fractured by closed manual manipulation. The animals were randomised to either treatment group receiving Enoxaparin (Rhone Poulenc Rorer, Ireland) 2mg (in 400µl saline SC-OD. Six animals from each group were euthanised after healing for 3, 7 and 14 days. All procedures were approved by the University Research Ethics committee. The fractured ribs were excised, fixed in neutral buffered formalin and decalcified in 5% EDTA for six weeks. The fracture callus was isolated and callus index was then determined.

Callus index = maximum diameter of callus (mm)

These specimens were then cut longitudinally and wax embedded for sectioning and immunohistochemical staining. After routine H&E and toluidine blue staining fibrin clot was measured and fracture healing was graded (1-10) as described by Huo. Blood vessel proliferation and immaturity index were determined by immunohistochemical staining with monoclonal antibodies for Ki 67 and α-smooth muscle actin respectively (Dako Chemicals). Medullary cell proliferation response was determined as the number of Ki 67 +ve cells/field.

Blood vessel immaturity index = α-smooth muscle actin negative vessels/Total vessel number

Cartilage and bone progenitor units were identified by characteristic appearance on α-smooth muscle actin and H&E staining and counted per field.

Results represent mean ± standard error of the mean, n=6 in each case, each specimen was stained in triplicate. Analysis of variance was used for statistical analysis and a p value <0.05 was considered significant (* indicates versus control at the same time following fracture).

Daily LMWH therapy resulted in a significant reduction in callus index compared to controls. Following 3 days thromboprophylaxis the intraf emergent fibrin clot was significantly increased and the medullary cavity cellular response significantly decreased. At 7 and 14 days following fracture total blood vessel number was markedly increased in the LMWH treated group while blood vessel immaturity index and vascular cell transformation index was significantly reduced. Ultimately fracture healing and callus maturity were significantly delayed following LMWH treatment at both 7 and 14 days (See Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Control Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>LMWH Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callus index</td>
<td>1.4</td>
<td>1.9*</td>
<td></td>
<td>1.4</td>
<td>1.9*</td>
<td></td>
</tr>
<tr>
<td>Fibrin clot (mm³)</td>
<td>1.5±0.2</td>
<td>0</td>
<td>2.8±0.4*</td>
<td>1.05±0.1*</td>
<td>0</td>
<td></td>
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<tr>
<td>Medullary proliferation</td>
<td>127±29</td>
<td>374±34</td>
<td>775±38</td>
<td>85±33*</td>
<td>244±56*</td>
<td>651±31*</td>
</tr>
<tr>
<td>Healing (Grade)</td>
<td>3</td>
<td>6</td>
<td>2*</td>
<td>4*</td>
<td></td>
<td></td>
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<tr>
<td>EC transformation</td>
<td>---</td>
<td>64±5</td>
<td>---</td>
<td>36±3*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Indices of vascularisation and fracture healing, comparing Control and those treated with low molecular weight heparin at days 3, 7 and 14 following fracture.

These studies are mandatory to fully establish the effects of these products on fracture healing.

In summary therefore, administration of a therapeutic dose of low molecular weight heparin significantly delayed endochondral ossification 7 and 14 days following fracture. This attenuation in bone repair is directly consequent on the clot resorbs. This correlates with previous reports from Weitz suggesting that fracture hematoma is markedly attenuated and does not begin to recover until the clot resorbs. These data suggest that the use of LMWH in the treatment of fractures will improve fracture healing.

References:
5. Greuber et al, Nature Medicine, June 1999