Introduction:

It has been demonstrated, that the systenical application of growth hormone (GH) results in enhancement of fracture healing (1,2). However, whether GH itself or its mainly liver-produced mediator insulin-like growth factor I (IGF-I) exerts the bone promotive effect at the fracture site remains unclear. In a model using small defects in rat mandibulae, it was demonstrated, that in combination with osteopromotive membranes locally as well as systemically administered GH accelerates defect healing (3). However, to our knowledge, there exist neither quantitative data about the effect of local administered GH in a fracture healing model, nor a comparison with the local administration of IGF-I in-vivo. Therefore, we performed a histomorphometrical analysis of the the callus in a femur osteotomy model in rats in order to determine, whether local administration of GH and its mediator IGF-I leads to different callus stimulation.

Methods:

The left femurs of 24 female Sprague-Dawley rats were osteotomized (creating a gap of 3 mm) after a monolateral external fixation device was applied. All procedures were carried out with the ethical permission from the animal rights protection authorities. In each animal a mini-osmotic pump with a flexible tube at the fracture site was implanted. Using this pump, 8 animals received 100 µg/kg bodyweight/day human GH (group III), 8 received 100 µg/kg bodyweight/day IGF-I (group II) and 8 received phosphate buffer as placebo (group I) via the flexible tube. The femurs were harvested after 21 days and processed for histomorphometrical analysis (4 µm decalcified serial slices). The sections were stained with Safranine-O stain combined with light-green stain. The regions of interest were digitized and processed using the KS 400 image analysis workstation (Zeiss, Oberkochen, Germany). With specially developed algorithms, the following parameters were evaluated: callus area (Cl.B.Ar.), mineralized callus area (Cl.Md.B.Ar.), cartilage area (Cg.Ar.) and the callus diameter (Cl.Wd.). Based on this measurements the callus bone density (Cl.B.Dn.) and the cartilaginous share of the callus area (Cg.Ar./Cl.B.Ar.%). The Mann-Whitney – U test was used to determine differences between the treatment groups.

Results:

One animal in I group and group III, respectively had to be excluded because of secondary dislocation of the fracture. The Cl.B.Ar. and the Cl.Md.B.Ar. was significantly higher in the GH - treated and the IGF-I-treated group compared to the placebo group (Tab. 1). The Cg.Ar. and the Cg.Ar./Cl.B.Ar.% was nearly doubled in the placebo group compared to group II and III. However, due to huge standard deviations, there was no significant difference. The structure of the callus, represented by the Cl.B.Dn. and the Cl.Wd., was not different between the three groups (Tab 1).

Table 1: Histomorphometrical parameters, the data are given ± standard deviation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
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<tbody>
<tr>
<td>Cl.B.Ar. (mm²)</td>
<td>7.75 ± 5.04</td>
<td>10.9 ± 3.24*</td>
<td>10.97 ± 2.03*</td>
</tr>
<tr>
<td>Cl.Md.B.Ar. (mm²)</td>
<td>5.04 ± 2.11</td>
<td>8.31 ± 2.38*</td>
<td>8.0 ± 1.21*</td>
</tr>
<tr>
<td>Cl.B.Dn. (%)</td>
<td>64.6 ± 9.93</td>
<td>76.47 ± 7.27</td>
<td>73.59 ± 6.43</td>
</tr>
<tr>
<td>Cl.Wd. (mm)</td>
<td>5.11 ± 0.81</td>
<td>5.58 ± 0.54</td>
<td>5.09 ± 1.11</td>
</tr>
<tr>
<td>Cg.Ar. (mm²)</td>
<td>0.41 ± 0.37</td>
<td>0.24 ± 0.23</td>
<td>0.25 ± 0.28</td>
</tr>
<tr>
<td>Cg.Ar./Cl.B.Ar.%</td>
<td>4.4 ± 3.4</td>
<td>2.11 ± 2.21</td>
<td>1.87 ± 1.65</td>
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</table>

* significant difference to placebo group (group I) p < 0.05

Discussion:

We sought to determine whether local administration of both GH or its mediator IGF-I lead to similar acceleration of fracture healing or whether these substances show a different effect on callus formation. Our results demonstrate, that after 21 days of fracture healing local application of both, GH and IGF-I increases hard callus formation nearly to an equal amount. Similarly, both substances change the callus composition in the same way towards a lower share of cartilage. The results propose, that GH may exert a direct, nonliver mediated effect on fracture healing.

One weakness of the study is, that the data left not judge, whether the lower callus area in the control group results from advanced remodeling. However, the slightly lower callus bone density and the higher share of cartilage in the placebo group argue against that assumption.

In conclusion, our findings in fracture healing may resemble the so called “dual effector theory”, which hypothesised a direct effect of GH on longitudinal bone growth additionally to the sytemical effect mediated by IGF-I (4,5).

References:

(1) Bak et al., Bone 11: 233, 1990.

Listing of additional author affiliation:

**Medical Research Lab.M and Medical Department M (Diabetes and Endocrinology), University of Aarhus, Aarhus Kommunehospital, aarhus C, Denmark