Heparin-like Polysaccharides Markedly Reduce Osteolytic Bone Destruction and Tumor Growth in a Mouse Model of Breast Cancer Bone Metastasis

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Results

In vitro coagulation

Table 1: The effects of K5-NSOS, fragmin and heparin in in vitro coagulation assays (two materials and methods for detail).

<table>
<thead>
<tr>
<th>Compound</th>
<th>aXa (IU/mg)</th>
<th>aIIa (IU/mg)</th>
<th>APTT (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>228</td>
<td>198</td>
<td>101</td>
</tr>
<tr>
<td>Fragmin</td>
<td>155</td>
<td>74</td>
<td>9.4</td>
</tr>
<tr>
<td>Heparin LEO</td>
<td>148</td>
<td>132</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Materials and methods

- In vitro coagulation assay: Anti-a and anti-X activities of fragmin, Heparin LEO and K5-NSOS were determined utilizing Chromogenic 2, 3-TM Thrombin assay kit according to the manufacturer’s protocol (Instrumentation Lab. Company). The effects of the test compounds fragmin, Heparin LEO and K5-NSOS on activated partial thromboplastin time (APTT) in citrated plasma were measured using 2, 3-TM Thrombin kit lyophilized silica kit (ILS Laboratories Scandinavia).

Aim of the study

We have evaluated the theoretical potential of two HLGAGs with low anticoagulant activity, a low-molecular-weight synthetic heparin (fragmin, dalteparin, Pharmacia), and a high-molecular-weight K5-derived heparin-like polysaccharide (K5-NSOS, prepared according to Casu et al. (1)), to inhibit osteolytic bone destruction and tumor growth in an experimental mouse model of breast cancer bone metastasis.

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- In vitro cell adhesion assay: MDA-MB-231(SA) cells were pre-incubated with HLGAGs and then transferred to wells (Real-Time Protein A coated, Pierce) coated with E- and P-selectin, and ICAM-1 Fc chimeras (2 μg/well; R&D Systems). After removal of non-adherent cells, cell proliferation was measured using a proliferation agent (Roche Diagnostics).

- Breast cancer bone metastases mouse model: MDA-MB-231(SA) human breast cancer cells (10⁷) were inoculated into the left cardiac ventricle of anesthetized (Ketamine/Xylazine), 5-week-old, female athymic nude mice. Treatment groups were dosed once daily with vehicle only (n=10), fragmin (5 mg/kg, s.c.‐i.p); 101) or K5-NSOS (3 mg/kg, i.‐p.). The animals were weighed, and the development of bone metastases was monitored weekly by radiography (Faxitron MOSD-DC2). Total lesion number and area in hind limbs per mouse were quantitated from the images using MetaMorph image analysis software (Molecular Devices Corporation). Total tumor area per bone in decalcified and H&E stained sections was determined using MetaMorph image analysis software.

- In vivo bone resorption assay: Human osteoclast precursor cells (PoieticsTM, Cambrex, Baltimore, MD) were cultured on bovine bone slices (Kendall Biocassette Diagnostics) for 10 days according to the manufacturer’s instructions. The test compounds were added after osteoclast differentiation was completed at day 7. Stimulation was performed by 3 μg/ml TIBA. Secreted TRACP was measured at day 7 as an index of the number of osteoclasts formed before the addition of test compounds. A secretion index was calculated by dividing the CTX values obtained at day 10 by the TRACP h values obtained at day 7 (4).

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Conclusions

These data demonstrate that HLGAGs can efficiently reduce osteolytic lesion area and tumor burden in bone. K5-NSOS is a potential antineoplastic and antiresorptive agent with low anticoagulant activity, and it could be further optimized as an anti-tumor agent.

References