Inhibition of Cellular Proliferation After Experimental Balloon Angioplasty by Low-Molecular-Weight Heparin

Hartmut Hanke, MD; Martin Oberhoff, MD; Sybille Hanke, MS; Stefan Hassenstein, MD; Joachim Kamenz, MS; Karl M. Schmid, MD; Eberhard Betz, MD; and Karl R. Karsch, MD

Background. The proliferative response induced by balloon angioplasty is known to be an important factor in the development of restenosis after successful coronary angioplasty.

Methods and Results. To study the effects of low-molecular-weight heparin (LMWH) on cellular proliferation after experimental balloon angioplasty, LMWH (3.9 kd, 400 anti-Xa units/kg/day) was given to 20 male New Zealand White rabbits. After an intimal fibromuscular plaque was induced by electrical stimulation in the right carotid artery, LMWH was applied during the 7 days after balloon dilatation. As the control group, 20 other rabbits underwent balloon angioplasty without application of LMWH. The vessels were excised 3, 7, 14, and 28 days after balloon treatment. During the final 18 hours before the rabbits were killed, bromodeoxyuridine was applied. Intimal wall thickness increased from 13±5 cell layers (preangioplasty control group) to 20±6 cell layers in the LMWH-treated group at 28 days (p<0.05). In contrast, histological examination of control animals 28 days after angioplasty revealed a significant increase to 35±15 cell layers (p<0.01). Immunohistological quantification showed a significant increase (p<0.001) of cells undergoing DNA synthesis at 3 (10.2±4.2%) and 7 (7.7±4.8%) days after balloon dilatation in control animals. In contrast, at 3 and 7 days after balloon treatment, the percentage of cells undergoing DNA synthesis in LMWH-treated rabbits was lower (3 days, 2.7±1.8%; 7 days, 1.9±0.3%) than the corresponding untreated controls but showed a significant increase (p<0.01) compared with the preangioplasty controls. The differences between the two groups were statistically significant, however (3 days, p<0.01; 7 days, p<0.05). As early as 14 days after angioplasty, the extent of cellular proliferation was normalized and was comparable to the preintervention levels in both groups.

Conclusions. Our data indicate that the proliferative response after balloon angioplasty can be reduced in vivo by early treatment with LMWH and thus encourage further clinical investigations. (Circulation 1992;85:1548–1556)

KEY WORDS • smooth muscle cells • heparin, low molecular weight • angioplasty • restenosis

 Percutaneous transluminal coronary angioplasty (PTCA) is a well-established method to reduce the severity of lesions in atherosclerotic coronary heart disease.1,2 Its primary success rate approaches 90%. The long-term success of PTCA, however, is severely limited by the high incidence of restenosis, which occurs in up to 30–40% of primary successfully treated patients.3–5 Several experimental7,8 and postmortem studies9–11 have demonstrated that restenosis after balloon angioplasty is caused by intimal proliferation of smooth muscle cells (SMCs) and platelet aggregation leading to mural thrombi.12,13 Subsequent expression of growth factors, activation of macrophages and leukocytes, and expression of a variety of mitogens also occurs.14–19

In the past, different pharmacological interventions were performed to reduce the incidence of restenosis. Up to now, all compounds that were considered to reduce the incidence of restenosis failed in clinical studies.20–23 It has been shown, however, that heparin inhibits cellular proliferation and intimal thickening in experimental in vivo and in vitro studies.24–26

The currently available clinical data on heparin and restenosis show conflicting results. In a preliminary study, Hirshfeld et al27 suggested that there may be a reduced incidence of restenosis after PTCA in patients with a prolonged heparin treatment after intervention, whereas in a study by Ellis et al,28 no reduction of restenosis was found. Clinical application of unfractionated heparin is limited, however, by the hazard of increased bleeding complications.29

The pharmacological profiles of low-molecular-weight heparins (LMWH) are known to differ from those of unfractionated heparin, with a longer half-life and higher

See p 1632
bioavailability. In vitro experiments demonstrated an improved inhibition of SMC proliferation in SMC cultures compared with unfractionated heparin.

A recent study showed that intimal proliferation of SMCs in vivo is a dynamic process during the first 7 days after balloon angioplasty, with a subsequent increase of intimal wall thickness within a period of 28 days after intervention. Thus, the rationale of this study was to investigate the influence of LMWH treatment during the first 7 days after balloon angioplasty on cellular proliferation by use of bromodeoxyuridine (BRDU) labeling in the identical animal model.

Methods

Animal Model

A predominantly fibromuscular plaque was produced by local weak electrical stimulation of the carotid artery wall in 55 male New Zealand White rabbits as described by Betz and Schlote. In brief, this model is based on the implantation of two graphite-coated gold electrodes in the adventitia of the common carotid artery under general anesthesia (8 mg metomidate HCl and 0.1 mg fentanyl base per kilogram of body weight). The electrodes were held in position by a Teflon cuff on either side of the artery. Thin, subcutaneously placed leads from the electrodes were connected to a small plastic socket attached to the skull. With an external stimulation unit, this arrangement allowed the local and transmural electrical stimulation of the right carotid artery under standardized conditions in order to produce plaques of comparable sizes before balloon treatment (Figure 1). Constant-current DC impulses (15 m sec/impulse, 0.1 mA, 10 Hz) were applied twice daily for 30 minutes and 15 minutes with a time interval of 8–10 hours between the stimulation cycles for a period of 28 days in each animal.

To induce a fibromuscular cholesterol-rich plaque, all animals received a commercially available 0.5% cholesterol diet (Altromin, Lage, FRG) during the 28 days of electrical stimulation. After balloon angioplasty, the rabbits were fed a standard diet without cholesterol (Altromin).

Study Protocol

After 28 days of electrical stimulation, transluminal balloon dilatation of the plaque was performed in 45 rabbits under general anesthesia. The rabbits were open-randomized into a pharmacologically nontreated control group (n=20), a LMWH-treated group (n=20), and an unfractionated heparin–treated group (n=5). Ten rabbits were electrically stimulated for 28 days and served as a control group without angioplasty.

Angioplasty was performed with a 2.0-mm balloon catheter (Advanced Cardiovascular Systems, Inc., Temecula, Calif.) introduced by direct arteriotomy into the exposed vessel and then advanced into the region of plaque under microscopic control. The angioplasty catheter was inflated to 5 atm two times for 30 seconds. Between the two dilations, the balloon was deflated for 30 seconds. After the deflated catheter was removed, the small incision of the arteriotomy was closed by 7-0 polypropylene sutures. To avoid bacterial infections, all animals were on antibiotic therapy for 3 days after intervention.

To study the time course of cells undergoing DNA synthesis, the animals were killed 3, 7, 14, or 28 days after transluminal angioplasty. Five rabbits were used in each group of LMWH-treated and control animals. In addition, to determine the effect of unfractionated heparin on cellular proliferation in this model, five rabbits received 900 IU/kg body wt standard heparin (Heparin-Natrium, Braun Melsungen AG, Melsungen, FRG) subcutaneously twice daily for 7 days after balloon treatment. These animals were killed on day 7 after angioplasty.

LMWH Application

The LMWH used in this study (LU 47311, Knoll, Ludwigshafen, FRG) had a mean molecular weight of 3.9 kd and a specific activity of 160 anti-Xa units/mg and 29 activated partial thromboplastin time (aPTT) units/mg.

Twenty animals received LMWH (2.5 mg/kg/day s.c., which equals 400 anti-Xa units/kg/day) for 7 days after angioplasty. The first subcutaneous injection of 200 anti-Xa units/kg was performed immediately after intervention. Subsequent injections of 200 anti-Xa units/kg were given every 12 hours from day 1 to day 7 after intervention in groups with 3-, 7-, 14-, and 28-day periods of observation. Plasma activity of LMWH and unfractionated heparin was determined by anti-Xa and aPTT assays in five animals before and after 7 days of LMWH or after 7 days of unfractionated heparin treatment.

The anti-Xa assay (Kabi Vitrum GmbH, Munich, FRG) was performed according to the method of Teien et al with the modification that lipoproteins were precipitated before measurement using fluorotrichloromethane (Aldrich-Chemie, Steinheim, FRG). The aPTT reagent (Actin FS, Electra 800) was from Baxter GmbH, Munich, FRG.

Bromodeoxyuridine Labeling

To determine the extent of cells undergoing DNA synthesis, BRDU, a thymidine analogue, was given to each animal 18 and 12 hours before excision of the vessels. As described earlier in detail, 100 mg BRDU/kg body weight and 75 mg deoxycytidine/kg body weight (both from Sigma GmbH, Deisenhofen, FRG) were given as subcutaneous neck depot 18 hours
<table>
<thead>
<tr>
<th>Study group</th>
<th>Animal</th>
<th>SMC layers</th>
<th>DNA synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control animals without LMWH treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Days after balloon angioplasty</td>
<td>1</td>
<td>7</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>21</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>16</td>
<td>9.3</td>
</tr>
<tr>
<td>Mean±SD (n=5)</td>
<td>15±8</td>
<td></td>
<td>10.2±4.2*</td>
</tr>
<tr>
<td>7 Days after balloon angioplasty</td>
<td>6</td>
<td>6</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>26</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>28</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>22</td>
<td>7.3</td>
</tr>
<tr>
<td>Mean±SD (n=4)</td>
<td>21±10</td>
<td></td>
<td>7.7±4.8*</td>
</tr>
<tr>
<td>14 Days after balloon angioplasty</td>
<td>11</td>
<td>28</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Vessel occlusion by thrombus</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>22</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>12</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>35</td>
<td>0.4</td>
</tr>
<tr>
<td>Mean±SD (n=4)</td>
<td>24±10</td>
<td></td>
<td>1.1±0.5</td>
</tr>
<tr>
<td>28 Days after balloon angioplasty</td>
<td>16</td>
<td>24</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>29</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>57</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>28</td>
<td>0.1</td>
</tr>
<tr>
<td>Mean±SD (n=4)</td>
<td>35±15†</td>
<td></td>
<td>0.4±0.2</td>
</tr>
<tr>
<td>Control animals (only 28 days electrically stimulated)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group without treatment</td>
<td>21</td>
<td>8</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>20</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>9</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>12</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>15</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>11</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>14</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>8</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>15</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>9</td>
<td>1.3</td>
</tr>
<tr>
<td>Mean±SD (n=10)</td>
<td>12±4</td>
<td></td>
<td>0.7±0.4</td>
</tr>
<tr>
<td>LMWH-treated animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Days after angioplasty</td>
<td>31</td>
<td>11</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>9</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>3</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>22</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>14</td>
<td>3.2</td>
</tr>
<tr>
<td>Mean±SD (n=5)</td>
<td>12±7</td>
<td></td>
<td>2.7±1.8†‡</td>
</tr>
<tr>
<td>7 Days after angioplasty</td>
<td>36</td>
<td>14</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>5</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>16</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>12</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>19</td>
<td>1.7</td>
</tr>
<tr>
<td>Mean±SD (n=5)</td>
<td>13±5</td>
<td></td>
<td>1.9±0.3†§</td>
</tr>
</tbody>
</table>
before the animals were killed. In addition to this neck
depot, 30 mg BRDU/kg body weight and 25 mg deoxy-
cytidine/kg body weight was injected intramuscularly 18
and 12 hours before perfusion fixation.

Perfusion Fixation

The dilated arteries were perfused in situ with 500 ml of
0.1 M cacodylate-buffered 2% paraformaldehyde solution
at a pressure of 60–80 mm Hg through a catheter inserted
into the left ventricle. The excised vessels were embedded
in paraffin and prepared for histological and immunohis-
tological examination. The embedded vessels were cut
into cross sections beginning at the caudal end of the
dilated region until maximal plaque size in the dilated area
was reached. Approximately 10–12 cross sections (4 µm
thick) were assessed and used for histological and immuno-
histological analyses.

The incorporation of BRDU occurring during the 18
hours of the labeling period in the DNA of replicating
cells allowed determination and quantification of cellular
proliferation in the dilated arterial segment. A mono-
clonal antibody against BRDU (Bio Cell Consulting,
Greilningen, Switzerland) was used to identify these
proliferated cells. Immunohistological detection of
BRDU-labeled cells was performed in cross sections
using the biotin–avidin method38,39 and combined stain-
ing with hemalaune.

For quantification of the percentage of cells under-
going DNA synthesis after angioplasty, all BRDU-
labeled cells were counted in the intima of four subse-
quent serial cross sections in the region of the maximal
plaque size. The percentage of cells undergoing DNA
synthesis in the cross-sectional intimal area was deter-
mined as the relation between BRDU-labeled cells and
the total intimal cell number in the histological cross
sections. As previously described,8 a calculated calibra-
tion curve was used as a reference to determine the
total number of intimal cells in each section. Histolog-
ical sections of the control and LMWH-treated animals
were examined in random sequence by an independent
investigator who was blinded to the type of treatment
protocol.

In addition, estimation of the small intestine mucosa
labeling index was used in each animal to control the
incorporation of BRDU in replicating cells. In all
animals, DNA synthesis was found in approximately
30% of all cells in the small intestine mucosa.

To identify neointimal cells as SMCs, immunohis-
tological staining (biotin–avidin method) with a mono-
clonal antibody against α-actin (Renner, Dannstadt, FRG)
in an additional cross section was performed. α-Actin is
known to be a highly specific marker of SMCs.40,41

In addition to the immunohistochemical staining of
BRDU and α-actin, embedded sections of the dilated
region were stained with hematoxylin and eosin.

The extent of intimal cell layers was determined by
counting the number of cell nuclei on the perpendicular
line between endothelium and lamina elastica interna at
the area of maximal plaque size.

### Table 1. Continued

<table>
<thead>
<tr>
<th></th>
<th>14 Days after angioplasty</th>
<th>Mean±SD</th>
<th>28 Days after angioplasty</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n=5) 15±6</td>
<td></td>
<td>(n=5) 20±6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0±0.5</td>
<td></td>
<td>0.6±0.6</td>
</tr>
<tr>
<td>SMC, smooth muscle cells; LMWH, low-molecular-weight heparin.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Statistically significant compared with control group without balloon angioplasty (p&lt;0.001, t test).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>†Statistically significant compared with control group without balloon angioplasty (p&lt;0.01, t test).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>§Statistically significant compared with corresponding pharmacologically (heparin/LMWH) untreated group (p&lt;0.01, ANOVA).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>¶Statistically significant compared with corresponding pharmacologically (heparin/LMWH) untreated group (p&lt;0.05, ANOVA).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‡Stenosis of more than 50% luminal reduction caused by intimal proliferation of smooth muscle cells.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>†Statistically significant compared with control group without balloon angioplasty (p&lt;0.001, t test).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
All arterial segments of the different groups that were used for analysis of proliferative activity and quantification of intimal cell layers displayed an equivalent composition of the lesion. Three vessels with total occlusion by thrombus formation were excluded from analysis (Table 1).

Statistical Evaluation

Results are expressed as mean±SD. The statistical significance of differences between normal and dilated carotid arteries was determined by unpaired Student’s t test. Differences in the extent of cells undergoing DNA synthesis and intimal thickening in control and heparin/LMWH-treated animals were compared by ANOVA. Differences were considered significant at a value of p<0.05.42

Results

Morphological Results

After 28 days of electrical stimulation, determination of intimal SMC layers in the region of maximal plaque size displayed a mean of 12±4 cell layers in the control group without angioplasty (Figure 2). Staining of α-actin displayed a concentric intimal proliferation of SMCs with the greatest plaque size below the anode. The actin filaments in the plaque were found to be arranged homogeneously. Several foam cells could be identified predominantly in the central part of the plaque.

Compared with the control group without angioplasty, a progressive increase of intimal cell layers in the LMWH-untreated group was found up to 35±15 cell layers (p<0.01) after 28 days after balloon angioplasty. In this group, a stenosis of more than 50% luminal reduction caused by neointimal proliferation was observed in only one animal. As shown by α-actin staining, this lesion contained predominantly SMCs (Figure 3). In addition, a total occlusion caused by thrombus formation was found in three animals of this group (Table 1).

Histological examination in the LMWH-treated animals revealed an increase of intimal wall thickness at 28 days after angioplasty (20±6 cell layers), which was significantly higher (p<0.05) than the control group without angioplasty.

A significant lesion caused by neointimal proliferation or thrombus formation did not occur in the LMWH group. The difference in maximal extent of intimal cell layers at 28 days after balloon angioplasty between the LMWH-treated and nontreated groups, however, was statistically not significant. The frequency of thrombotic occlusions in the dilated arterial segments was not significantly higher (by Fisher’s exact test) in the control group with angioplasty than in LMWH-treated animals.

Determination of intimal cell layers in animals treated with unfractionated heparin displayed a mean of 14±4 cell layers at 7 days after angioplasty, which was not significantly different from the control group without intervention (Table 1).

Quantification of Cells Undergoing DNA Synthesis

In control animals without angioplasty, quantification of cellular proliferation in the intima revealed an extent of cells undergoing DNA synthesis of 0.7±0.4%.

In the animals that underwent angioplasty but did not receive LMWH treatment, immunohistological quantification displayed a significant (p<0.001) increase of cells undergoing DNA synthesis at day 3 (10.2±4.2%) and day 7 (7.7±4.8%) after balloon treatment (Figure 4). Fourteen and 28 days after dilatation, the extent of intimal SMC proliferation was normalized.

In contrast, intimal proliferation in the LMWH-treated group was reduced and had a moderate but significant increase of cells entering the S phase during the first 7 days (3 days, 2.7±1.8%, p<0.01; 7 days, 1.9±0.3%, p<0.01) compared with the preinterventional measurement (Figure 4B). Fourteen and 28 days after dilatation, the extent of cells undergoing DNA synthesis was already comparable to the baseline level.

The difference in cellular proliferation between the dilated control group and the LMWH-treated group

![Figure 2. Histological cross section of a rabbit carotid artery after 28 days of electrical stimulation and an additional 0.5% cholesterol diet (control group without interventional or pharmacological treatment) shows a fibromuscular plaque in the intima (bar=50 μm; hemalaune and eosin stain).](http://circ.ahajournals.org/lookup/doi/10.1161/01.CIR.85.4.1552)
activity of SMCs within the first 7 days after intervention.8,20 It is also known that heparin and its low-molecular-weight fragments can inhibit proliferation of vascular SMCs in vitro and in vivo,25,26,31,43–46 but little is known about the mechanisms by which heparin inhibits SMC proliferation. Recent studies suggest that heparin and its fragments interact with endothelial cells, SMCs, growth factors, platelets, and blood coagulation factors antithrombin III and Xa.44,47–49 The pharmacological profile of LMWHs is different from that of unfractionated heparin.29,30,50 Thus, in recent years, LMWHs have attracted increasing clinical interest as antithrombotic compounds. Clinical investigations, however, have focused on the prevention of deep vein thrombosis after abdominal surgery.51

In addition to these antithrombotic properties, it has been shown that LMWH inhibits endothelial growth to a lesser extent than unfractionated heparin does in vitro.52,53 It is speculated that the modulation of SMC proliferation might be induced by binding of several substances that interact with the endothelial lining (e.g., histamine, serotonin, thrombin, platelet-derived growth factor,54,55 effect on components of the fibrinolytic system, platelets,56 protein synthesis,49 and a direct inhibition of stimulated SMCs in the G1 phase of mitosis.43,46

Growth-arrested SMCs in cell culture are 50–100 times more sensitive to the antiproliferative effects of heparin than are exponentially growing cells in culture.44 Thus, it seems adequate and important to start with LMWH treatment before SMCs are activated by the vessel wall injury caused by PTCA.

In the present in vivo study comparing LMWH-treated and nontreated rabbits after balloon angioplasty, LMWH therapy resulted in a significantly reduced increase of intimal cells undergoing DNA synthesis in the early stage after dilatation.

Our study is limited, however, in regard to a further differentiation of the cell types involved in this process. It has been shown that macrophages within atherosclerotic lesions are capable of proliferation. In hypercholesterolemic rabbits, simultaneous thymidine autoradiography and immunostaining revealed that approximately 30% of the labeled cells were macrophages and 45% were SMCs.57 In addition, proliferating endothelial cells58 and lymphocytes59 might be also detected by thymidine or BRDU labeling after balloon angioplasty in experimental models.

In a recent study,8 we have shown a significant increase in the number of intimal cells undergoing DNA synthesis only within the first 7 days after balloon dilatation. Thus, LMWH administration in the present study was terminated 7 days after balloon angioplasty. In addition, it could be demonstrated that a significant increase of intimal cell layers occurred during the first 28 days after balloon dilatation.

No significant increase of intimal cell layers occurred from 28 to 42 days after intervention.8 Thus, the current study was terminated 28 days after balloon dilatation.

Determination of the anti-Xa activity in the LMWH-treated group displayed significantly increased levels of anti-Xa activity compared with the values before LMWH treatment. In addition, no changes were observed in aPTT values before and during LMWH treatment.
Figure 4. Panel A: Histological cross section of a carotid artery at 7 days after balloon angioplasty without low-molecular-weight heparin treatment. Avidin–biotin staining of bromodeoxyuridine indicates a large number of cells undergoing DNA synthesis (arrow) predominantly below the luminal surface (bar=50 μm; nuclear counterstained with hemalaune). Panel B: Cross section of a carotid artery at 7 days after balloon angioplasty with an additional administration of low-molecular-weight heparin. Detection of bromodeoxyuridine shows only few labeled cells in the intimal layer below the luminal surface (bar=50 μm; nuclear counterstained with hemalaune).

The LMWH used in our experimental setting has been investigated in human cell cultures and demonstrated a significant inhibition of SMC proliferation without an effect on endothelial cells in vitro. Only limited information is available, however, about the effect of LMWH in vivo after balloon angioplasty. In a preliminary experimental study, Pow et al. reported a reduced incidence of restenosis after balloon angioplasty in rabbits by histological and angiographic criteria.

In addition to these results, we found an inhibition of cellular proliferation in the early stage after intervention. The reduced proliferative activity of SMCs resulted in only a moderate increase of intimal wall thickness after a 28-day control period after intervention. However, the difference in intimal cell layers after 28 days between the LMWH-treated and nontreated group was not statistically significant. Thus, although LMWH treatment resulted in a significant inhibition of cellular proliferation at 3 and 7 days after angioplasty, the effect on overall intimal wall thickness seems to be limited. This might be explained in part by migration of SMCs from the media in the intimal layer, as suggested by Clowes et al.

In the current study, unfractionated heparin was administered in one group for 7 days after dilatation to compare the effect of unfractionated heparin on cellular proliferation with the antiproliferative activity of LMWH. In agreement with several other in vivo studies, a significant inhibition of cells undergoing DNA synthesis compared with the control group without pharmacological treatment was found. Determination of the aPTT before and during unfractionated heparin treatment, resulting in a significant prolongation of the aPTT time, has demonstrated plasma levels.

<table>
<thead>
<tr>
<th>Table 2. Plasma Anti-Xa Levels and aPTT Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study group</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Unfractionated heparin (n=5)</td>
</tr>
<tr>
<td>LMWH (n=5)</td>
</tr>
</tbody>
</table>

aPTT, activated partial thromboplastin time; LMWH, low-molecular-weight heparin.
Values are mean±SD.
*Statistically significant compared with corresponding levels before LMWH/unfractionated heparin treatment (p<0.01, t test).
†Statistically significant compared with corresponding levels before LMWH/unfractionated heparin treatment (p<0.001, t test).
comparable to full heparin treatment in patients, as shown by a significant prolongation of the aPTT time. Anti-Xa activity was highly increased in this group, however, demonstrating that heparin was administered in the maximum possible dosage.

Despite reduced interaction with blood coagulation, as indicated by an increase of anti-Xa activity without prolongation of the aPTT time, the significant inhibition of cellular proliferation observed in a human equivalent dosage of the compound supports the concept of prevention of restenosis by LMWH treatment in the early stage after PTCA.

In conclusion, our study shows that LMWH is useful for inhibition of cellular proliferation in the early stage after experimental angioplasty. The impact of LMWH treatment on the incidence of restenosis that occurs after angioplasty in humans, however, has to be determined in future clinical investigations.

Acknowledgments

The authors gratefully acknowledge the excellent technical assistance of Rosemarie Barth and Mechthild Holz in the immunohistochemical methods and histopathological preparations.

References

5. Roubin GS, King SB III, Douglas JS Jr: Restenosis after percutaneous coronary angioplasty: Emory University Hospital experience. Am J Cardiol 1987;60:39B–43B
41. Vandekerckhove J, Weber K: The complete amino acid sequence of actins from bovine aorta, bovine heart, bovine fast skeletal muscle, and rabbit slow skeletal muscle. Differentiation 1979;14:123–133
Inhibition of cellular proliferation after experimental balloon angioplasty by low-molecular-weight heparin.
H Hanke, M Oberhoff, S Hanke, S Hassenstein, J Kamenz, K M Schmid, E Betz and K R Karsch

Circulation. 1992;85:1548-1556
doi: 10.1161/01.CIR.85.4.1548

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/85/4/1548

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/