Local and systemic delivery of low molecular weight heparin stimulates the reendothelialization after balloon angioplasty

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Abstract

Objective: Recent investigations revealed the importance of endothelial cell integrity and function in the pathogenesis of restenosis after angioplasty. Agents which stimulate reendothelialization may prevent restenosis after interventional procedures. The results of in vitro studies suggested that heparin and low molecular weight heparin administration may enhance the recovery of the endothelium. In this study the extent of endothelial denudation and the occurrence and time course of endothelial regeneration after experimental balloon angioplasty followed by subcutaneous or local delivery of low molecular weight heparin was investigated.

Methods: A total of 102 rabbits were included in the study. An atheromatous plaque was induced by electrical stimulation in the right carotid artery of the animals. All animals underwent balloon angioplasty. Thirty-two rabbits received no further medical treatment. Twenty-five rabbits received subcutaneous low molecular weight heparin reviparin 400 anti-Xa-units/day during the following 7 days. In 25 animals the dilated arterial segments were treated locally with reviparin 1500 anti-Xa-units/4 ml, 2 atm using a porous balloon 2.5 mm, 35 holes, diameter 75 µm. Twenty animals served as control group without intervention. The vessels were excised 3, 7, 14, 28 and 56 days following intervention. Sections were stained with an antibody against von Willebrand factor and PECAM 1 to confirm the endothelial origin of the lining cells. After bromodeoxyuridine labeling, the extent of proliferation was determined by using a monoclonal antibody. In addition, morphometric analysis of the intimal and medial area was performed.

Results: Three days after balloon angioplasty histomorphological analysis showed a reduction of about 60% of the preinterventional endothelial cell number in all three groups. Already one week after intervention there was a significantly higher number of endothelial cells in both groups of low molecular weight heparin treated animals compared to the untreated group sc group 144 ± 33, local group 142 ± 32 versus untreated 79 ± 17 endothelial cells, p ≤ 0.05. This significant difference was maintained during the following four weeks and demonstrated a 2-fold increase in endothelial proliferation in the heparin treated animals compared with the untreated group. In addition, immunohistological examination showed a significant decrease in smooth muscle cell proliferation in the sc and local reviparin treated animals and a subsequent reduction of intimal thickening.

Conclusion: Local delivery of low molecular weight heparin promotes reendothelialization and contributes to the inhibition of smooth muscle cell proliferation. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Endothelium; Von Willebrand factor; PECAM1; Local drug delivery; Low molecular weight heparin; Rabbits

1. Introduction

Normal endothelium covers the vessel surface as a closed cell layer and produces a semipermeable barrier that regulates exchange and transport of substances through the vessel wall. As a paracrine organ it produces a non-thrombogenic vessel surface and inhibits platelet aggregation. Endothelial cells (ECs) produce prostacyclin, endothelium-derived relaxing factor (EDRF), heparin-like molecules, such as heparan sulfates [1], and tissue-Plas-
minogen-Activator which activates the local plasmin-synthesis and leads to the dissolution of thrombus [2].

In addition, the endothelium plays a key role for the control of vasoreactivity. Several studies showed that vasorelaxation in response to acetylcholine requires an intact endothelium, and this response was attributed to endothelium-derived relaxing factor [3]. Additionally, EDRF has also been shown to inhibit platelet aggregation and has been identified as nitric oxide derived from L-arginine in endothelial cells [4]. However, the endothelium also produces substances responsible for vasoconstriction such as endothelin-1 and angiotensin II [2].

Furthermore, the endothelium has a key role in the control of migration and proliferation of smooth muscle cells. In coculture systems, endothelial cells inhibited the proliferation of smooth muscle cells by synthesis of heparinoids [5,6]. In addition, EDRF was identified as a potent inhibitor of smooth muscle cell growth [7]. Beside that, the endothelium is involved in subintimal inflammatory processes [8] and modifies lipoproteins [9].

Several different mechanisms can injure or destroy the endothelium. In the present study balloon angioplasty leads to severe denudation with subsequent platelet adhesion to the subendothelium due to interaction with collagen-receptors and von Willebrand factor (vWF) [8]. After aggregation and activation, the platelets release numerous substances, e.g. adenosine diphosphate, serotonin, thromboxane A2, thrombin and histamine [2]. The release of these molecules can lead to severe vasoconstriction and thrombus formation [10] and leads to stimulation and invasion of monocytes. The activation of macrophages and smooth muscle cells results in subsequent neointima formation [11]. Recent investigations have demonstrated the importance of endothelial cell function in the pathogenesis of restenosis after angioplasty. Agents which stimulate the regrowth of vascular endothelium play a key role in the prevention of restenosis due to their inhibitory effects on smooth muscle cell proliferation. Cell culture studies have demonstrated that the antiproliferative properties are comparable or even greater for the lower molecular weight heparins (LMWH) than unfractionated heparin due to their longer half-life and higher bioavailability [12,13].

Thus, the aim of the present study was to examine the effect of local and systemic delivery of LMWH on the reendothelialization following experimental balloon angioplasty in vivo.

2. Methods

2.1. Animal model

A total of 102 male New Zealand White rabbits (2.8–3.4 kg BW) were used in this study. In 92 rabbits an intimal plaque was induced using the electrostimulation model as previously described [14]. In brief, this model is based on the implantation of two graphite-coated gold electrodes in the adventitia of the common carotid artery. The electrodes are held in position by a Teflon cuff on either side of the artery. Thin, subcutaneously placed leads from the electrodes are connected to a small plastic socket attached to the skull. This arrangement allows for local and transmural electrical stimulation of the right carotid artery under standardized conditions in order to produce plaques of comparable sizes before intervention. Constant-current DC impulses (15 ms/impulse, 0.1 mA, 10 Hz) are applied twice daily using an external stimulation unit for 30 min with a time interval of 8–10 h between the stimulation cycles for a period of 28 days in each animal. To induce a fibromuscular cholesterol-rich plaque, all animals receive an 0.5% cholesterol diet (Altromin, Lage, Germany). The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

2.2. LMWH

The low molecular weight heparin reviparin (Knoll, Ludwigshafen, Germany), a partly sulphated and N-acetylated glycosaminoglycan, used in this study had a mean molecular weight of 3.9 kd (range 3500–4500). In vitro experiments with human endothelial and smooth muscle cells using reviparin by Hämmerle et al. could demonstrate that reviparin given in a concentration of 42 μg/ml resulted in a significant inhibition of smooth muscle cell proliferation and migration with a simultaneous stimulatory effect on endothelial cell growth [13]. A solution of 375 anti-Xa-units/ml was used for local delivery. The solution contains the active substance, NaCl and water for injection. The therapeutic concentration of reviparin after local delivery was confirmed in a previous study with tritium-labelled reviparin [15].

The subcutaneously treated animals received reviparin (2.5 mg/kg/day sc, 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 h from day 1 to day 7 after intervention. Anti-Xa plasma levels were measured in this study to demonstrate adequate concentrations in the sc treated group. The anti-Xa assays (Kabi Vitrum, Munich, Germany) were performed with the modification that lipoproteins were precipitated before measurement using fluorotrichlormethane (Aldrich-Chemie, Steinheim, Germany).

2.3. Local drug delivery device

A 135-cm long 2.5 mm SI III porous balloon catheter (ACS, Santa Clara, CA, USA) with 35 pores in a design of
7 rows of 5 pores each was used in this study. Each pore has a diameter of 75 ± 15 μm. The device has an inner lumen accepting a 0.014-in. guide wire and a proximal port for balloon inflation (Fig. 1). The drug delivery catheter was prepared with a 0.9% saline solution before it was advanced to the preformed plaque. The balloon inflation and fluid delivery was performed with a low pressure pump (DVI, CA, USA). A volume of 4.0 ml was delivered locally using an inflation pressure of 2 atm.

2.4. Study protocol

Transluminal balloon angioplasty of the preformed plaque was performed in 82 rabbits. Ten rabbits were not operated and served as a baseline control group. Ten rabbits were electrically stimulated and served as control group without balloon angioplasty. Following adequate anesthesia with intramuscular metomidate-HCl (8 mg/kg BW) and fentanyl-base (0.1 mg/kg BW) angioplasty was performed with a 2.0 mm balloon catheter (Micro-Hartzler, Guidant, CA, USA). The balloon was introduced by direct arteriotomy into the exposed vessel, advanced into the region of plaque, and then inflated to 5 atm twice for 30 s.

The rabbits were open-randomized in a pharmacologically nontreated control group (BA alone, n = 32), a subcutaneously treated group (SC, n = 25) and a local LMWH (n = 25). The animals received subcutaneous reviparin (400 anti-Xa-units/day) during the following 7 days in the subcutaneously treated group. In the local LMWH group, the porous balloon was advanced into the region of the predilated plaque, and 4 ml of reviparin-solution (375 anti-Xa-units/ml) was delivered with a pressure of 2 atm during a time period of approximately 30 s. After the catheter device was removed, the incision of the arteriotomy was closed carefully by 7-0 polypropylene microsutures under microscopic control. In order to avoid bacterial infections, all rabbits were on antibiotic therapy during the following 3 days.

2.5. Tissue analysis

The rabbits were sacrificed 3, 7, 14, 28 or 56 days after balloon treatment with an overdose of intravenous metomidate-HCl. The carotid arteries were perfused in situ with 500 ml 0.1 M cacodylate-buffered 2% paraformaldehyde solution under physiological pressure. A 3–4-cm segment of the treated carotid artery with the teflon cuff was excised, and the proximal and distal ends were marked with sutures. The excised vessels were embedded in paraffin and prepared for histological and immunohistological 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 immunohistochemical analysis. In addition to the immunochemical staining, embedded sections of the dilated region were stained with hematoxylin and eosin.

Fig. 1. Porous SI III balloon, demonstrating jet streaming using a fluorescent agent.
Staining for factor VIII-related antigen (goat antihuman factor VIII-related antigen, Atlantic Antibodies, Scarborough, ME, USA) and PECAM-1 1:10 (Dako) were performed to confirm the endothelial origin of the cells using an immunoperoxidase technique with rabbit anti-goat immunoglobulin G and avidin-biotin complex. To provide a quantitative parameter of intimal proliferation, morphometric analysis was performed on vascular rings. The absolute number of endothelial cells was counted and related to the length of the endothelial layer (luminal perimeter). The technique of the immunohistological determination of proliferating smooth muscle cells has been described previously and is based on staining with monoclonal antibodies against bromodeoxyuridine (BrdU) [16].

2.6. Quantitative histopathology

All sections were quantitatively analyzed by computerized morphometry. The sections were projected onto a digitizing pad (Summagraphics, Seymour, CT, USA) by a side tubus affixed to the stereoscope (Olympus, Hamburg, Germany). The luminal perimeter, the intimal and medial area were traced manually under stereoscopic control using standard software (Bioquant, Bilany Consulting, Düsseldorf, Germany) and the intima-to-media ratio (ratio of the cross-sectional area of the intima to that of the media) was calculated. Three adjacent sections were used to count the number of endothelial cells, and the mean was calculated. In addition, the number of ECs per mm luminal perimeter was calculated to eliminate the influence of vascular remodeling (intimal thickening, vessel enlargement) after injury on the study results. The reendothelialization factor was calculated as the increase in the number of endothelial cells per mm luminal perimeter during 28 days after balloon angioplasty. To obtain the number of proliferating SMC cells, the number of BrdU-positive cells in the intimal and medial layer was determined and related to the total cell number.

2.7. Scanning electron microscopy

Vessel segments for electron microscopy were fixed in 2% glutaraldehyde (Paesel, Germany) in 0.1 mol/l cacodylate buffer pH 7.4 for 1 h. The tissue was then washed several times in the same buffer, postfixed in 1% buffered osmium tetroxide for 1 h, washed again and dehydrated in ethanol. The specimens were then treated with saturated uranyl acetate in 70% ethanol for 2 h. After the completion of dehydration in absolute ethanol and propylene oxide, the tissue was embedded in Araldite (Serva). Semithin and

| Table 1
| Results of morphometric evaluation of the endothelium |
|----------------|----------------|----------------|----------------|
|                | Baseline control | 28 days ES | BA | sc LMWH | Local LMWH |
| n = 10         | n = 10           | n = 10     | n = 5 | n = 5 | n = 5 |
| 173 ± 20 ECs   | 158 ± 41 ECs     | 54 ± 17 ECs/mm | 54 ± 17 ECs/mm | 54 ± 17 ECs/mm | 54 ± 17 ECs/mm |
| 3 days after intervention | 3.74 ± 0.68 mm | 3.04 ± 0.74 mm | 3.04 ± 0.74 mm | 3.04 ± 0.74 mm | 3.04 ± 0.74 mm |
| 7 days after intervention | 59 ± 32 ECs | 56 ± 27 ECs | 61 ± 42 ECs | 61 ± 42 ECs | 61 ± 42 ECs |
| 14 ± 8 ECs/mm | 17 ± 7 ECs/mm | 17 ± 7 ECs/mm | 17 ± 7 ECs/mm | 17 ± 7 ECs/mm | 17 ± 7 ECs/mm |
| 14 days after intervention | 79 ± 17 ECs | 144 ± 33 ECs | 142 ± 32 ECs | 142 ± 32 ECs | 142 ± 32 ECs |
| 28 days after intervention | 4.71 ± 0.79 mm | 4.2 ± 1.47 mm | 2.78 ± 1.37 mm | 2.78 ± 1.37 mm | 2.78 ± 1.37 mm |
| 5 ± 5 | 36 ± 9 ECs/mm | 52 ± 14 ECs/mm | 52 ± 14 ECs/mm | 52 ± 14 ECs/mm | 52 ± 14 ECs/mm |
| 28 days after intervention | 90 ± 32 ECs | 98 ± 33 ECs | 178 ± 47 ECs | 178 ± 47 ECs | 178 ± 47 ECs |
| 3.5 ± 1.0 mm | 2.97 ± 1.38 mm | 3.15 ± 0.8 mm | 3.15 ± 0.8 mm | 3.15 ± 0.8 mm | 3.15 ± 0.8 mm |
| 26 ± 6 ECs/mm | 39 ± 16 ECs/mm | 63 ± 37 ECs/mm | 63 ± 37 ECs/mm | 63 ± 37 ECs/mm | 63 ± 37 ECs/mm |
| 56 days after intervention | 89 ± 24 ECs | 162 ± 15 ECs | 238 ± 59 ECs | 238 ± 59 ECs | 238 ± 59 ECs |
| 3.82 ± 1.39 mm | 3.5 ± 0.96 mm | 3.51 ± 0.78 mm | 3.51 ± 0.78 mm | 3.51 ± 0.78 mm | 3.51 ± 0.78 mm |
| 25 ± 10 ECs/mm | 48 ± 10 ECs/mm | 70 ± 20 ECs/mm | 70 ± 20 ECs/mm | 70 ± 20 ECs/mm | 70 ± 20 ECs/mm |
| 130 ± 53 ECs | 160 ± 25 ECs | 207 ± 13 ECs | 207 ± 13 ECs | 207 ± 13 ECs | 207 ± 13 ECs |
| 3.21 ± 1.06 mm | 4.65 ± 0.82 mm | 3.95 ± 0.4 mm | 3.95 ± 0.4 mm | 3.95 ± 0.4 mm | 3.95 ± 0.4 mm |
| 40 ± 8 ECs/mm | 34 ± 5 ECs/mm | 53 ± 4 ECs/mm | 53 ± 4 ECs/mm | 53 ± 4 ECs/mm | 53 ± 4 ECs/mm |

Baseline control indicates control without any intervention; 28 days ES, 28 days of electrostimulation; BA, balloon angioplasty group without further treatment; sc LMWH, subcutaneously treated group; local LMWH, locally treated group. The first line demonstrates number of treated animals, the second line absolute number of endothelial cells (ECs), the third line length of luminal perimeter and the fourth line the number of endothelial cells per mm luminal perimeter. The data are given as mean ± ISD. *p ≤ 0.05 vs. untreated group. †p ≤ 0.05 vs. sc LMWH group.
ultrathin sections were stained with Toluidine Blue and lead citrate respectively. Ultrathin sections were examined with Zeiss EM 10 and Zeiss EM 902 microscopes and photographed.

2.8. Statistical evaluation

All values are expressed as mean ± 1 SD. The results for balloon angioplasty, sc treated animals and local drug

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Fig. 2. Light micrograph of a rabbit carotid artery (control without any intervention) showing morphological features of endothelial cells. Normal endothelial cells demonstrate a granular staining for PECAM 1 and line the luminal surface of a control vessel. PECAM 1-staining. Magnification ×500.

Fig. 3. Light micrograph of a rabbit carotid artery after 28 days of electrical stimulation. Endothelial cells that are polygonal in shape and to some extent orientated towards the vessel lumen. Black staining is derived from the graphite coated cuff electrode. PECAM 1-staining. Magnification ×500.
delivery treated vessels were compared using the Tukey-Kramer test [17]. Differences were considered to be statistically significant at \( p \leq 0.05 \).

3. Results

3.1. Morphology and morphometric evaluation of the endothelium

The results of the morphometric evaluation are summarized in Table 1. In animals without electrostimulation (baseline control-group, \( n = 10 \)), the luminal surface was formed by a continuous line of elongated and flat ECs (Fig. 2). The electrostimulation control-group represents ten animals which were electrostimulated for 28 days without further treatment. In all animals histology confirmed the presence of a continuous layer of ECs. In contrast to the baseline control group about 60% of the endothelial cells after electrical stimulation were irregular in size and polygonal in shape (Fig. 3).

Three days after intervention a severely injured endothelial layer could be observed in all animals (Fig. 4). Large areas with loss of ECs were seen in all specimens. In all three groups the remaining cells were irregular in size, polygonal in shape and lacked the normal longitudinal orientation with blood flow (Fig. 5). The amount of endothelial cell loss due to balloon injury was comparable.

Fig. 4. Histological cross section of carotid artery at day 3 after BA without LMWH treatment. Extensive areas of endothelial cell loss are present. The few remaining cells are polygonal and have an irregular size. PECAM 1-staining. Magnification \( \times 500 \).

Fig. 5. Scanning electron microscopy of two endothelial cells protruding into the lumen surface 3 days after balloon angioplasty without treatment. The scale bar represents 1.1 \( \mu \)m.
in all three groups and was calculated as about 60% of the preinterventional cell number. Seven days after intervention the arteries still revealed large areas of endothelial cell loss in all groups. The remaining cells were irregular in shape and many of the cells were orientated towards the vessel lumen. However, in all groups there were small foci of regenerated endothelium with regular cell size and longitudinal orientation. The animals treated with sc LMWH showed a significant increase of the absolute number of ECs and the ECs/mm. Fourteen days after intervention the luminal surface was almost completely covered by ECs in most vessels. Many endothelial cells

Fig. 6. Untreated carotid artery at day 14 after BA. In this group there are still foci of denuded endothelium. PECAM 1-staining. Magnification ×500.

Fig. 7. Carotid artery with subcutaneous treatment at day 14 after BA. PECAM 1-staining. Magnification ×500.
protruded into the lumen surface (Figs. 6–8). In the local LMWH treated animals the absolute number of ECs and ECs/mm were significantly increased. Twenty-eight days after intervention the morphologic evaluation revealed an intact endothelial cell lining with focal areas of polygonal-shaped and irregular-sized cells not aligned with blood flow in all groups. In contrast to the animals treated with balloon-angioplasty, the LMWH treated animals showed a more dense arrangement of the ECs. The local LMWH treated animals showed a significant increase in the absolute number of ECs and ECs/mm compared to the animals treated with balloon-angioplasty and the sc treated animals. Fifty-six days after intervention histology demonstrated a continuous flat sheet of ECs in all animals. Most of the cells were longitudinally orientated, regular in size and shape. However, there were still small foci of cells with polygonal shape and without longitudinal orientation.

3.2. Intimal and medial proliferation and morphometric evaluation

The results are shown in Table 2. Three days after intervention the sc treated animals showed a significant reduction of proliferation compared to the untreated and local LMWH treated group. However, 7 days after intervention there was a relevant decrease in intimal SMC proliferation in the animals treated with sc and local LMWH. No significant difference in proliferation was observed during the following time period. Medial proliferation was not significantly affected by the treatment during the whole observation period. The calculation of the intima-to-media ratio displayed a significant difference between the untreated and local LMWH treated animals 56 days after intervention. The sc treated animals showed only a trend towards a reduction of intimal area. There were no difference in the extent of deeper vessel trauma in all three groups. Only one animal in the local treated group showed signs of small injuries due to fluid jets with rupture of the internal elastic lamina.

3.3. Reendothelialization factor

At 4 weeks after angioplasty the reendothelialization factor in the untreated group was calculated as 1.8-fold increase in the number of endothelial cells compared to the group three days after intervention. In contrast a reendothelialization factor of 2.8-fold after subcutaneous LMWH treatment and a 4.1-fold increase after local delivery of LMWH was observed.

3.4. Plasma activity of LMWH

The average plasma anti-Xa-activity was 1.8 ± 1.2 anti-Xa-units 30 min after local delivery of LMWH. In the sc
treated animals the plasma activity was 0.4 ± 0.2 anti-Xa units/ml at day 7 after balloon angioplasty.

4. Discussion

In 1981, Reidy and Schwartz demonstrated that reendothelialization already starts as early as a few hours after denudation [18]. They described, that type and extent of denudation are important determinants for the process of reendothelialization. Following a small injury only the endothelial cells migrate rapidly from the edges of the injured area in an attempt to close the defect. In contrast, after a larger injury endothelial cells have not only to migrate but also to increase the total cell number by proliferation. Obviously the process of proliferation takes a longer time period, leads to subtypes of small endothelial cells and may result in incomplete reendothelialization [19,20].

Several studies demonstrated a variety of proliferation kinetics of endothelial cells after denudation procedures. Endothelial cells of rat carotid arteries showed an initial proliferation rate of 2 mm per week [20], whereas endothelial cells of the rat aorta showed a reduced proliferation rate of only 0.4 mm per week [19]. Furthermore, it was shown in rat carotid arteries that even after 3 months denuded areas could be observed. In these animals the process of reendothelialization was incomplete even after one year. It was additionally shown in this study that the process of endothelial proliferation came to a rest after 6 weeks [21]. In femoral arteries of rabbits a slow reendothelialization after 7 days and a complete reendothelialization 4 weeks later [22]. Two other studies in rabbits showed corresponding results with complete reendothelialization after 2 [23] and 4 weeks [24].

In human atherectomy studies only a few endothelial cells were observed in the early period after PTCA [25]. The reason for these observations is probably the previ-

<table>
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<th>Table 2</th>
<th>Results of morphometric evaluation of intimal and medial proliferation, intima-to-media ratio and intimal and medial area</th>
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<td>Baseline control</td>
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<td></td>
<td>0.1 ± 0.1%</td>
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<td>0.03 ± 0.02 mm²</td>
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<td>3 days after intervention</td>
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<td>5.5 ± 5.2%</td>
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<td>0.40 ± 0.23</td>
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<td>7 days after intervention</td>
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<td>0.83 ± 0.34</td>
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<td>0.20 ± 0.06 mm²</td>
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<td>0.25 ± 0.04 mm²</td>
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<td>14 days after intervention</td>
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<td>0.21 ± 0.07 mm²</td>
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<td>0.25 ± 0.01 mm²</td>
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<td>28 days after intervention</td>
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<td>0.8 ± 0.5%</td>
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<td>0.48 ± 0.14 mm²</td>
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<td>56 days after intervention</td>
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<td>0.46 ± 0.18 mm²</td>
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<td>0.42 ± 0.06 mm²</td>
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Baseline control indicates control without any intervention; 28 days ES, 28 days of electrostimulation; BA, balloon angioplasty group without further treatment; sc LMWH, subcutaneously treated group; local LMWH, locally treated group. The first line demonstrates percentage of proliferating intimal smooth muscle cells (ratio of BrdU-positive cells to total cell population of the intima), the second line the percentage of medial smooth muscle cells (ratio of BrdU-positive cells to total cell population of the media), the third line the intima-to-media ratio, the fourth line the intimal area and the fifth line the medial area. The data are given as mean ± 1SD. *p ≤ 0.05 vs. baseline control, † p ≤ 0.05 vs. untreated BA group and ‡ p ≤ 0.05 vs. local LMWH group.
ously performed PTCA and the manipulation with the atherectomy device at the lesion site [26]. In a post-mortem study in 11 patients who died during the first month after PTCA, no endothelial cells were observed. In contrast, all 6 patients who had died after one to 19 months after PTCA, showed a distinct reendothelialization [27]. From these observations it can be speculated that humans have a comparable but probably a somewhat delayed proliferation kinetic of endothelial cells.

Several studies investigating the time course of smooth muscle cell proliferation after balloon angioplasty demonstrate an increase of proliferation in the early stage after balloon-injury [22,23]. Especially during the early stage, the endothelial layer plays an important role in smooth muscle cell growth control. In vitro data show that endothelial cells have an inhibitory effect on smooth muscle cell proliferation [5,6]. Possible mechanisms might be the endothelial production of heparin proteoglycans [5] or the release of NO [28]. Several studies in the rat model of arterial balloon injury have shown an inverse relation between reendothelialization and SMC proliferation [29–31]. In this study, we demonstrate that subcutaneous and local treatment with reviparin manifest antiproliferative effects on SMC in vivo. The early growth inhibitory effect results in a subsequent reduction of neointimal growth represented by the decrease in the intima-to-media ratio. There was no relevant effect on medial SMC proliferation. In addition, the results demonstrate an advantage of local delivery compared to systemic delivery. These findings suggest that the increased reendothelialization may thus contribute to the antiproliferative effect of reviparin.

Heparin is a heterogeneous mixture of sulphated polysaccharides with a mean molecular weight of 15 000 dalton. The anticoagulatory effect of antithrombin III is increased dose-dependently and factor X is inactivated. Additionally, heparin binds several proteins, e.g. platelet-factor 4, vitronectin, fibronectin, lipoprotein and vWf. Using depolymerisation LMWH can be produced with molecular weights of 4000–6500 dalton. LMWHs have a higher bioavailability, a longer half-life and a lower incidence of side effects [32,33].

Heparin and low molecular weight heparin have been reported to induce endothelial growth. Thornton et al. demonstrated that heparin together with EGF promotes endothelial cell growth [34]. Terranova et al. could show a chemotactic influence of heparin on endothelial cells [35]. Fibroblast growth factor (FGF) was isolated as a heparin-binding endothelial mitogen. Both aFGF and bFGF induce proliferation of endothelial cells, fibroblasts and smooth muscle cells [19,36,37]. A dose dependent synergy of heparin and FGF on the proliferation of endothelial cells was demonstrated, and reinforce the concept that heparin plays an important role for the internalization and prevention of the degradation of growth factors [36,37]. Recently, Kang et al. found that a selective stimulation of endothelial growth and inhibition of smooth muscle cell proliferation could be achieved using a specific combination of aFGF and heparin [38].

In the present study, significant differences in the time course of reendothelialization were observed between the animals treated with LMWH and the untreated group. The animals which received LMWH showed as early as 7 days after injury a significantly higher number of endothelial cells and a more intact endothelium compared to untreated animals. This effect was additionally augmented in those animals in whom LMWH was locally delivered. This significant difference was maintained during the following 28 days. However, two months after intervention there was no significant difference between all three groups. The reendothelialization factor during 28 days was calculated as 2.8-fold after subcutaneous treatment and 4.1-fold after local LMWH treatment. These findings represent a twofold increase in endothelial proliferation following local LMWH application during 28 days compared to the untreated animals. This effect is most probably due to the high concentration of LMWH delivery directly at the site of injury. From a clinical standpoint it is encouraging that a single application of LMWH was sufficient to improve reendothelialization after injury. In contrast to our results, Hardhammar et al. showed in a study with heparin-coated coronary stents that endothelial growth on the stent struts was inhibited [39]. This might be explained by the fact that the effect on endothelial proliferation differs considerably between the various heparin preparations [13]. Furthermore, the high local heparin concentration on the stent struts may have an inhibitory effect on endothelial growth.

Several of the endothelial cells after balloon angioplasty showed morphological differences from normal endothelial cells with irregular size, polygonal shape and lack of the normal orientation with blood flow. These findings are in correspondence with recent observations of other investigators [22]. However, due to the fact that only electron microscopy on cross sections and no en-face views of the vessel surface were performed this finding is of descriptive nature only.

Furchgott et al. demonstrated that an intact endothelium is required for normal endothel-dependent vasorelaxation [3]. The reendothelialization after injury seems to be closely connected to the improvement of endothelial function [40–42]. In a study investigating the vasodilator-function of the endothelium 2 and 4 weeks after denudation in rabbit iliac arteries, it was shown that the endothelial function was reduced after injury compared to the control group [26]. Thus, the increased reendothelialization after LMWH treatment found in the present study may play an important role for the restoration of endothelial cell function. Whether the favorable effects of reendothelialization in the present study also indicate functional improvement remains to be determined.

The electrostimulation model allows the induction of a plaque which contains smooth muscle cells, leucocytes and cholesterol [43]. The maintenance of the endothelial lining
throughout the 28 days of electrostimulation is the major advantage of this model and allows for the investigation of the pathophysiology of the endothelium.

In conclusion, the present study demonstrates that in vivo systemic and local heparin administration enhanced the recovery of the morphological appearance of the endothelium and may, therefore, improve endothelial function. This might be a therapeutic option to accelerate reendothelialization after interventional procedures and may explain the promising results achieved in early clinical studies using local delivery of heparin and low molecular weight heparin [44,45].

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