Vascular Injury and Time Course of Smooth Muscle Cell Proliferation After Experimental Holmium Laser Angioplasty

Stefan Hassenstein, MD; Hartmut Hanke, MD; Joachim Kamenz, MS; Martin Oberhoff, MD; Sybille Hanke, MS; Reimer Rießen, MD; Karl K. Haase, MD; Eberhard Betz, MD; and Karl R. Karsch, MD

Background. In vitro experiments have shown that holmium laser energy can effectively ablate even calcified plaque in human arterial vessels. Because high-energy densities from holmium lasers can easily be transmitted through quartz fibers, this solid-state laser has been suggested as an alternative intraluminal treatment of atherosclerotic plaque.

Methods and Results. To develop an intimal plaque, 35 New Zealand White rabbits underwent electrical stimulation of their right carotid artery for 28 days. Subsequently, in 25 rabbits, holmium laser angioplasty (wavelength, 2.12 μm; pulse duration, 150 μsec; energy density, 350 mJ/mm²) was performed. To study the morphological results, the vessels were excised after 7, 14, 28, and 42 days. Cross sections were analyzed in regard to laser-specific injury. Staining of α-actin was used to identify smooth muscle cells (SMCs). After bromodeoxyuridine labeling, the extent of proliferation (number of cells undergoing DNA synthesis) was determined by using a monoclonal antibody. Holmium laser ablation resulted in an initial decrease of the numbers of intimal cell layers in the early group (7 days after treatment: 5±1 cell layers with 76±39 μm; control: 13±3 cell layers with 144±44 μm). Quantification of SMCs undergoing DNA synthesis in the intima (control: 51±19 cells/mm²; p=0.003) and 14 days (281±139 cells/mm², p=0.011). Integrity of the internal elastic lamina was disrupted in all animals after intervention. Seven and 14 days after treatment, a considerable reduction of medial cell nuclei was found in 10 of 12 animals. SMC proliferation in the medial layer was increased within the first 2 weeks after laser ablation (168±113 cells/mm²; control: 8±4 cells/mm²; p=0.023). Six weeks after holmium laser angioplasty, SMC proliferation had returned to control levels in the intima and remained increased in the medial layer. This proliferative response resulted in a significant increase of intimal thickening within 6 weeks after laser ablation (30±6 cell layers, 375±97 μm resp.; p=0.001 each).

Conclusions. Holmium laser treatment leads to considerable vessel wall injury and results in SMC proliferation in the intimal and medial layer with a maximum of proliferative activity within the first 2 weeks. Subsequently, this results in considerable intimal and medial hyperplasia within 6 weeks after treatment. (Circulation 1992;86:1575–1583)

KEY WORDS • smooth muscle cells • holmium laser • bromodeoxyuridine • atherosclerosis • lasers • angioplasty

Percutaneous coronary excimer laser angioplasty has been suggested as an alternative treatment of atherosclerotic lesions.1–5 However, there are inherent problems in excimer laser ablation. Fiber optic transmission of short laser pulses with sufficient energy densities to ablate calcified plaque leads to enormous power densities that could destroy the fiber.6 Furthermore, the ablative efficacy of excimer laser ablation is limited.2,4

These limitations are thought to be overcome by the use of an alternative laser energy, i.e., holmium lasers. The pulsed holmium laser appears suitable because the energy of this mid infrared laser is ubiquitously absorbed in tissue and feasible ablation of atherosclerotic plaque occurs at thresholds between 100 and 400 mJ/mm².7–11 The holmium laser is of particular interest since transmission of even high energies is possible through non-toxic standard quartz fibers.12 Using intracoronary holmium laser energy, Geschwind et al.13 reported successful ablation in eight of 10 procedures in a first clinical study. The major clinical limitation of all available techniques for treatment of atherosclerotic heart disease is the high incidence of restenosis, which will occur in as many as 30% of all primary successfully treated patients. It is well known that the increase of intimal thickness at the site of intervention is primarily due to smooth muscle cell (SMC) proliferation14,15 and can be ob-
served after balloon dilatation and excimer laser angioplasty.16–18
This biological response of proliferating intimal and medial cells may result in the occurrence of restenosis. Although clinical trials for the evaluation of coronary angioplasty using holmium lasers have been initiated, little is known about the effect of holmium laser energy application on adjacent arterial tissue and subsequent SMC proliferation in experimental in vivo studies.
Thus, the aim of the present in vivo study was to analyze the 1) laser-specific injury and 2) the time course and extent of proliferating SMCs after experimental holmium laser ablation.

Methods

Animal Model
Before laser ablation was performed, an intimal atheromatous plaque was produced with the electrostimulation method first reported by Betz and Schlote.19
Briefly, two graphite-coated gold electrodes were fastened to the adventitia of the right common carotid artery of 35 animals under general anesthesia (intra-muscular administration of 8 mg metomidate-HCl and 0.1 mg fentanyl base per kilogram of body weight). The electrodes (5 mm long, 0.1 mm thick, and 0.2 mm wide) were held in position with a flexible Teflon cuff on diametrically opposed sites of the artery. Subcutaneous leads from the electrodes were connected to a small socket attached to the skull. Twice daily, two leads from an external stimulation unit were plugged into this socket. Weak DC impulses (15-msec impulse, 0.1 mA, 10 Hz) were delivered for 30 minutes in the morning and 15 minutes in the afternoon for a total of 28 days in each animal. During the stimulation procedure, the animals were conscious and unrestrained. To produce atheromatous plaques, the rabbits were fed a 0.5% cholesterol diet (Altromin, Lage, FRG) during their stimulation period.20 This procedure resulted in an intimal atheroma below the anode in all animals.
All experiments were performed in accordance with the position of the American Heart Association on research animals use.21

Laser System
A prototype pulsed holmium:yttrium-argon-garnet (YAG) laser (LISA Inc., Katlenburg, FRG) (wavelength, 2.12 μm; pulse duration, 150 μsec; repetition rate, 1.5 Hz) was used. The laser was supplied with a red diode laser coupled into the output by a beam splitter to allow better local targeting during the procedure. The laser beam was focused with a lens into an 800-μm core-diameter low-OF quartz fiber (CeramOptec Inc., Bonn, FRG). Transmitted energy fluence was adjusted at 350 mJ/mm² and measured before and after treatment with a conventional power meter (RBM Inc., Munich, FRG).

Study Protocol
A total of 35 male New Zealand White rabbits (3.2±0.3 kg body wt) were used in this study. After 28 days of electrical stimulation, the cholesterol diet was changed to a standard diet (Altromin). Two hours before laser irradiation, a bolus of 700 units heparin/kg body wt (B. Braun Inc., Melsungen, FRG) was given subcutaneously. The right carotid artery was prepared under general anesthesia. The arterial blood flow was stopped by setting two clamps at least 4 cm caudal and 2.5 cm cranial to the Teflon cuff. The bare fiber was introduced into the lumen by small transversal arteriotomy under stereoscopic control. With the red diode laser working as a control light that could be detected through the vessel wall, the fiber tip was moved cranial to the region of plaque. In 25 rabbits, laser energy was delivered while the fiber was slowly advanced. Irradiation was started 5 mm distal and stopped 5 mm proximal of the cuff. Within 20 seconds, the entire distance of 15 mm (0.75 mm/sec) was irradiated. Additional irradiation was applied during withdrawal (15 mm in 10 seconds, 1.5 mm/sec) of the fiber tip. After a second advancement, the fiber was withdrawn and removed. A total of 75 pulses of holmium laser light was delivered within 50 seconds to each vessel. To reestablish arterial blood flow, the arteriotomy was closed with 7-0 polypropylene sutures.
For the next 3 postoperative days, a broad-spectrum antibiotic powder was supplied. In addition, the rabbits received 900 units heparin/kg body wt s.c. twice daily for the next 3 postoperative days.

The rabbits were killed with an intravenous overdose of metomidate-HCl and fentanyl base on day 7, 14, 28, or 42 after laser treatment. Before excision, all carotid arteries were perfused in situ under physiological pressure (80–100 mm Hg) with 500 ml of 0.1 M cacodylate-buffered 2% paraformaldehyde solution. After in situ fixation, the vessels were excised and immersion-fixed in 2% cacodylate-buffered paraformaldehyde for at least 6 hours.

A sample of small intestine was excised for determination of the incorporation of bromodeoxyuridine (BrdU) into replicating cells.
Ten electrostimulated rabbits were used as controls. Five of these animals underwent the same procedure as described above without energy delivery to determine possible mechanical injury by the bare fiber (sham operated). The remaining five control animals were killed without laser treatment immediately after the stimulation period to analyze the plaque resulting from electrical stimulation only.

Application of BrdU
BrdU labeling was performed in all rabbits to quantify the amount of proliferating SMCs at different time intervals after laser ablation.16,17,22 Eighteen hours before the rabbits were killed, a subcutaneous neck depot consisting of 100 mg BrdU/kg body wt and 75 mg 2'-deoxycytidine (d-cyt)/kg body wt (both from Sigma Inc., Deisenhofen, FRG) was given. In addition, intramuscular injections (30 mg BrdU/kg body wt, 25 mg d-cyt/kg body wt) were given 18 and 12 hours before the vessels were excised.

Tissue Analysis
Histological examination. After removal of the Teflon cuff, the specimens were excised and embedded in paraffin. The specimens were cut in series beginning at the caudal end of the region irradiated by holmium laser energy to the maximal extent of the plaque. The obtained 4-μm-thick cross sections were used for histolog-
ical and immunohistological examination. For morphometric evaluation, the sections were stained with standard hemalaune and hematoxylin and eosin. The irradiated vessel wall sections were examined for laser-specific alterations such as carbonization, perforation, dissection, and fragmentation of the internal elastic lamina.8,11,23–28

The technique of immunohistological examination has been described previously16,17 and is based on staining with avidin-biotin29,30 using a monoclonal antibody against BrdU31 (Bio Cell Consulting, Grellingen, Switzerland). This technique allows identification of all proliferating cells in the vessel wall, which entered the S phase during the 18 hours of the labeling period. In addition, immunohistological staining (fluorescein isothiocyanate–labeled immunofluorescence [Sigma Inc.]) of the α-actin isoform of vascular smooth muscle (monoclonal antibody by Renner Inc., Dannstadt, FRG) was performed to identify the proliferated cells in the intima as SMCs.

In addition, estimation of the small intestine mucosal-labeling index was performed in each animal to control the incorporation of BrdU in replicating cells.

**Morphometry.** All sections containing BrdU-positive cells were quantitatively analyzed by computerized morphometry. The sections were projected by a side tubus affixed to the stereoscope (Olympus Inc., Hamburg, FRG) onto a digitizing pad (Summagraphics Inc., Seymour, Conn.). The luminal perimeter, internal and external elastic lamina, length of the disrupted internal elastic lamina, and medial area without SMC nuclei were traced manually under stereoscopic control. The thickness of the medial and intimal layers was determined at the region of maximal plaque size. The area of the intima, media, and residual lumen was calculated using standard software (BIOQUANT, Bilaney Consulting, Düsseldorf, FRG).

The number of intimal cell layers was taken as a quantitative measure. It was obtained by counting the number of cell nuclei on the perpendicular line between the intraluminal surface and internal elastic lamina at the area of maximal plaque size.

The medial area without SMC nuclei was determined by morphometry and calculated as percentage of the total medial area:

\[
\text{Medial area without SMCs} = \frac{\text{Total medial area}}{\text{Total medial area}} \times 100(\%)
\]  

(1)

Vessels with thrombotic occlusions were not considered for this analysis. The length of the disrupted internal elastic lamina (IEL) was calculated as the proportion of the total IEL:

\[
\text{Disrupted IEL} = \frac{\text{Total IEL}}{\text{Total IEL}} \times 100(\%)
\]  

(2)

The severity of the lesion was calculated as:

\[
\frac{\text{Intimal area} \times 100}{\text{Intimal area} + \text{lumen area}}(\%)
\]  

(3)

All BrdU-positive cells were counted separately in the intimal and medial cross-sectional area to determine the extent of the proliferative response after holmium laser treatment. The proliferating cells per area (PCA) were obtained for both layers separately:

\[
\text{PCA}_{\text{intima}} = \frac{\text{BrdU-cells}_{\text{intima}}}{\text{Intimal area}} \times \left(\frac{1}{\text{mm}^2}\right)
\]  

(4)

\[
\text{PCA}_{\text{media}} = \frac{\text{BrdU-cells}_{\text{media}}}{\text{Medial area}} \times \left(\frac{1}{\text{mm}^2}\right)
\]  

(5)

In addition, the total numbers of cells were separately counted in the intimal and medial areas. The number of proliferating cells was calculated as the proportion of the total cell number in percentage for both layers.

**Determination of Serum Cholesterol Level**

Venous blood was collected from all animals when they were killed. Total serum cholesterol was measured by standard enzymatic protocol.32 To obtain normal values in six animals, total serum cholesterol was estimated before the 0.5%-cholesterol diet was started.

**Statistical Analysis**

All values are expressed as mean±SD. The significance of differences between control and laser-treated vessels was determined using two-tailed Student’s t test. The value of p was calculated between the control and the laser-treated animals and reported exactly.33 Differences were considered significant at p≤0.05.34

**Results**

**Histology**

**Laser-specific injury.** Transmural perforation was observed in only one vessel. This animal, however, survived and was killed 28 days after intervention. The small perforation was covered by the Teflon cuff, and an occlusive thrombus was found at the site of perforation. In one animal, an aneurysm was noted. Separation between the intimal and medial layers was found in 10 of 25 laser-treated vessels (Figure 1) and in none of the control vessels.

An area without SMC nuclei in the medial layer was observed in all laser-treated vessels 7 days after intervention with a mean extent of 73±31% (control: 1.5±0.3%; p=0.002). The area without SMCs was found to be decreased to 37.7±27.7% (p=0.019) 14 days after laser treatment. At day 28, the medial area without SMC nuclei was 28.5±7.5% (p=0.001) and decreased further to 7±3% (p=0.005) at day 42 after treatment (Figure 2).

Disrupted internal elastic lamina could not be found in the control or sham-operated group. In all laser-treated groups, however, the internal elastic lamina was disrupted.

At 7 days after holmium laser angioplasty, 20.5±18.7% of the total internal elastic lamina was disrupted. Two weeks after laser treatment, 18.3±10.0% of the internal elastic lamina was disrupted. Four weeks after laser angioplasty, the proportion of disrupted internal elastic lamina was 16.6±3.8%, and after 6 weeks, it was 29.3±9.9%. There was no statistical difference in the percentage of disrupted internal elastic lamina at the different time periods.
FIGURE 1. Photomicrograph of cross section of a right carotid artery 1 week after holmium laser angioplasty. The lumen is at the top and right. At the right, loss of smooth muscle cells can be detected in medial layers (arrow 1). Fibrin is located between medial layers (arrow 2). At the top and left, the original structure of the medial layer is disrupted (arrow 3). Hematoxylin and eosin staining; original magnification, ×32.

Signs of carbonization were not observed in any artery.

Intimal thickening. After 28 days of electrical stimulation, in all control animals an intimal atheromatous plaque was found beneath the anode. In these five animals, the plaque consisted of 13±3 cell layers, and mean intimal wall thickness was 144±44 μm. In the sham-operated group, the plaque consisted of 14±3 cell layers, and the intimal wall thickness was 161±20 μm 7 days after intervention. There was no significant difference between the control group and the sham-operated group in regard to intimal cell layers (p=0.424) and intimal wall thickness (p=0.416).

In the treatment group at 7 days after laser ablation, the remaining plaque had 5±1 cell layers, and the intimal wall thickness was 76±39 μm (p=0.049). Fourteen days after holmium laser angioplasty, the plaque consisted of 16±8 cell layers, and mean intimal wall thickness was 205±113 μm (p=NS). Four weeks after holmium laser angioplasty, the plaque consisted of 22±2 cell layers, and mean intimal wall thickness was 317±59 μm (p=0.001). Six weeks after intervention, the plaque consisted of 30±6 cell layers, and the intimal wall thickness was 375±97 μm (p=0.001) (Figure 3).

Morphometric analysis revealed a reduction in severity of stenosis from 24±7% (control) to 14±3% (p=0.054) 7 days after laser angioplasty. Two weeks after treatment, stenosis was 35±12% (p=NS); after 28 days, it was 45±4% (p=0.001), and it was 58±18% (p=0.004) at 6 weeks.

A lesion of >50% of luminal area was found in four of the laser-treated arteries. In three of these vessels, the lesion was predominantly due to intimal proliferation of SMCs (two lesions after 42 days and one after 28 days), and in one vessel, a partially recanalized thrombus was found (animal 7).

On histological examination, thrombi were found in nine of 25 animals. The thrombi in six rabbits resulted in an occlusion that was partially recanalized in one (animal 7); in three of these animals, only mural thrombi with <50% luminal reduction were found (Table 1).

SMC proliferation after holmium laser angioplasty. In all animals, the incorporation of BrdU into replicating cells was tested by examination of the small intestine mucosa-labeling index. Approximately 30% of all cells were stained BrdU-positive.
The amount of proliferating cells per squared millimeter in the intimal layer of the control group was 51±19 and showed no significant difference from the sham-operated group (47±17 cells/mm²) (p=0.776).

Seven days after intervention, a significant increase in the number of BrdU-positive cells was observed with 216±74 cells/mm² (p=0.003) in the intimal layer. The maximum number of BrdU-positive cells was found 14 days after intervention (281±139 cells/mm², p=0.03) (Figure 4). After 4 weeks, the number of proliferating cells per squared millimeter remained increased (177±120 cells/mm², p=0.071). After 6 weeks, the number of proliferative cells remained significantly increased (25±10 cells/mm², p=0.043) (Table 2).

No significant differences were found in the medial layers between the control (8±4 cells/mm²) and the sham-operated group (11±6 cells/mm², p=0.430). Seven days after treatment, a significant increase of BrdU-positive cells was observed (161±122 cells/mm², p=0.037) in the medial layer. Fourteen days after intervention, the number of SMCs in the media was 168±113 cells/mm² (p=0.023). At 28 days after intervention, the number of proliferating cells decreased to 70±33 cells/mm² (p=0.033). Six weeks after ablation, 24±19 cells/mm² were found in the medial layer (p=0.158, NS) (Table 2).

The proportion of BrdU-positive cells to all cells in the control animals was 0.8±0.2% in the intimal layer and 0.6±0.2% in the medial layer. The maximum number of replicating cells was found after 14 days in the intimal (10±5%, p=0.005) and medial layers (12±8%, p=0.021). Six weeks after intervention, the proportion of proliferating cells remained increased in the intimal

**TABLE 1. Histomorphological and Immunohistochemical Findings in All Animals**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Days after intervention</th>
<th>Intima wall thickness (μm)</th>
<th>Stenosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>141</td>
<td>Control</td>
<td>124</td>
<td>10.3</td>
</tr>
<tr>
<td>144</td>
<td>123</td>
<td>21.4</td>
<td></td>
</tr>
<tr>
<td>145</td>
<td>124</td>
<td>29.3</td>
<td></td>
</tr>
<tr>
<td>148</td>
<td>107</td>
<td>29.2</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>227</td>
<td>26.4</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Sham operated</td>
<td>131</td>
<td>23.5</td>
</tr>
<tr>
<td>28</td>
<td>185</td>
<td>30.4</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>173</td>
<td>18.3</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>162</td>
<td>24.6</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>155</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>144±44</td>
<td>24.1±7.4</td>
</tr>
<tr>
<td>80</td>
<td>14</td>
<td>198</td>
<td>34.5</td>
</tr>
<tr>
<td>81</td>
<td>28</td>
<td>Occlusive thrombus formation</td>
<td></td>
</tr>
<tr>
<td>82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>323</td>
<td>Occlusive thrombus formation</td>
<td></td>
</tr>
<tr>
<td>134</td>
<td>317</td>
<td>45.1</td>
<td></td>
</tr>
<tr>
<td>135</td>
<td>90</td>
<td>19.8</td>
<td></td>
</tr>
<tr>
<td>136</td>
<td>97</td>
<td>26.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>205±113</td>
<td>34.8±11.8</td>
</tr>
<tr>
<td>83†</td>
<td>28</td>
<td>Occlusive thrombus formation</td>
<td></td>
</tr>
<tr>
<td>84*</td>
<td>271</td>
<td>45.6</td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>259</td>
<td>38.9</td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>389</td>
<td>48.3</td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>370</td>
<td>44.1</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>297</td>
<td>50.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>317±59</td>
<td>45.5±4.5</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>Occlusive thrombus formation</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>366</td>
<td>45.0</td>
<td></td>
</tr>
<tr>
<td>7‡</td>
<td>536</td>
<td>89.1</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>377</td>
<td>57.8</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>295</td>
<td>48.5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>303</td>
<td>51.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>375±97</td>
<td>58.4±17.8</td>
</tr>
</tbody>
</table>

*Mural thrombus formation.*
††Transmural vessel perforation.
‡‡Recanalized occlusion.

layer with 1.3±0.4% (p=0.044); in the medial layer, the number was 2.1±1.6% (p=0.088, p=NS) (Table 2).

No linear correlation between the SMC proliferation and the progressive increase of SMC layer accumulation could be observed.

**Serum Cholesterol Level**

Before the 0.5%-cholesterol diet was started, mean serum concentration of total cholesterol was 39±17
mg/dl. After 28 days of feeding the diet, serum cholesterol concentration increased to 1,155±186 mg/dl. At this time, the cholesterol diet was changed to the standard diet. Within 42 days of feeding the normal diet, serum cholesterol concentration decreased to 95±57 mg/dl (Table 3).

Discussion

Holmium Laser-Induced Vessel Wall Injury

The aim of this study was to evaluate the potential of holmium laser energy as an alternative for percutaneous angioplasty in an experimental animal model. Although several in vitro investigations have been performed, little is known about the acute and chronic in vivo effects of vascular application of holmium laser energy.

Threshold energy density necessary for effective ablation of atherosclerotic plaques has been determined previously in in vitro experiments. To ensure effective ablation, an energy threshold of 350 mJ/mm² was used in the present study. This energy could easily be transmitted through the bare fiber used in these experiments.

In in vitro experiments, holmium laser energy resulted in considerable thermal damage of the adjacent tissue depending on the energy densities used. In contrast, White et al. observed only minimal evidence of thermal injury in recanalized swine iliac arteries immediately after laser irradiation using energy densities between 255 and 795 mJ/mm². Thus, special attention was paid in regard to specific laser-related vascular injury.

In our experiments, a considerable number of vessels with fissuring off of the intimal and medial layers was found. As a sign of increased vascular injury, the internal elastic lamina was disrupted in all animals regardless of the time interval between treatment and tissue analysis. Furthermore, the medial area without SMC nuclei was considerably increased in the early groups (7 and 14 days after intervention). Both observations probably are the result of considerable thermal and/or acoustic injury of the arterial wall during the

**Table 2. Proliferating Cells in the Intimal and Medial Layer**

<table>
<thead>
<tr>
<th>Days</th>
<th>PCA (1/mm²)</th>
<th>PCPC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intima</td>
<td>Media</td>
</tr>
<tr>
<td>Control</td>
<td>51±19</td>
<td>8±4</td>
</tr>
<tr>
<td>Sham operated</td>
<td>47±17</td>
<td>11±6</td>
</tr>
<tr>
<td>7</td>
<td>216±74</td>
<td>161±122</td>
</tr>
<tr>
<td>14</td>
<td>281±139</td>
<td>168±113</td>
</tr>
<tr>
<td>28</td>
<td>177±120</td>
<td>70±33</td>
</tr>
<tr>
<td>42</td>
<td>25±10</td>
<td>24±19</td>
</tr>
</tbody>
</table>

PCA, proliferating cells per mm²; PCPC, proliferating cells in percent.

**Table 3. Total Serum Cholesterol Level**

<table>
<thead>
<tr>
<th>Days</th>
<th>Serum cholesterol level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39±17</td>
</tr>
<tr>
<td>Sham operated</td>
<td>1,130±175</td>
</tr>
<tr>
<td>7</td>
<td>1,033±197</td>
</tr>
<tr>
<td>14</td>
<td>517±180</td>
</tr>
<tr>
<td>28</td>
<td>205±45</td>
</tr>
<tr>
<td>42</td>
<td>95±57</td>
</tr>
</tbody>
</table>
Holmium laser energy application since comparable changes were not found in the sham-operated group.

In our study, successful ablation of atheromatous plaque was demonstrated by the decrease of intimal thickness and number of cell layers 7 days after intervention. Furthermore, as a sign of severe arterial injury and in concordance with the results of White et al., who found arterial dissections into the adventitial layer in four of 16 recanalized arteries, separation between the intimal and medial layers was found in 10 of 25 vessels. However, aneurysm formation or perforation appears to be a rare event after holmium laser angioplasty and was observed only in one case.

Our results indicate that the application of holmium laser light leads to considerable vascular injury with considerable reduction of medial SMCs, fracturing of the internal elastic lamina, and a high incidence of dissections. Thus, even at an energy range slightly above the ablation threshold, intraluminal holmium laser energy application results in severe vascular injury (Figure 1) and can be compared with type III grade following the classification of Ip et al.

**Time Course of SMC Proliferation and Incidence of Restenosis After Holmium Laser Ablation**

Several experimental and human postmortem studies have demonstrated the predominant role of proliferating SMCs in the development of restenosis after vessel wall injury. In contrast to a preliminary report by Aretz et al., who could not detect significant intimal hyperplasia in two dogs 1 and 3 weeks after holmium laser angioplasty using very high energies (4,800 and 8,000 mJ/mm²), the results of our study demonstrate a considerably increased number of intimal cell layers 28 days after the intervention treatment. The early and high increase of SMC proliferation resulted in a subsequent increase of intimal thickening as early as 6 weeks after laser ablation.

Furthermore, in contrast to excimer laser angioplasty previously investigated in the same experimental model, the proliferative activity of SMCs in the medial layer after holmium laser angioplasty was considerably increased and prolonged. This may be the result of the reduction of SMCs in the medial layer early after treatment. However, the comparison of both studies is limited due to the different application devices. In the current study, a bare fiber was used with a tip area of 0.5 mm², whereas in the excimer laser study, ablation was performed with a multifiber catheter. Comparable multifiber catheters for the holmium laser, however, were not available for use in the present study.

SMC migration and mitotic activity are controlled by a variety of stimulating and inhibiting mediators. Among the factors that are known to be important, the endothelial cells and mitogens derived from platelets have been investigated in several experimental studies. Intact endothelial cells are capable of inhibiting SMC proliferation. Furthermore, there is experimental evidence that injured endothelial cells can stimulate the mitogenic activity of adjacent SMCs. In an experimental model of balloon angioplasty, it has been shown that endothelial injury leads to platelet adhesion and aggregation. Subsequent degranulation releases mitogens like platelet-derived growth factor or transforming growth factor β, by injured SMCs and activation of macrophages with released macrophage-derived growth factor, these responses are thought to stimulate migration and proliferation of SMCs in the area of vessel wall injury.

Compared with the animals that were only electrically stimulated, the sham-operated animals of our study showed no statistically significant differences in regard to plaque size or number of proliferating SMCs. Thus, the observed effect of considerable SMC proliferation probably is the result of holmium laser energy application on the preformed atheromatous plaque.

**Limitations of the Model**

Non-specific electrical stimulation appears to be a very artificial method of inducing an intimal arterial plaque. However, migration of cells can be directed by electrical fields and tissue damage. It is well known that electrical stimulation causes a transient increase of transmural electrical resistance and leads to enhanced permeability of the endothelial cell for macromolecules beneath the anode. Given the functional alteration of endothelial cells, this model can be classified to produce vascular injury type I as described by Ip et al. Subsequently, SMCs protrude and migrate through pores of the internal elastic lamina into the subendothelial space. Using this animal model under standardized conditions, fibromuscular plaques of comparable size can be produced by maintaining the endothelial integrity. This is in contrast to the experimental balloon injury technique in which the plaque is produced by complete denudation of the arterial endothelium. By feeding a 0.5%-cholesterol diet, an accumulation of cholesterol occurs within the stimulated region. This results in the development of an atheroma instead of a fibromuscular plaque. This plaque might be comparable to the early stage of atherosclerotic lesions in humans. However, the model is limited because we cannot produce calcified plaques.

**Study Implications**

Holmium laser ablation resulted in a severe arterial injury in the intimal and medial layers with considerable and persistent disruption of the internal elastic lamina. Furthermore, the proliferative vessel wall response is pronounced and leads to a considerable increase of intimal cell layers within the 6-week follow-up period. In view of these results, the clinical use of holmium laser angioplasty for human applications appears to be limited.

**Acknowledgments**

We greatly appreciate the helpful and stimulating discussions of Joachim Rupp, PhD, and Manfred Wehrmann, MD. We also thank Ms. Rosemarie Barth and Ms. Rosemarie Weidler for their excellent technical assistance in conducting the in vivo experiments. We also thank Ms. Rosemarie Barth and Ms. Regine Baur for help, advice, and assistance with immunohistochemical methods.

**References**


44. Schneider MD, Parker TG: Cardiac myocytes as targets for the action of peptide growth factors. Circulation 1990; 81:1443–1456


46. Lindner V, Reitn U: Proliferation of smooth muscle cells after vascular injury is inhibited by an antibody against basic fibroblast growth factor. Proc Natl Acad Sci U S A 1991; 88:3739–3743
56. Strohschneider T, Betz E: Densitometric measurement of increased endothelial permeability in atherosclerotic plaques and inhibition of permeability under the influence of two calcium antagonists. *Atherosclerosis* 1989;75:135–144
58. Kling D, Holzschuh T, Strohschneider T, Betz E: Enhanced endothelial permeability and invasion of leukocytes into the artery wall as initial events in experimental arteriosclerosis. *Int Ang 1987;6*: 21–28
Vascular injury and time course of smooth muscle cell proliferation after experimental holmium laser angioplasty.
S Hassenstein, H Hanke, J Kamenz, M Oberhoff, S Hanke, R Riessen, K K Haase, E Betz and K R Karsch

_Circulation_. 1992;86:1575-1583
doi: 10.1161/01.CIR.86.5.1575

_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/86/5/1575

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/