Acute Biological Response to Laser Balloon Angioplasty in the Atherosclerotic Rabbit

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Laser balloon angioplasty with Nd:YAG energy has been proposed as a method to seal intimal dissection and prevent elastic recoil after balloon angioplasty. To better define the vessel response to laser balloon angioplasty, its effects on luminal diameter, Indium-111 labelled platelet deposition, and histology were studied in 10 atherosclerotic rabbits. Balloon angioplasty was performed in both iliac arteries and was followed by laser balloon angioplasty in only one iliac artery. The nonlased artery served as a control. Single (15-35 W for 20 sec) or repetitive laser pulses (12–25 W for 20 sec ×3) were used. Platelet deposition was quantified 2 hr after the intervention. Lumen diameter (mm) increased following balloon angioplasty from 0.99 ± 0.47 (mean ± SD) to 1.92 ± 0.43 and 0.89 ± 0.46 to 1.99 ± 0.57 in the balloon and laser-treated arteries, respectively (P < 0.001 for both groups for comparisons to baseline, P = NS for between groups comparison). Laser balloon angioplasty resulted in a further increase in luminal diameter to 2.42 ± 0.53 (P < 0.02) when compared to the post balloon angioplasty diameter. Platelet deposition (10⁶/cm vessel) was higher following laser balloon angioplasty (26.9, 10.2–189; median range) than after balloon angioplasty (10.6, 3.4–30), P < 0.001. Histologic evidence of laser “sealing” was present in only one artery. Thus although laser balloon angioplasty results in an improved lumen diameter, it is accompanied by increased platelet deposition. In the atherosclerotic rabbit model, abolition of vascular recoil rather than “sealing” seems to be the most important advantage of laser balloon angioplasty over conventional balloon angioplasty.

INTRODUCTION

Laser balloon angioplasty with Nd:YAG energy using the Spears (USCI, Billerica, MA) balloon may seal intimal dissection and/or prevent vascular elastic recoil after balloon angioplasty [1]. The sealed, smoothed vascular surface may result in decreased platelet accumulation and therefore in less restenosis. This laser system has been used percutaneously for coronary angioplasty in humans, with satisfactory acute results [2], specially in the treatment of abrupt closure following angioplasty [3]. However, the restenosis rate has not been shown to be diminished compared to conventional balloon angioplasty [4,5]. Because the biological response of atherosclerotic vessels to laser balloon angioplasty in vivo has

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not been well defined, we assessed the acute effects of laser balloon angioplasty in atherosclerotic vessels using angiography. Indium-111 labeled platelets, and histology. In addition, we compared the results obtained with laser balloon angioplasty to those obtained with conventional balloon angioplasty.

MATERIALS AND METHODS

Experimental Protocol

Ten male New Zealand rabbits, weighing 2.5–3.0 kg, were used. Experimental atherosclerosis was induced by a high cholesterol diet (2% cholesterol, 10% peanut oil) and balloon deendothelialization as previously described [6]. In brief, after 2 weeks of a high cholesterol diet, the animals were anesthetized by pentobarbital (25 mg/kg given I.V.) and their iliac arteries deendothelialized by a 3F Fogarty catheter inserted through the popliteal artery. The animals recovered from anesthesia and were continued on the atherogenic diet for 6 additional weeks.

All the procedures performed in the study were in accordance with institutional guidelines and followed the American Heart Association guidelines for use of animals in research.

Platelet labeling. Twenty-four hours prior to the final procedure, 20–25 ml of blood was drawn from the animal for autologous platelet radioactive labeling, as previously described [7]. Briefly, Indium-111 tropolone was prepared from indium-111 chloride by the addition of 25 μg of tropolone dissolved in 25 μl of saline to 250 μCi of indium-111 chloride. This solution was mixed with 0.5 cc of platelet poor plasma (PPP). Platelets were harvested by two successive centrifugations. The isolated pellet of platelets was resuspended in 2 ml of PPP to obtain a platelet-rich plasma concentrate (PRP). The indium–tropolone complex was added to the PRP and the mixture incubated at 37°C for 20 minutes. Free indium-111 was removed by washing with 2 ml of PPP. The final pellet of labeled platelets was resuspended in 4.5 ml of PPP and injected into the animal after a low-spin centrifugation to remove any microaggregates. The labeling procedure lasted for ~2 hours. An average efficiency (percentage of indium-111 activity incorporated into the platelets) of 57 ± 4 (mean ± 1SE), was obtained. The average injected activity was 174 ± 34 μCi (mean ± 1SE) and an average of 1,368 × 10⁹In¹¹¹ labeled platelets/μl were injected in a total volume of 4.5 ml.

On the day of the procedure, the animals were anesthetized with IV pentobarbital and a right carotid artery cutdown was performed. Following heparinization with 1,000 IU, a 4 Fr Swan-Ganz catheter was inserted and its balloon inflated immediately before the bifurcation of the descending aorta in order to obstruct the vessel. Baseline iliac angiography was performed by injecting peripherally Renographin 76, using a Precise Optics (P150, Bay Shore, NY) fluoroscopy unit and recording on 3/4" Umatic videotape.

Conventional balloon angioplasty of both iliac arteries was followed using a 3.0 mm angioplasty balloon (Simplus, USCI) inflated 3 times in each artery for 30 sec at 5 Atm, with 30 sec intervening between the inflations. Repeat iliac angiography was performed, followed by randomization to laser balloon angioplasty of one of the iliac arteries. The contralateral artery served as a control.

Laser protocol. Laser angioplasty was performed using a 3.0 mm laser-balloon catheter with a 100 μ fiber optic. The balloon was inflated with a non-ionic contrast mixed with D₂O. In the first 6 rabbits, single laser pulses were given for 20 sec (35 W for 5 sec, 25 W for 5 sec, and 15 W for 10 sec) generated from a Nd:YAG power source (Quantronics, Smithtown, NJ). Following the absence of the expected sealing effect on histology and the change in the laser parameters used clinically [2], in the last 4 rabbits repetitive (×3) pulses were used for 20 sec each: 25 W for 5 sec, 15 W for 5 sec, and 12 W for 10 sec. During the laser energy delivery, continuous flushing of the central lumen of the balloon catheter was performed using normal saline at a room temperature. However, in this small animal model no perfusion was possible proximal to the balloon through a guiding catheter.

Immediately following the laser procedure, final angiography was performed. Two hr after the angioplasty procedure, the animals were sacrificed with pentobarbital overdose and in situ fixed by pressure perfusing for 15 min with 4% formaldehyde in 0.1 M Sodium Phosphate buffer (pH 6.9–7.1) at a controlled pressure of 100 mmHg. After fixation, the surrounding connective tissue and adventitia were removed and the length of each artery measured. For platelet quantification, blood sampling was performed at the beginning of the experiment, before the angioplasty procedure and before sacrificing the animal.
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Platelet quantification. Tissues and bloods were counted for 5 min in a Packard Auto-Gamma 5650 counter (United Technologies). Platelet deposition on each treated artery (× 10^5/cm of vessel length) was calculated from the blood platelet count and indium-111 counts on the arterial wall and in the blood as previously described [7].

Histology. The presence of mural thrombus and extravascular damage was assessed macroscopically. Light microscopy was used to document the presence of a dissection or medial tear. From each arterial segment, cross sections were stained with hematoxylin and eosin as well as Heidenhain's Weigert-van Gieson stain. A zone of adherence within the neointima or media was considered as evidence of sealed dissection, as previously described by Hiehle et al. [8].

Angiographic analysis. The recorded angiograms were magnified (4×) and the minimal diameter of the lumen was measured with a light pen using as a reference the diameter of a fully inflated 3.0 mm angioplasty balloon. This method is reproducible within 0.2 mm in our laboratory.

Statistical methods. Luminal vessel diameter values are expressed as mean±SD and platelet values as median and range. The Students t-test (paired or unpaired as appropriate) and the nonparametric Wilcoxon test were used, respectively.

RESULTS

There were no differences in angiographic, platelet, and histologic findings between the segments that received single vs. repetitive laser pulses. Therefore, the laser treated segments were considered as a single group and compared to the conventionally balloon angioplasty segments.

Angiography

There was no vessel perforation or acute closure in the entire series. Vessel luminal diameter at baseline angiography, following conventional balloon angioplasty and following laser balloon angioplasty in control and laser treated arteries, is presented in Table 1. There was no difference in vessel luminal diameter at baseline angiography between groups. Following conventional balloon angioplasty, vessel luminal diameter increased significantly in both groups. Laser balloon angioplasty resulted in further increase in luminal diameter. In the balloon-only treated arteries, the diameter at final angiography was similar to the immediate postballooning angiography. Although the laser-treated arteries had a larger diameter than the balloon alone-treated arteries at final angiography, this difference was not statistically significant.

Table 1. Vessel luminal diameter (mm, mean ± SD) at baseline angiography, post balloon angioplasty (BA), and at final angiography in control and laser balloon (LBA)-treated arteries

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<th>Baseline</th>
<th>Post-BA</th>
<th>Final angiography</th>
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<tr>
<td>Control (BA only)</td>
<td>1.99 ± 0.47</td>
<td>1.92 ± 0.43*</td>
<td>2.10 ± 0.33</td>
</tr>
<tr>
<td>Laser group (BA+LBA)</td>
<td>1.99 ± 0.57*</td>
<td>2.42 ± 0.53**</td>
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<td><strong>P</strong>&lt; 0.001, when compared to baseline.  <strong>P</strong>&lt; 0.02, when compared to post-BA.</td>
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Platelet Deposition

The individual platelet deposition in each of the treated arteries is displayed in Figure 1. There was significantly higher platelet deposition in the laser-treated segments compared to the control balloon-treated segments: 26.9 (range 10.2–189) vs. 10.6 (range 3.4–30), (P < 0.001). In addition, in each individual animal, platelet deposition in the laser-treated segment was always higher than in the contralateral balloon alone-dilated segment.
Histologic Findings

Macroscopically, there was evidence of intraluminal thrombosis in 4 out of 10 of the laser-treated arteries and in 2 out of 10 of the balloon alone-dilated arteries. In 2 laser-treated arteries, there was also evidence of extravascular damage and thrombus formation. However, we were unable to differentiate whether this was caused by the laser treatment or by a small perforation undetected by angiography.

On light microscopy, all the studied segments showed the characteristic appearance of the balloon-dilated atherosclerotic vessel: a thick neointima separated from the media and tears or dissection involving the intima and or the media. A typical example from a balloon dilated artery, which was subsequently treated with laser application, is shown in Figure 2. In only one out of 10 laser-treated arteries, visible dissection appeared to be sealed by laser irradiation, (Fig. 3).

DISCUSSION

Angiography

In this study, a significant improvement in the vessel luminal diameter was observed with the application of laser energy following conventional balloon angioplasty. Previous studies [1,8] have proposed that the mechanism of this improvement is the abolition of elastic recoil of the vessel and the prevention of intimal flaps from interfering with flow. Although quantification was difficult, an angiographic appearance of a smoother luminal surface was apparent in at least a few cases. Jenkins et al. [9] showed that laser balloon angioplasty in normal rabbit iliac arteries increases luminal diameter acutely and, at a moderate laser dose, chronically. In our study, there was a tendency toward increased final diameter in the laser vs. the conventional balloon angioplasty-treated segments. However, this did not reach statistical significance, probably due to the small number of the arteries studied.

Platelet Deposition: Histologic Findings

Despite the increased diameter and improved angiographic appearance in the laser-treated segments, platelet deposition was significantly higher following laser irradiation. The absence of any histologic improvement in all but one laser-treated segment may explain the lack of an apparent effect on platelet deposition. Even in
that single experiment in which evidence of histologic sealing appeared to be present, platelet deposition was 6-fold higher in the laser-treated than the conventionally treated artery. Tissue preparation for histology was similarly meticulous in laser- and nonlaser-treated arteries, so that the presence of similar dissections between them and absence of sealing at the lased sites cannot be attributed to the technique used. The lack of sealing in the rabbit model in our study is in discordance with the favorable bail out record of laser balloon angioplasty in humans [3]. Hiehle et al. [8] have described the in vitro thermal fusion of human atheromatous plaque-arterial wall separations with Nd:YAG laser. However, in half of the segments of whole artery in which intraluminal Nd:YAG laser radiation from an inflated balloon was applied, no adhesion was seen. Differences in the composition of the human atherosclerotic plaque and the atherosclerotic rabbit plaque and consequent dissections post angioplasty in the two settings may account for this discrepancy.

It is possible that the injury to the vessel wall caused by the simultaneous angioplasty and laser energy is more severe than the one produced by angioplasty alone or that there was activation of thrombosis and platelet deposition by the thermal energy. Thermal trapping of labelled platelets within the vasa vasorum and at the luminal surface could have contributed, although we believe to a minor degree, to the observed high platelet numbers at the laser treated sites. The influence of heating the arterial wall with different laser systems on its thrombogenicity is not well defined. Borst et al. [10] described in vitro a loss of platelet adhesion after heating with Nd:YAG laser native and cultured human subendothelium to 100°C. Lawrence et al. [11] recently reported that in the atherosclerotic rabbit aorta, excimer did not change, whereas erbium:YAG irradiation and catalytic thermal angioplasty reduced platelet adhesion. In contrast, Ragimov et al. [12] found no significant changes in platelet adhesion to the canine femoral artery after hot tip or Nd:YAG irradiation induced thermal injury, compared to mechanically deendothelialised arterial segments, but significant reductions after argon and excimer laser injury. In addition, previous work from our laboratory has shown reduced
thrombi and platelet accumulation with excimer vs. thermal angioplasty in normal coronary arteries in the swine [13].

The energy doses used in this study may be too traumatic for the vessel wall. The effects of lower energy doses on the above parameters is therefore worthy of study. Although wall temperature was not measured in our experiments, previous work has shown that it is increased to ~100°C by radial Nd:YAG irradiation [14].

The laser system we used consisted of a single helically coiled fiber, which may distribute laser energy nonuniformly in “hot spots.” Systems trying to provide more uniform energy distribution may be accompanied by better angiographic, platelet, and histologic results.

Conclusions

In the atherosclerotic rabbit model, improved vessel diameter with high levels of platelet deposition is seen following laser balloon angioplasty. The absence of histologic sealing suggests that the abolition of elastic recoil represents the most important advantage of this present laser angioplasty system over conventional balloon angioplasty.

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