Laser Balloon Angioplasty: Potential for Reduction of the Thrombogenicity of the Injured Arterial Wall and for Local Application of Bioprotective Materials

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Mitigation of adverse biologic reactivity after balloon angioplasty is necessary before the incidence of restenosis can be appreciably reduced. A brief review of experimental evidence supports the hypothesis that the thrombogenicity of the injured arterial wall can be reduced by a suitable level of thermal denaturation or cross-linking of thrombogenic proteins. In addition, the concept of local pharmacologic therapy, which can be provided with laser balloon angioplasty at the site of arterial injury, is introduced. Preliminary in vitro and in vivo data suggest that guide catheter-injected albumin-heparin conjugates fabricated as water-insoluble microspheres remain adherent to the injured luminal surface and deeper arterial layers after physical trapping by the inflated balloon and subsequent laser/thermal exposure. The combination of initially adequate luminal morphology, reduction of the thrombogenicity of the injured arterial wall and application of local pharmacologic therapy with laser balloon angioplasty may eventually prove helpful in reducing the incidence of restenosis.

(J Am Coll Cardiol 1991;17:179R-88R)

Background

The cause of restenosis after percutaneous transluminal coronary angioplasty (1-12) is unclear. But thrombus formation, cellular proliferation and deposition of extracellular material (such as collagen) in response to arterial injury have been implicated in the pathogenesis of this common problem. As an additional potential cause, an inadequate immediate luminal result after balloon angioplasty very likely is contributory in some cases (13). In recent years, laser balloon angioplasty (14-28) has been found to achieve an adequate luminal result in most patients (>80%) in whom impending or overt acute closure occurred during conventional balloon angioplasty. Moreover, an adequate luminal result (<50% residual stenosis) was achieved in all but 1 of 210 consecutive elective cases of laser balloon angioplasty. The simultaneous application of heat from continuous wave neodymium yttrium-aluminum-garnet (Nd:YAG) laser irradiation (1.06 μm) and pressure during balloon inflation remodels the arterial lumen more effectively than does the use of pressure alone, as a result of thermal fusion of separated tissues, reduction of arterial recoil (including loss of vasoconstrictor potential) and desiccation of preexisting thrombus with remodeling of the residue into a thin film at the luminal surface.

As an energy source, Nd:YAG laser radiation is particularly useful because the depth of thermal penetration achievable is approximately 3 mm and the increased absorption of the radiation by thrombus compared with other arterial tissues results in selective desiccation of thrombus when a laser dose that is adequate to seal a dissection is used. However, the technologic challenges involved in modifying the pattern of laser energy emitted from a fiber optic filament to achieve a cylindrically uniform pattern of emitted radiation and in the control of the thermal responses of adjacent tissues are considerable and have been only partially surmounted clinically.

Although technologic refinements of a continuous wave Nd:YAG laser-based balloon angioplasty system may further improve the acute luminal result in the setting of a poor balloon angioplasty result, clinical evidence (29-32) has recently accrued that, irrespective of the type of technology applied, a highly satisfactory immediate angiographic luminal result is insufficient to predict a satisfactory long-term result. Undoubtedly, adverse biologic reactivity, including thrombus formation, cellular proliferation and deposition of extracellular matrix material, needs to be addressed if the incidence of restenosis after initially successful angioplasty is to be greatly reduced. Appropriate application of laser balloon angioplasty may have utility in this regard.

Potential Reduction of Thrombogenicity

Role of thrombus formation in restenosis. After denudation of the endothelium by conventional balloon angioplasty and exposure of subendothelial tissues to blood, platelet
adhesion, activation and aggregation occur along with potential activation of intrinsic and extrinsic coagulation pathways (33-40). Microthrombi or overt thrombi may form either transiently or subacutely until the endothelial layer has regenerated. Deep mural tears are particularly thrombogenic, perhaps as a result of the combination of tissue exposure and abnormal local flow patterns, such as turbulence and flow separation. The frequent finding of fresh and organized thrombus in arterialized specimens from restenosis lesions supports the potential importance of thrombus formation in the pathogenesis of restenosis. Because thrombin and platelet-derived growth factor within thrombus would be expected to stimulate proliferation of smooth muscle cells (41-46), the mass effect of intraluminal thrombus may be accentuated by an associated hyperplastic response. Currently available platelet inhibitors, as well as heparin, at clinically used doses appear inadequate to prevent thrombus formation after arterial injury (47).

**Thermal laser to reduce thrombogenicity.** At least five groups of investigators (48-52) have provided experimental evidence that thermal treatment of the luminal surface at subvaporization thresholds reduces the thrombogenicity of exposed tissues. Zeiher and Bonzel (48) showed that the degree of radiolabeled platelet deposition on the arterial luminal surface of an in vitro preparation after balloon angioplasty is reduced by the use of a radio frequency-heated balloon that produces a peak temperature of approximately 80°C. Sinclair et al. (49) demonstrated by scanning electron microscopy that the amount of acute fibrin and overt thrombus formation on the luminal surface of dog coronary arteries subjected to in vivo laser balloon angioplasty appears to be reduced compared with that in arteries treated with conventional balloon angioplasty. Because of the nonuniform spatial distribution of radiation from the laser balloon angioplasty device, peak estimated temperatures at the luminal surface ranged from 60° to 90°C. Recently, with the use of radiolabeled platelets and scanning electron microscopy, Abela et al. (50) showed that platelet deposition at the luminal surface of normal dog arteries was markedly reduced at laser/thermal angioplasty sites compared with that at balloon angioplasty sites. Peak surface temperatures were estimated to be approximately 80°C. Using an in vitro deendothelialized umbilical artery model, Bors et al. (51) demonstrated that the percent surface covered with platelets was only 5% in laser-exposed regions, in which peak temperatures of 100°C were measured with a thermographic camera, whereas control regions demonstrated a 31% value. Regions of peak temperatures of 55°C, however, demonstrated a slightly increased value of 40%. Similarly, in our laboratory (52), when a subsurface peak temperature of 100°C was achieved during continuous wave Nd:YAG laser exposure of thrombus in vitro, the thrombogenicity of this tissue, as measured by fibrinogen consumption and thrombin/anti-thrombin III complex generation, was markedly reduced. In contrast, a temperature of only 70°C was ineffective.

Consistent with these in vitro observations, preliminary clinical evidence suggests that laser balloon angioplasty treatment of thrombus compares favorably with published results of conventional angioplasty in terms of acute and subacute thrombotic complications. In the only discordant study, Alexopoulos et al. (53) found that radiolabeled platelet counts were higher in athroesclerotic rabbit iliac arteries treated with laser balloon angioplasty than in arteries treated with conventional balloon angioplasty. However, the number of animals studied was small, and conventional intraluminal flushing with saline solution was not performed during balloon inflation, so that radiolabeled platelets injected before inflation may have been thermally bonded to tissues.

**Mechanism of thrombogenicity with thermal laser.** Thermal denaturation or cross-linking of thrombogenic proteins may be an important mechanism for reducing the thrombogenicity of exposed tissues. Regarding thermal treatment of thrombus, we have found that the thermal history required to inhibit thrombin activity in vitro is similar to that required to reduce the thrombogenicity of thrombus with laser exposure. Similarly, thermal denaturation of experimental aortic fibrillar collagen has been shown by Gentry et al. (54) to inhibit platelet adhesion and initiation of coagulation, which would ordinarily occur with exposure of blood to collagen (55-59). and commercial collagen-based hemostatic pads are inactivated by heat (60). Similar changes in laser balloon angioplasty-treated tissues may occur because structural alteration of collagen has been shown by Schober et al. (61) to provide a mechanism for laser-induced anastomosis of vessels and, therefore, very likely also provides a mechanism for laser balloon angioplasty-induced fusion of separated tissue layers. The correlation between laser-induced tissue fusion (21) and reversible optical properties of the arterial wall is likewise consistent with the occurrence of structural alterations of collagen during heating. The protein cross-linking effects of heat have been shown to be similar to those induced by glutaraldehyde (62), and use of the latter reduces the thrombogenicity of heterograft porcine values (63.64) and heterologous vascular grafts (65).

**Issues to be addressed.** Available evidence suggests that a significant reduction of the thrombogenicity of thrombus and tissues injured by angioplasty is feasible with an appropriate level of thermal energy. However, further studies are needed to define the relation between the thermal history of thrombus and mechanically injured arterial tissues and their thrombogenicity. Moreover, should any attempt be made to correlate observed changes in the thrombogenicity of intact arterial tissue after thermal exposure with biochemical alterations of specific molecules within the tissue, a variety of potentially important issues may have to be addressed. In the case of collagen, a multiplicity of factors may have a slight effect on the degree of denaturation of collagen during heating of tissue (66-69), including collagen type and concentration, water content, pH, glucose concentration, rate of heating and level of isometric tension applied to collagen...
fibers during heating. One also has to consider that, in vivo, thermal reactions may be more complicated than denaturation or cross-linking of collagen itself. For example, the complex Maillard reaction (70), in which nonenzymatic thermal reactions occur between proteins and carbohydrates and between oxidized lipids and proteins, may affect the thermal stability of collagen. Finally, it is possible that thermal denaturation of other potentially important proteins at the luminal surface of the mechanically injured arterial wall, such as fibronectin, laminin, von Willebrand factor and thromboplastin, could affect tissue thrombogenicity.

Application of a Bioprotective Material With Laser Balloon Angioplasty

Mechanisms of neointima formation. Although platelets and thrombus may play an important role in neointima formation, other mechanisms may also be operative (71-73). For example, circulating monocytes adhere to the injured luminal surface and transform into macrophages that enter the arterial wall. An inflammatory cell reaction may then result from a variety of macrophage secretory products, such as lymphokines. In addition, macrophages stimulate fibroblast activity at least in part from release of mitogens similar to platelet-derived growth factor. Smooth muscle cells and endothelial cells are also capable of releasing similar growth factors after injury, thereby potentially further amplifying smooth muscle cell proliferation by platelets and thrombus. In addition to cellular proliferation, inflammatory/proliferative responses may increase the mass of the extracellular matrix compartment (74). If newly synthesized collagen within the latter is deposited at the luminal surface before endothelial cell repair is complete, the thrombogenicity of the luminal surface could continue to be problematic for many months.

Local application of bioprotective combined medium/drug to prevent restenosis. To eliminate thrombogenic responses and markedly reduce both proliferative responses and collagen deposition after arterial injury, pharmacologic therapy will probably be necessary. Unfortunately, many potentially useful agents cannot be taken orally and are associated with a significant incidence of serious side effects if administered systemically for the anticipated few months required for completion of arterial healing.

We recently investigated the experimental feasibility of using thermal energy during laser balloon angioplasty to induce adherence of intraluminally injected microencapsulated drugs to the luminal surface and deeper layers (fracture planes, vasa vasorum) of the mechanically injured arterial wall. As shown schematically in Figure 1, a drug-encapsulating material injected proximally during balloon inflation would be physically trapped and juxtaposed under pressure at the luminal surface and within the tissue by the inflated balloon. The application of heat by exposure of the material and tissues to laser radiation could then theoretically be used to induce adherence of the material to tissues as a result of a transient phase change and formation of molecular cross-links. By use of an encapsulating medium that is relatively water insoluble, a semipermanent coating might thereby be applied.

As an example of a potentially useful encapsulating medium/drug combination ("bioprotective material"), heparin has been both covalently bound to and encapsulated within albumin microspheres. Albumin is particularly attractive as an encapsulating medium because this water-insoluble material has been used to semipermanently coat prosthetic vascular surfaces to reduce their thrombogenicity (75). In addition, virtually any type of water-soluble drug can be encapsulated (76), the technology for making albumin microspheres for injectable drug delivery systems is well established, microspheres <3 μm can be fabricated easily (77) and the half-life of diffusion of a drug from the microspheres can be varied from minutes to many months, depending on the degree of cross-linking induced either thermally or chemically during the preparation of the microspheres (78). For some drugs, such as heparin, covalent binding to albumin is also possible, and heparin/albumin conjugates greatly reduce the thrombogenicity of prosthetic vascular grafts (79,80). Theoretically, microspheres entrapped within thermally sealed fissures and vasa vasorum would provide a depot for relatively slow release of the drug, and microspheres bound to the luminal surface would provide a drug that could be immediately available at this location.

Alternative approaches. Although physical trapping of a candidate bioprotective material at the luminal surface and within deeper layers by inflating the balloon during intravascular injection of the bioprotective material with subsequent heating appears to be a practical approach to induce adherence of the material to tissue, the possibility exists that transfer of a sleeve of a bioprotective material from the inflated balloon surface to the luminal surface with heat might represent an alternative useful approach. Likewise, rupture of pressure-sensitive microcapsules during balloon inflation for local release of a bioprotective material along with a biocompatible adhesive might obviate the need for thermal induction of adherence of a bioprotective material to tissue. However, the feasibility of these latter two approaches has not been demonstrated. The feasibility of the use of a perforated balloon for local delivery of drugs to the arterial wall at high pressure was recently demonstrated by Wolinsky and Thung (81). However, water-soluble drugs or nonaggregating insoluble materials would have to be used to prevent embolization of the distal vessel and side branches. Such agents, when injected by this approach, disappear quickly from the luminal surface. With the appropriate use of laser balloon angioplasty, the insoluble nature of the applied drug carrier and thermal induction of its adherence to tissue may allow much longer persistence. Moreover, by inducing thermal adherence of a bioprotective material as a thin film with laser balloon angioplasty to tissues at multiple levels,
swelling of tissue, which might accompany high pressure injection to physically deliver a drug through perforations in a balloon to the arterial wall, would not be a problem.

Potential classes of available drug carriers and drugs. A number of other drug carriers could also potentially be used, such as high molecular weight carbohydrates (82), endogenous platelets (83) and red blood cells (84). Potential classes of drugs that could be encapsulated or bound to a carrier include anticoagulants, collagen inhibitors and antiproliferative agents. If a thermally labile drug were encapsulated, alternative approaches to be considered would include the potential use of a carrier with a low bonding temperature (for example, liposomes) or the use of the combination of a chromophore at the surface of the albumin microsphere and pulsed laser radiation of an appropriate wavelength to selectively heat the microsphere surface, thereby sparing the encapsulated drug. However, many drugs, such as heparin and hirudin, are unaffected by the peak temperatures used to date to induce albumin microsphere adherence to tissue. Considering the potential for heparin to inhibit both cellular proliferation and coagulation (85) and the potent antithrombin effect of hirudin (86), including that for thrombin bound to fibrin, a combination of the two drugs may have utility.

In Vitro Studies

Thermal precipitation of drug carriers in solution (Table I). Initially, the concept of thermal precipitation or coagulation of a potentially useful drug carrier, such as albumin or a high molecular weight carbohydrate in solution, onto the luminal surface of injured arterial tissue was investigated in a manner to simulate an in vivo application. The media of
normal porcine aortic sections was manually split into two layers, and a 250 mg/ml solution of human serum albumin was placed on 2 × 2 cm sections of the exposed, "injured" intramural surfaces. A sheet of polyethylene terephthalate (laser angioplasty balloon material) was then applied to the surface &DE slide at a tissue pressure of approximately 1 to 2 atm. A continuous wave Nd:YAG laser dose of 68 W for 30 s was delivered by fiber optics over a nominal spot size on the tissue surface of approximately 4 cm², which was estimated with thermistor measurements to produce peak temperatures of 90° to 100°C. Control sections were treated in the same manner but received a sham laser dose. Similarly, a 200 mg/ml solution of hydroxyethyl starch (Sigma) was applied to porcine aortic segments, followed by irradiation with a continuous wave Nd:YAG laser dose of 68 W for 30 s. The sections were then rinsed in water to remove any unbound material. Adherence of albumin was detected by the binding of bromocresol green to albumin, and adherence of starch was detected by iodine staining. The sections were then vigorously washed in water for 5 min to determine whether the carrier was bound to the surface.

**Albunm microspheres.** To test the possibility that albumin microspheres can be thermally bonded to arterial tissue, standard techniques (78) for preparing albumin microspheres were used with minor modifications. From a 250 mg/ml solution of human serum albumin, 2 ml was added to 30 ml of cottonseed oil at 4°C while stirring with a 1 in. (2.54 cm) propeller-type stirrer at 1,600 rpm or sonicating with a 3 mm titanium probe at 20 kHz. The resultant emulsion was added in drops to a bath of cottonseed oil (100 ml) stirred at approximately 800 rpm with a magnetic stirrer. The temperature of the oil bath was raised to 150°C over a 15 min period and held at this temperature for 5 min while stirring. After cooling and centrifugation at 2,000 g for 10 min, the albumin microspheres were washed several times with diethyl ether to remove the oil. The microspheres were then suspended in 10 ml of phosphate-buffered saline solution (Fig. 2). For some studies, 50 mg of a dye (Balsam's kit, Polysciences) that fluoresces red or ultraviolet excitation was dissolved in 0.5 ml of butyl benzoate (Eastman Kodak) and added to the albumin solution; the mixture was sonicated and added to the cottonseed oil bath. The resultant albumin microspheres exhibited intense red microscopic fluorescence. Other albumin microsphere preparations contained either fluorescein isothiocyanate-albumin (Sigma) label or entrapped heparin and fluorescein isothiocyanate-heparin (Polysciences) label so that the resultant microspheres demonstrated intense green fluorescence on ultraviolet excitation.

**Adherence of microsphere preparations (Table 2).** With all batches of different microsphere preparations, firm adherence of a layer of microspheres was readily inducible either with a laser exposure as just described or with simple exposure of a thin film of a suspension of microspheres on porcine aortic sections placed in an oven at 100°C for 30 to 60 s. In contrast, application of pressure alone during laser exposure resulted in no detectable adherence. Vigorous washing of the thermally treated tissue with water for 5 min did not have a detectable effect on the intensity of microscopic fluorescence. By scanning electron microscopy, a confluent layer of albumin microspheres appeared to be firmly adherent (with no appreciable detachment during scanning electron microscopic processing) to the luminal surface by a thin film of coagulum.

**Heparin activity.** To determine whether heparin would be inactivated by temperatures used for fabrication of albumin microspheres or for inducing adherence of albumin microspheres to tissue, a solution of heparin (1,000 U/ml) was boiled for 1 min. As assessed by thrombin-activated clotting times, no inhibition of heparin activity was noted. In addi-

<table>
<thead>
<tr>
<th>Carrier</th>
<th>Mode of Heating</th>
<th>Adherence of Carrier</th>
<th>Persistence of the Adherent Layer After Washing With Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSA 250 mg/ml</td>
<td>None</td>
<td>Note when stained with BCG</td>
<td>NA</td>
</tr>
<tr>
<td>HSA 250 mg/ml</td>
<td>CW Nd:YAG; 68 W × 30 s over 4 cm²</td>
<td>Yes when stained with BCG</td>
<td>No</td>
</tr>
<tr>
<td>HES 200 mg/ml</td>
<td>None</td>
<td>None when stained with iodine</td>
<td>NA</td>
</tr>
<tr>
<td>HES 200 mg/ml</td>
<td>CW Nd:YAG; 68 W × 30 s over 4 cm²</td>
<td>Yes, 3 μm granules visible on iodine</td>
<td>Yes</td>
</tr>
</tbody>
</table>

BCG = bromocresol green; CW Nd:YAG = continuous wave neodymium yttrium-aluminum-garnet; HES = hydroxyethyl starch; HSA = human serum albumin; NA = not applicable.
tion, albumin microspheres were prepared with heparin present in the aqueous phase and thermal cross-linking was performed at 130°C for 15 min. The microspheres were washed with diethyl ether to remove excess oil and then with phosphate-buffered saline solution to remove any untrapped heparin. As assayed by thrombin-activated clotting times, no inhibition of heparin activity was found after disruption of the microspheres by sonication to release the encapsulated heparin.

Hirudin activity. The potential effect of temperatures used for thermal bonding of albumin microspheres to tissue was also evaluated for hirudin, which is a specific and potent inhibitor of thrombin. Thrombin-activated clotting times were determined before and after heating a solution of hirudin (Sigma) at 97°C for 30 s. No inhibition of activity was noted. Although the effect of higher temperatures on hirudin activity requires further study if albumin microspheres are prepared with thermal cross-linking methods, chemical cross-linking methods could be used, if necessary, as an equally acceptable approach for preparation of albumin microspheres (78) when thermally labile drugs need to be encapsulated.

In Vivo Studies

Experimental model. Six adult mongrel dogs were anesthetized with pentobarbital, and the common carotid and superficial femoral arteries were surgically exposed. A 0.035 in. (0.89 cm) guide wire with a 1 mm sharp metal burr was introduced into the lumen by means of an arteriotomy and passed back and forth approximately 12 times over a 1 to 2 cm length, while opposing walls of the artery were coped manually. In this manner, multiple nonperforating mural tears within each lumen were invariably created, simulating the mural disruption frequently accompanying clinical angioplasty procedures. After arteriotomy repair and reestablishment of blood flow in this model, multiple nonoccluding mural thrombi were noted adjacent to each tear within 2 h of the injury, despite heparin (1,000 U) administration at the time of arterial exposure. A suspension of albumin microspheres was injected into each artery immediately before and during inflation of a 3 mm laser angioplasty balloon at the site of injury. A continuous wave Nd:YAG laser (1.06 μm) exposure of 500 J over 20 s was given to ipsilateral carotid and femoral arteries; a sham laser exposure was given to control contralateral arteries. The arteriotomy was repaired with 5-0 Prolene sutures to reestablish blood flow, and the arteries were examined either immediately or 1 h or 4 h after the procedure. By visualization of the red fluorescence of the albumin-encapsulated dye, no loss of prominent albumin adherence could be appreciated qualitatively over a period of 4 h of reestablishment of blood flow. Adherence of albumin in cryosections of arteries was noted both at the luminal surface and within deeper planes corresponding to the location of mural tears (Fig. 3).

Treatments with albumin and heparin conjugates. To test the feasibility of applying in vivo albumin microspheres with heparin both covalently bound and encapsulated, conjugates of albumin and heparin were prepared as described by Hennink et al. (79). Bovine serum albumin (1.25 g) was

Table 2. Thermal Binding of Albumin Microsphere Preparations

<table>
<thead>
<tr>
<th>Type of Preparation</th>
<th>Substrate</th>
<th>Mode of Heating</th>
<th>Adherence of Microspheres</th>
<th>Persistence of the Adhered Layer After Washing With Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS labeled with red fluorescent dye</td>
<td>Human atheromatous plaque</td>
<td>None</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>MS labeled with red fluorescent dye</td>
<td>Human atheromatous plaque</td>
<td>CW Nd:YAG; 68 W x 30 s over 4 cm² area</td>
<td>Fluorescent patches of microspheres on the surface</td>
<td>Yes</td>
</tr>
<tr>
<td>MS labeled with red fluorescent dye</td>
<td>Mechanically injured porcine aortic media</td>
<td>None</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>MS labeled with red fluorescent dye</td>
<td>Mechanically injured porcine aortic media</td>
<td>Constant temperature oven; 100°C for 60 s</td>
<td>Fluorescent patches of microspheres on the surface</td>
<td>Yes</td>
</tr>
<tr>
<td>MS with FITC-albumin label</td>
<td>Mechanically injured porcine aortic media</td>
<td>None</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>MS with FITC-albumin label</td>
<td>Mechanically injured porcine aortic media</td>
<td>CW Nd:YAG; 68 W x 30 s over 4 cm² area</td>
<td>Patches of microspheres on the surface and within deeper layers</td>
<td>Yes</td>
</tr>
<tr>
<td>MS with entrapped heparin (50 U/mg of protein) with FITC-heparin label</td>
<td>Mechanically injured porcine aortic media</td>
<td>None</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>MS with entrapped heparin (50 U/mg of protein) with FITC-heparin label</td>
<td>Mechanically injured porcine aortic media</td>
<td>CW Nd:YAG; 68 W x 30 s over 4 cm² area</td>
<td>Patches of microspheres on the surface and within deeper layers</td>
<td>Yes</td>
</tr>
</tbody>
</table>

FITC = fluorescein isothiocyanate; MS = albumin microspheres; other abbreviations as in Table 1.
dissolved in water (18 ml) and lyophilized sodium hepaim salt (500,000 U) (Sigma) was added; FITC-albumin (60 µl) tag was added to this solution. Heparin and albumin were coprecipitated from solution by acidification and then redissolved. Eight 300 µl aliquots of 1-ethyl-3-(dimethylaminopropyl)-carbodiimide (32.5 mg/ml) were added stepwise over 4 h and the pH adjusted to 7.5. The coupling reaction was allowed to occur with gentle stirring with a magnetic stirrer over 20 h at room temperature. The solution was then dialyzed against Tris-HCl (0.025 M) for 2 h (pH 7.5). Albumin microspheres were then fabricated with the conjugate as just described and washed with diethyl ether. After resuspending the washed centrifugal pellet, the microspheres were suspended in phosphate-buffered saline solution. Although the estimated mean size of the microspheres was relatively large (approximately 50 to 100 µm), the preparation was adequate for testing the in vivo thermal inducibility of adherence.

After a protocol identical to that described, laser balloon angioplasty was performed immediately after intraluminal injection of the suspension in one carotid and two superficial femoral arteries that had been surgically exposed and mechanically injured. The remaining carotid artery served as a 4 h control artery for injury alone. Twenty-four hours after arteriotomy repair and reestablishment of blood flow, all three arteries were found to be widely patent on surgical exposure and the treated segments were removed for fluorescence microscopic examination. All three segments dem-
onstraled prominent adherence of the microspheres over most of the dilated segment (Fig. 4) at sites of overt mechanical injury, with no qualitative loss of material compared with the amount noted immediately after treatment in prior studies. There was no evidence of thrombus formation at the surface of the microspheres, consistent with possible retained heparin activity. No anticoagulant therapy had been given after the procedure, and thrombus formation was noted in mechanically injured adjacent regions where microspheres were not applied. The large size of the microspheres and lack of a surfactant in the preparation may have accounted for some agglomeration of the particles, which could be seen through the thin arterial wall during balloon inflation, so that a nonuniform density of microspheres was present during laser exposure. With the use of smaller microspheres (for example, <10 μm) and perhaps a suitable surfactant, the uniformity of microsphere density between the balloon and luminal surface should be improved and the thickness of the albumin layer would be expected to be much smaller. Despite the use of a relatively coarse microsphere preparation, the results suggest that semipermanent laser balloon angioplasty-induced adherence of albumin microspheres to the injured arterial wall with retention of pharmacologic activity of an entrapped drug is feasible.

Conclusions

It is fortuitous that the peak temperatures required to induce adherence of albumin microspheres are similar to those required to adequately fuse tissues together and to reduce arterial recoil; moreover, the Nd:YAG laser dose required to achieve these effects results in appropriately higher temperatures when thrombus is present, so that the latter tissue is selectively desiccated and its thrombogenicity reduced. Therefore, it appears possible that a single laser dose could be chosen for simultaneous improvement of luminal morphology and dimensions, reduction of thrombogenicity and induction of adherence of a bioprotective material. A great deal of basic research is required, however, to carefully define the effect of the thermal history on thrombogenicity and application of a bioprotective material, as well as to characterize candidate materials, such as albumin microspheres in terms of half-life, rate and mechanism of drug release. In the short term, successful local application of an anticoagulant at the site of angioplasty should help to improve the safety of the procedure. Reduction of the incidence of restenosis might also be achievable with such therapy, but greater insight regarding the mechanisms of restenosis may be necessary before a rational attempt to address this problem with alternative local pharmacologic therapy can be properly made.

References


JACC Vol. 17, No. 6
May 1991:1798-888

SPARIS ET AL.
LASER BALLOON ANGIOPLASTY
187B


