Decreased Platelet Deposition and Smooth Muscle Cell Proliferation After Intramural Heparin Delivery With Hydrogel-Coated Balloons

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**Background** In vitro and in vivo studies have demonstrated both anticoagulant and antiproliferative effects of heparin. The purpose of this study was to assess the effect of local intramural delivery of heparin, using heparin-coated hydrogel balloons, on platelet deposition and early smooth muscle cell proliferation after in vivo balloon angioplasty.

**Methods and Results** The effects of local heparin delivery were assessed during balloon angioplasty of porcine peripheral arteries. All balloon dilatations were performed with oversized hydrogel balloons coated with a known quantity of heparin. Balloon dilatations in contralateral vessels with uncoated hydrogel balloons served as study controls. The pharmacokinetics of heparin delivery were assessed using [3H]-Heparin to quantitate heparin wash-off from the balloon surface, heparin delivery to the arterial wall, and intramural persistence of drug. Platelet deposition at 1 hour after balloon injury was quantified using [11]In-labeled platelets. Smooth muscle cell proliferation was assessed 6 to 7 days after angioplasty with immunohistochemical staining for proliferating cell nuclear antigen. [3H]-Heparin wash-off from the hydrogel balloon surface occurred rapidly, with approximately 95% of the heparin coating disappearing within 10 seconds in the intact circulation. Approximately 2% of heparin on the balloon surface was delivered intramurally at the time of angioplasty. Intramural heparin dissipated rapidly, although small amounts of intramural heparin could still be detected for at least 48 hours. In comparison to control vessels, there was less [11]In-platelet deposition (P<0.002) and less medial smooth muscle cell proliferation (P<0.05) in heparin-treated vessels.

**Conclusions** Local intramural delivery of heparin at the time of balloon angioplasty with heparin-coated hydrogel balloons results in intramural deposition of drug that persists for at least 48 hours. This in vivo technique significantly decreases platelet deposition and early smooth muscle cell proliferation after angioplasty injury. *(Circulation. 1994;90: 433-441.)*

**Key Words** • balloon angioplasty • platelets • smooth muscle cells

Acute vascular closure and late restenosis are two major problems that continue to confront interventional cardiology. In part, mechanical factors such as vascular dissection and vessel recoil play a role in causing these suboptimal angioplasty results. Newer adjunctive angioplasty techniques, including intracoronary stents, coronary atherectomy, and laser angioplasty, have been developed to minimize these mechanical problems. Equally important, however, biological factors such as intracoronary thrombus formation and smooth muscle cell hyperplasia can lead to abrupt closure and to restenosis, respectively. To date, neither mechanical devices nor systemic pharmacological therapy has been completely effective in combating these problems.

Localized intramural delivery of therapeutic agents during balloon angioplasty has been proposed as a potential treatment for abrupt closure and for the prevention of chronic restenosis. Localized drug delivery theoretically is advantageous because it produces high intramural concentrations of a therapeutic agent at the angioplasty site while minimizing circulating drug levels that may cause undesirable systemic side effects. Prior attempts to deliver drugs intramurally have involved the use of various techniques including perforated balloon catheters, double-balloon catheters, polymeric-coated stents, iontophoresis, and periadventitial drug delivery.

A novel catheter-based drug delivery system that has been developed recently involves the use of hydrogel-coated balloons. This system uses a standard polyethylene angioplasty balloon coated with a hydrogel polymer into which various drugs can be absorbed. Balloon inflation results in polymer contact with the vessel wall and diffusion of a given drug from the hydrogel polymer into the vessel wall. Previous work from our laboratory has documented the efficacy of this system in locally delivering the marker agent horseradish peroxidase and fluoresceinated heparin.

The purpose of the present study was to assess the effects of local heparin delivery during in vivo balloon...
angioplasty of normal porcine peripheral arteries with heparin-coated hydrogel balloons. Specifically, the study was designed with three goals: first, to evaluate the pharmacokinetics of heparin-coated hydrogel balloons in terms of the quantity of heparin delivered and its intramural persistence; second, to assess the effect of locally delivered heparin on platelet deposition after balloon angioplasty; and third, to assess the effect of locally delivered heparin on early smooth muscle cell proliferation after balloon angioplasty.

Methods

Equipment

The drug delivery balloon system that was used in this study consists of a standard 4-cm polyethylene balloon angioplasty catheter coated with hydrogel (Hydro-Plus, Boston Scientific). The hydrogel consists of a network of cross-linked polyacrylic acid chains that are adherent to the balloon surface. Upon exposure to aqueous solution, water is absorbed into the hydrophilic hydrogel lattice structure, the hydrogel swells, and any drugs in solution are also absorbed into the matrix. The thickness of the coating ranges from 5 μm when dry to 25 μm when fully saturated with water. At the time of angioplasty, the hydrogel is compressed against the vessel wall, expressing any agents from the hydrogel into the vessel wall.

Loading of Heparin onto Hydrogel-Coated Balloons

Hydrogel balloons ranging in size between 6 and 8 mm were coated with a known quantity of porcine intestinal mucosal heparin (Elkins-Sinn, Inc). For each balloon, heparin was pipetted onto the inflated balloon surface in 25-μL aliquots. The inflated balloon was then suspended in air and allowed to dry. This process was repeated until the total required volume was loaded.

For evaluation of effects on platelet deposition and smooth muscle cell proliferation, 3000 U of heparin was loaded onto each balloon in a total volume of 300 μL of heparin at a concentration of 10 000 U/mL. For pharmacokinetic studies, 3H-heparin (New England Nuclear) was obtained with an initial specific activity of 320 U/mCi and diluted as necessary with heparin (10 000 U/mL) before balloon loading. Amounts of 3H-heparin loaded on the balloon surface ranged between 0.01 and 75 μCi (1000 U heparin). Control balloons consisted of hydrogel-coated balloons dipped in saline.

Angioplasty Drug Delivery Protocol

Angioplasty with heparin-coated or control, saline-coated hydrogel balloons was performed on the iliac and carotid arteries of 25 anesthetized, 3- to 4-month-old (20 to 30 kg) male Yorkshire swine. Animals were premedicated with Telazol (200 mg IM) and atropine (2 mg IM) and ventilated with ethrane 1% to 3%. Shortened 1 to 2 cm, 9F introducer sheaths (USCI) were inserted into both carotid and both iliac arteries approximately 10 cm distal to the aorta by direct arterial exposure using standard sterile surgical technique. To prevent thrombosis of the vascular sheaths, all animals received a single bolus of 200 U/kg of heparin at the onset of surgery and 100 U/kg each hour thereafter while anesthetized. Additional systemic heparin was not administered to animals undergoing the platelet deposition studies described below.

Before angioplasty, intravascular ultrasound was performed on all carotid and iliac arteries to assess arterial diameter and to localize the site of balloon inflation measured from the origin of the vessel from the aorta. Imaging was performed using a 20-MHz, 6.2F catheter (Boston Scientific) with an imaging console adapted for 20-MHz operation and 360° scans (Diasomics).

For each artery dilated, a heparin-coated or control balloon was rapidly inserted through the sheath in a retrograde fashion to the site of angioplasty in either the carotid or iliac artery and immediately inflated at 4 atm of pressure for 5 minutes. All balloon dilations were performed with a balloon to artery ratio of approximately 1.3:1 as determined by intravascular ultrasound. Longer inflations were not used because of the potential for ischemia when long inflation durations are performed for clinical angioplasty. All animal studies conformed to the guiding principles of the American Physiological Society.

Pharmacokinetics of Local Heparin Delivery

Persistence of Heparin on the Balloon Surface

Persistence of heparin on the hydrogel balloon surface in the intact circulation was evaluated in two pigs using 40 hydrogel balloons (7-mm diameter) coated with 1000 U of 3H-heparin. After heparin loading, balloons were inserted through a 9F introducer sheath into the blood stream for time periods ranging between 1 and 120 seconds. Balloons were then removed, excised from their shaft, and immersed in Solvable (New England Nuclear) for 12 hours. Fifteen milliliters of Aquasol (New England Nuclear) then was added, and after incubation for 12 hours, specimens were counted in a scintillation counter and reported as decays per minute (dpm) after subtraction of background counts. Four balloons coated with 3H-heparin were evaluated at each of the times listed in Table 1. Three additional balloons enclosed in a protective 7F introducer sheath (USCI) were inserted through the 9F sheath and maintained within the circulation for 5 minutes. After removal from the bloodstream, the 3H-heparin–coated balloons were pushed out of the protective sheath, and scintillation counting was performed. The quantity of 3H-heparin remaining on balloon surfaces after exposure to the intact circulation was compared with the two control balloons that were loaded with the same amount of 3H-heparin but not exposed to the bloodstream.

Quantitation of Intramural Heparin Delivery

To quantitatively evaluate 3H-heparin delivery to the arterial wall, angioplasty was performed in three pigs as described above using 10 balloons coated with 1000 U of 3H-heparin. Vessel segments were excised within 1 minute after balloon

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Intramural 3H-Heparin (% of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.52</td>
</tr>
<tr>
<td>2</td>
<td>1.35</td>
</tr>
<tr>
<td>3</td>
<td>3.51</td>
</tr>
<tr>
<td>4</td>
<td>0.62</td>
</tr>
<tr>
<td>5</td>
<td>3.53</td>
</tr>
<tr>
<td>6</td>
<td>2.18</td>
</tr>
<tr>
<td>7</td>
<td>1.31</td>
</tr>
<tr>
<td>8</td>
<td>5.48</td>
</tr>
<tr>
<td>9</td>
<td>0.21</td>
</tr>
<tr>
<td>10</td>
<td>3.32</td>
</tr>
</tbody>
</table>

Mean 2.30
SD 1.63

Ten vessels were removed within 1 minute after angioplasty using 3H-heparin–coated hydrogel balloons. The quantity of 3H-heparin detected within the vessel wall is expressed as a percentage of the quantity of heparin initially loaded onto the balloon.

**TABLE 1. Delivery of Heparin to the Arterial Wall**
deflation, immersed in Solvable, and prepared for scintillation counting as described above.

**Persistence of Intramural Heparin**

To quantify the intramural persistence of heparin after delivery using a hydrogel-coated balloon, angioplasty was performed as described above in six pigs with 22 balloons coated with 1000 U of \(^3\)H-heparin. Vessel segments were excised at various time points up to 48 hours after angioplasty, immersed in Solvable, and prepared for scintillation counting as described above.

**Effect of Local Heparin Delivery on Platelet Deposition**

Nine anesthetized pigs were used to assess platelet deposition after heparin delivery. Platelet deposition was evaluated as described by Johnstone et al\(^{11}\) using \(^{111}\)In-labeled platelets. \(^{111}\)In-tropolone was prepared by mixing 50 \(\mu\)g of tropolone (Sigma) in normal saline solution with 0.5 mCi of \(^{111}\)In-chloride (New England Nuclear). Forty-three milliliters of whole blood was withdrawn into 7 mL of ACD saline (Malinckrodt) and centrifuged at 180g for 15 minutes. Platelet-rich plasma (PRP) was removed and centrifuged at 1600g for 10 minutes. The platelet pellet then was resuspended with \(^{111}\)In-tropolone and 2 mL of ACD saline solution for 20 minutes and centrifuged for 10 minutes at 1600g. The pellet was resuspended in 5.5 mL of PPP and centrifuged at 100g for 10 minutes to remove any macroaggregates. The supernatant containing \(^{111}\)In-labeled platelets was then removed and reinfected intravenously.

Angioplasty was performed from 1 to 16 hours after reinflection of labeled platelets. Balloon angioplasty using balloons coated with 3000 U of heparin was performed in 14 iliac or carotid arteries. Balloon dilatation using the same-sized, saline-coated hydrogel balloon was performed in the contralateral vessel and served as the control. Four vessel pairs were not used for technical reasons.

One hour after angioplasty the dilated arterial segment was excised, flushed gently with saline, stripped of adventitia, and counted in a gamma well counter. A symmetric 50-keV window centered around the 247-keV peak was used to quantitate \(^{111}\)In. Blood samples were obtained after angioplasty for determination of free and cell-bound \(^{111}\)In activity and for measurement of platelet counts and activated partial thromboplastin time (PTT).

**Smooth Muscle Cell Proliferation Studies**

To evaluate the effect of local heparin delivery on smooth muscle cell proliferation, vessel segments from seven pigs were examined by immunohistochemical staining for proliferating cell nuclear antigen (PCNA).\(^{12-14}\) Nine iliac or carotid vessels initially underwent angioplasty with heparin-coated balloons loaded with 3000 U of heparin. Contralateral vessels underwent angioplasty with same-size, saline-coated hydrogel balloons. After angioplasty, arteriotomy sites were repaired, the animal was allowed to waken and was returned to its cage. After 6 to 7 days, each animal was killed, and the carotid and iliac vessels were removed. Angioplasty was not performed on five vessel pairs for technical reasons.

Excised arterial segments were fixed for 12 hours in 10% buffered formalin. Four cross-sectional specimens then were taken from the site of angioplasty for quantitative immunohistochemistry and embedded in paraffin. Tissue sections were stained with PC10,\(^ {15}\) a monoclonal antibody to PCNA\(^ {16}\) (DAKO), using a sensitive labeled avidin-biotin technique.\(^ {17}\) For quantitative analysis, immunostaining was performed using diaminobenzidine (DAB) as chromogen and counterstaining with methyl green. In addition to immunohistochemical staining, adjacent tissue sections were also stained with hematoxylin and eosin.

To determine the relative contributions of smooth muscle cells and other cells such as macrophages, selected sections were stained with monoclonal anti-smooth muscle actin antibody 1A4\(^ {18}\) (Sigma) and PC-10 using the histostain-DS kit for double labeling (Zymed Laboratories, Inc). Fast blue and aminoaethycarbazole, respectively, were used as chromogens.

Quantitation of PCNA was performed using the Cell Analysis-200 System by a single observer unaware of the vessel assignment to the control or heparin-treated group.\(^ {13}\) Threshold levels of nuclear labeling (methyl green staining) were determined using control vessel sections labeled with nonimmune serum. Threshold levels of PCNA staining (DAB) were chosen to include all visually evident PCNA staining. Threshold levels were maintained constant for all sections of a given pair of vessels. Regions of interest (at 400-fold magnification) were selected to include four representative regions of the media from evenly spaced quadrants in four separate sections from each artery. The section of vessel demonstrating the most proliferation (PCNA staining) was used for all comparisons between heparin-treated and control vessels.

**Data Analysis and Statistics**

The presence of \(^3\)H-heparin detected either on the balloon surface or within the dilated vessel was expressed as a percentage of the \(^3\)H-heparin originally loaded onto the balloon.

**TABLE 2. Persistence of \(^3\)H-Heparin in the Vessel Wall**

<table>
<thead>
<tr>
<th>No. of Vessel Segments</th>
<th>Time of Removal After Angioplasty</th>
<th>% Heparin Remaining in Vessel Wall</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>&lt;1 min</td>
<td>2.30</td>
<td>1.63</td>
</tr>
<tr>
<td>4</td>
<td>7.5 min</td>
<td>1.97</td>
<td>0.88</td>
</tr>
<tr>
<td>4</td>
<td>10 min</td>
<td>0.86</td>
<td>1.21</td>
</tr>
<tr>
<td>4</td>
<td>30 min</td>
<td>1.10</td>
<td>0.88</td>
</tr>
<tr>
<td>4</td>
<td>90 min</td>
<td>0.48</td>
<td>0.50</td>
</tr>
<tr>
<td>3</td>
<td>24 h</td>
<td>0.093</td>
<td>0.081</td>
</tr>
<tr>
<td>3</td>
<td>48 h</td>
<td>0.00088</td>
<td>0.00086</td>
</tr>
</tbody>
</table>

Persistence of heparin within the vessel wall was evaluated after angioplasty performed with \(^3\)H-heparin-coated hydrogel balloons. The amount of heparin remaining within the arterial wall was expressed as a percentage of the original quantity of heparin loaded onto the balloon.
Platelet deposition was expressed as the calculated number of platelets deposited at the angioplasty site. The number of platelets deposited was calculated by multiplying the number of $^{111}$In cpm in the vessel by the number of platelets per milliliter of blood and dividing by the $^{131}$I cpm per milliliter of blood. The overall percentage reduction in platelet deposition was determined from the ratio of the difference between the average number of platelets deposited on the control vessels and the average number of platelets deposited on the heparin-treated vessel divided by the average number on the control vessels. To calculate the average reduction in platelet deposition, the reduction in platelet deposition was calculated for each of the vessel pairs individually, and these percentages were then averaged. PCNA staining was expressed as the percentage of the total nuclear area staining with PC-10.

All values are expressed as mean±SD. All intergroup comparisons between paired vessels were performed with the Wilcoxon signed rank test. A value of $P<.05$ was considered significant.

**Results**

**Heparin Wash-off From the Balloon Surface**

$^{3}$H-heparin washed off the uninflated balloon surface rapidly once introduced into the bloodstream. After 8 seconds in the circulation, 96% of the heparin coating was lost. After 120 seconds, only 1% of the originally loaded heparin remained on the balloon surface. Of note, this rapid wash-off could be prevented with the use of a protective sleeve. In the three balloons that were introduced into the circulation inside a protective sleeve, 85% of the heparin remained on the balloon surface after exposure to the bloodstream for 5 minutes. (See Fig 1.)

**Heparin Delivery to the Arterial Wall**

When vessels were removed within the first minute after angioplasty, 2.3±1.6% of $^{3}$H-heparin loaded onto the balloon surface was intramurally delivered to the angioplasty site during balloon angioplasty. After removal of balloons after balloon inflation and heparin delivery, less than 1% of the $^{3}$H-heparin remained on the balloon surface. (See Table 1.)

**Persistence of Intramural Heparin**

Rapid loss of intramurally delivered $^{3}$H-heparin was noted after local delivery. Approximately 1% of the heparin originally coated on the balloon was detected intramurally during the first 30 minutes after angioplasty. Even smaller amounts of intramural heparin persisted for up to 30 minutes after local delivery. One-milliliter blood samples revealed levels of $^{3}$H-heparin below detectable limits at all of the time points. Similarly, uninjured vessel segments showed no detectable $^{3}$H-heparin. (See Table 2.)

**Platelet Deposition**

Platelet deposition was analyzed in a total of 14 vessel pairs, consisting of 6 iliac and 8 carotid pairs. In 7 vessel pairs, heparin delivery occurred before dilatation of the contralateral control vessel; in the remaining 7 pairs, the control vessel was dilated before heparin delivery in the contralateral artery. Balloon to artery diameter ratios were similar in the control vessels ($1.40±0.11$) and the heparin treated vessels ($1.36±0.13$, $P=NS$).

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**Table 3. Effect of Local Heparin Delivery on Platelet Deposition**

<table>
<thead>
<tr>
<th>Vessel Pair</th>
<th>Vessel</th>
<th>Control Vessel</th>
<th>Heparin Vessel</th>
<th>Reduction, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Iliac</td>
<td>4.19x10⁸</td>
<td>2.92x10⁸</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Carotid</td>
<td>7.14x10⁸</td>
<td>9.67x10⁷</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>Iliac</td>
<td>2.86x10⁸</td>
<td>1.60x10⁷</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>Carotid</td>
<td>3.27x10⁷</td>
<td>3.23x10⁷</td>
<td>1.3</td>
</tr>
<tr>
<td>5</td>
<td>Carotid</td>
<td>6.26x10⁸</td>
<td>1.48x10⁷</td>
<td>76</td>
</tr>
<tr>
<td>6</td>
<td>Carotid</td>
<td>4.15x10⁸</td>
<td>1.64x10⁷</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>Iliac</td>
<td>9.28x10⁷</td>
<td>1.27x10⁷</td>
<td>−37</td>
</tr>
<tr>
<td>8</td>
<td>Carotid</td>
<td>1.83x10⁷</td>
<td>1.60x10⁷</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>Carotid</td>
<td>7.20x10⁹</td>
<td>7.76x10⁹</td>
<td>89</td>
</tr>
<tr>
<td>10</td>
<td>Iliac</td>
<td>1.67x10⁸</td>
<td>3.92x10⁷</td>
<td>77</td>
</tr>
<tr>
<td>11</td>
<td>Carotid</td>
<td>6.34x10⁷</td>
<td>3.16x10⁷</td>
<td>50</td>
</tr>
<tr>
<td>12</td>
<td>Iliac</td>
<td>5.89x10⁹</td>
<td>2.27x10⁷</td>
<td>62</td>
</tr>
<tr>
<td>13</td>
<td>Carotid</td>
<td>3.91x10⁷</td>
<td>2.90x10⁷</td>
<td>26</td>
</tr>
<tr>
<td>14</td>
<td>Iliac</td>
<td>1.76x10⁸</td>
<td>8.35x10⁷</td>
<td>52</td>
</tr>
</tbody>
</table>

Mean 7.32x10⁸ 1.43x10⁹ 81

SD 1.87x10⁹ 1.99x10⁹

The number of platelets deposited on vessels after angioplasty with a saline-coated balloon (control vessel) was compared with the number of platelets deposited on the contralateral vessel that underwent angioplasty with a heparin-coated balloon (heparin vessel). Local heparin delivery reduced platelet deposition in 13 of the 14 vessel pairs.

*P=.002.
In 13 out of 14 vessel pairs, there was less platelet deposition in heparin-treated vessels than in control vessels. One vessel pair demonstrated little change (<2%) between heparin treatment and control, and a single vessel pair showed a marked increase in platelet deposition in the heparin-treated vessel. Mean platelet deposition was 7.3±1.9×10⁶ in control vessels and 1.4±2.0×10⁶ platelets in heparin-treated vessels (P=.002); overall, platelet deposition was reduced by 81%. (See Table 3.)

The average reduction of platelet deposition per vessel pair was 45±36%. The reduction in platelet deposition was similar whether the heparin-coated or the control balloon angioplasty was performed first. The average reduction in platelet deposition in those vessel pairs where the heparin-coated balloon was used first was 42±44% and similarly was 48±28% when the control balloon was used first.

Baseline activated PTTs in seven animals measured 14.2±2.0 seconds. After a single heparin-coated balloon inflation the PTT rose to 31±15 seconds. In three animals with both carotid and iliac dilatations, the PTT rose to >51±35 seconds after the second balloon inflation.

**Smooth Muscle Cell Proliferation**

A total of 9 vessel pairs (5 iliac, 4 carotid) were analyzed by quantitative immunohistochemical staining of PCNA (Table 4). The balloon to artery diameter ratio was 1.29±0.17 in the control vessels and 1.27±0.16 in the heparin-treated vessels (P=NS). In 8 of 9 vessel pairs, the heparin-treated angioplasty site demonstrated less medial cellular proliferation than the contralateral control vessel (Fig 2). Overall, in control vessels, 12.7±9.7% of nuclear area demonstrated staining for PCNA compared with 8.5±7.1% of nuclear area in heparin-treated vessels (P=.03). Double labeling with both anti-PCNA and anti-smooth muscle actin antibody demonstrated the smooth muscle cell origin of proliferating cells within the media (Fig 3).

**Discussion**

This study demonstrates, in a nonatherosclerotic porcine model, that hydrogel-coated balloons can deliver heparin intramurally at the time of balloon angioplasty. Although wash-off of heparin from the balloon surface and dissipation of intramural heparin occurred rapidly, small amounts of heparin delivered by this technique did persist at the angioplasty site for at least 48 hours. More importantly, locally delivered heparin decreased both early platelet deposition and PCNA-identified smooth muscle cell proliferation at the angioplasty site when compared with contralateral vessels that underwent similar injury.

**Pharmacokinetic Studies**

Despite inefficient delivery to the arterial wall and rapid disappearance of locally delivered heparin, small amounts of intramural heparin did persist for at least 48 hours. This persistence of heparin at the angioplasty site is consistent with previous evidence that heparin adheres to components of the damaged arterial wall.19-21 In vitro, heparin has been shown to bind to smooth muscle cells as well as to components of the extracellular matrix including fibronectin, collagen, von Willebrand factor, and basic fibroblast growth factor. In addition, heparin is taken up and internalized by smooth muscle cells.22 Similarly, in an in vivo rat model, fluoresceinated heparin was deposited specifically at sites of arterial injury.23 We have shown previously that fluorescein-labeled heparin can be detected for at least 24 hours after local delivery with hydrogel-coated balloons.9

Only a small fraction of the amount of radioactive heparin that was originally loaded onto balloons could be detected intramurally. In part, this low efficiency of intramural delivery may have been related to heparin...
Fig 2. Immunohistochemical staining of proliferating cell nuclear antigen. Immunohistochemistry was performed on an iliac vessel 1 week after angioplasty with a saline-coated hydrogel balloon (A). The contralateral vessel underwent angioplasty injury with a heparin-coated balloon (B). Proliferating cell nuclear antigen staining with diaminobenzidine (brown) was reduced in the heparin-treated vessel. Nuclei are stained with methyl green (magnification x400).
washes-off from the balloon before heparin-hydrogel contact with the arterial wall. While heparin persistence in the vessel wall may be related to specific binding of heparin to wall components, it is also possible that heparin-binding sites have a nonsaturable component or were not fully saturated with the amount of H-heparin loaded on the balloon surface. If so, then loading larger amounts of heparin would result in a larger amount of heparin in the wall and possibly more pronounced effects on platelet deposition and smooth muscle cell proliferation.

Platelet Deposition

Heparin inhibits coagulation by accelerating the action of antithrombin III. However, in addition to inhibiting circulating coagulation factors, heparin has also been shown to have local anticoagulant effects at sites of arterial injury. As described above, heparin binds to thrombogenic components of the damaged arterial wall such as collagen, von Willebrand factor, and fibronectin. Araki et al\(^\text{13}\) recently demonstrated that arterial segments exposed to heparin were rendered less thrombogenic. The demonstration of both systemic and local heparin anticoagulant effects suggests that intra-arterial heparin administration at the site of injury may increase the antithrombotic effect of the drug.

Coincident with these findings, the present study demonstrated a reduction in platelet deposition in several cases even in the absence of an effectively prolonged PTT. Moreover, since the systemic dissemination of heparin would have equivalent effects on both the heparin-treated vessel and the contralateral vessel, the observed reduction in platelet deposition is likely to represent a specific effect of local heparin delivery rather than an effect of systemic heparin therapy.

Antiproliferative Effects

Smooth muscle cells contribute to intimal hyperplasia and restenosis after vascular injury by proliferation, migration, and synthesis of extracellular matrix. Numerous studies have documented the antiproliferative effects of heparin. In vitro, heparin has been shown to inhibit both smooth muscle cell proliferation\(^24-29\) and migration.\(^30,31\) Similarly, in vivo, heparin inhibits smooth muscle cell proliferation and intimal thickening in the balloon-injured rat carotid artery,\(^26\) in a rabbit model of vascular injury,\(^26\) and after intravascular stent placement in swine.\(^32\) Conversely, protamine, an inhibitor of heparin, augments the proliferation seen after vascular injury.\(^33\)

The mechanism by which heparin inhibits smooth muscle cell replication is currently under investigation. In part, the effect of heparin may be secondary to decreasing platelet adherence and preventing the activation of smooth muscle cells in response to thrombus formation. In addition, heparin has been shown to displace basic fibroblast growth factor, a potent stimulator of smooth muscle cell migration and proliferation, from the arterial wall.\(^34\)

Achieving adequate tissue levels of antiproliferative agents may be essential to reduce the response to injury. Discrepancies between therapy for restenosis in human studies and in animal studies may be explained in part by differences in effective dosage. Edelman et al\(^\text{35}\) recently demonstrated that in vivo periventricular heparin delivery significantly reduced smooth muscle cell proliferation, whereas similar results have not been seen in humans with systemic therapy.\(^36,37\) Similarly, systemic angiotensin-converting enzyme inhibitors produced marked reductions in intimal proliferation in a small animal model of restenosis,\(^38\) but human studies have not resulted in similar reductions.\(^39\) The dosage of systemic angiotensin-converting enzyme inhibitor in that animal study as well as the dosage of drugs in other small animal studies of therapy for restenosis were much higher than those achievable in human studies.\(^40-42\) This suggests that larger than normal concentrations of drug, if delivered locally to the site of injury, may have benefits not realized with systemic therapy. Our results support the concept that local delivery has...
important effects that are localized to the site of delivery and may achieve more effective therapy than could be achieved with systemic therapy.

In this study we have evaluated, in isolation, the role of smooth muscle cell proliferation in the response to vascular injury. Clinical restenosis, however, involves other processes as well, such as the migration of smooth muscle cells into the intima and matrix deposition. There is evidence that heparin reduces smooth muscle cell migration in vitro, and the reduction of thrombus formation might also reduce smooth muscle cell migration in vivo by reducing the contribution of factors such as platelet-derived growth factor and thrombin. This study did not address the role of matrix accumulation in clinical restenosis. Matrix accumulation may be reduced along with reduced smooth muscle cell proliferation or migration or might in fact be enhanced, as suggested by Snow et al. by inhibiting the expression of proteases required for matrix degradation.

**Technical Aspects of Hydrogel Delivery**

The specific method of local delivery entails several practical considerations. Several other devices for local delivery are currently in the early stages of development. Heparin and other agents have previously been delivered to the vessel wall by a perforated balloon catheter. The coated balloon used in the present study has several theoretical benefits. First, only a single hydrogel balloon is required both to perform angioplasty and for drug delivery. More importantly, the perforated balloon catheter has been shown to result in significant trauma from fluid jets, whereas the hydrogel-coated balloon results in no additional vessel trauma beyond that required for the angioplasty itself.

While this study demonstrated an extremely rapid wash-off of heparin from the hydrogel balloon surface, this problem was overcome by rapid deployment and inflation of the balloon. In addition, protection of the balloon surface with a protective sleeve prevented this rapid wash-off. Measures to decrease drug loss will need to be addressed in the clinical application of local hydrogel balloon delivery.

**Study Limitations**

In this study, both platelet deposition and smooth muscle cell proliferation were each evaluated at only a single time point after balloon injury. Previous studies have demonstrated that the platelet response to vessel injury is self-limited and that platelet deposition is maximal early after vessel injury and then falls off rapidly. The decreased platelet deposition observed in this study was noted at 1 hour after heparin delivery, at a time when only a small fraction of the heparin remained in the wall. It is therefore possible that early platelet aggregation immediately after balloon injury is partially inhibited by local heparin, resulting cumulatively in less platelets at the angioplasty site at later times. We have not addressed, however, whether there is a later convergence or even rebound of platelet deposition.

Similarly, there is evidence that smooth muscle cell proliferation is a self-limited process with maximal proliferation occurring within the first week and that critical mediators of proliferation such as oncogenes are triggered with self-limited expression early after injury. A reduction in smooth muscle cell proliferation was found in this study at the time of expected peak proliferation, the subsequent course of smooth muscle cell proliferation was not evaluated.

**Conclusions**

This study demonstrates, in vivo, a physiological effect of a catheter-based local drug delivery system and supports the concept that local delivery may yield benefits in the absence of systemic effects. Localized heparin delivery using heparin-coated hydrogel balloons reduces platelet deposition after balloon-induced vascular injury and therefore may have further applications by decreasing intracoronary thrombus formation after balloon angioplasty. Equally important, local heparin delivery using this technique reduces smooth muscle cell replication and may have significant effects on chronic restenosis.

**Acknowledgment**

This study was supported in part by a grant from the Beatrice Fox Auerbach Foundation.

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Decreased platelet deposition and smooth muscle cell proliferation after intramural heparin delivery with hydrogel-coated balloons.

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_Circulation_. 1994;90:433-441
doi: 10.1161/01.CIR.90.1.433

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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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:
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