Inhibition of Platelet Deposition and Lysis of Intracoronary Thrombus During Balloon Angioplasty Using Urokinase-Coated Hydrogel Balloons

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Background  Conventional balloon angioplasty of intracoronary thrombus is associated with a high incidence of abrupt closure, distal embolization, and no-reflow phenomenon. The purpose of this study was to assess a new technique for treating intracoronary thrombus consisting of the local delivery of urokinase directly to the angioplasty site with urokinase-coated hydrogel balloons.

Methods and Results  We assessed local urokinase delivery using hydrogel balloons in four protocols. First, we evaluated the pharmacokinetics of urokinase delivery in vitro using 125I-labeled urokinase to measure drug loading onto hydrogel balloons, drug retention by the hydrogel polymer during blood exposure, and drug transfer from the balloon surface to the arterial wall during balloon dilatation. Second, we measured 125I-urokinase washoff from the hydrogel balloon in the intact circulation and intramural drug delivery during in vivo balloon angioplasty in 10 anesthetized New Zealand rabbits. Third, we assessed the effect of local urokinase delivery on 111In-labeled platelet deposition after balloon angioplasty in vivo in 13 porcine carotid or iliac arteries dilated with urokinase-coated balloons and compared them with contralateral control arteries dilated with saline-coated balloons. Finally, we determined the clinical efficacy of urokinase-coated balloons in 15 patients with intracoronary thrombus, including 7 who demonstrated abrupt thrombotic closure after conventional angioplasty. Between 241 and 1509 U urokinase could be loaded onto hydrogel balloons ranging in size from 2 to 8 mm. In vitro and in vivo studies demonstrated that hydrogel balloons absorbed significantly more urokinase and demonstrated less drug washoff than nonhydrogel balloons (P < .01). Similarly, both in vitro and in vivo studies demonstrated urokinase transfer from the hydrogel to the arterial wall during balloon angioplasty, with greater intramural drug deposition with larger balloons (P < .01). Local urokinase delivery after in vivo pericentral angioplasty decreased 111In-labeled platelet deposition by 47% compared with contralateral control vessels (P = .03). Use of urokinase-coated balloons in patients with intracoronary thrombus resulted in thrombus dissolution and reversal of abrupt closure in all cases, without evidence of distal embolization.

Conclusions  With the use of hydrogel-coated balloons, urokinase can be delivered locally to an angioplasty site. This technique decreases platelet deposition after in vivo balloon angioplasty and is efficacious in treating intracoronary thrombus in patients, including those with abrupt thrombotic closure. (Circulation. 1994;90:1979-1988.)

Key Words  • drug delivery  • balloon angioplasty  • hydrogel  • urokinase

The optimal method for treating intracoronary thrombus during coronary angioplasty is currently unknown. Conventional angioplasty of thrombus and thrombus-containing stenoses is associated with an increased incidence of abrupt thrombotic closure, distal embolization, and no-reflow phenomenon.1-5 Compared with angioplasty of nonthrombotic lesions, percutaneous transluminal coronary angioplasty in this circumstance leads to a higher rate of myocardial infarction, emergency coronary artery bypass, and death. Alternative techniques for dealing with intracoronary thrombus have included new adjunctive mechanical devices for extracting or mechanically modifying thrombus6-14 as well as various pharmacological regimens of antiplatelet, antithrombin, and thrombolytic agents.15-25

The purpose of this study was to assess a new technique for treating intracoronary thrombus consisting of a catheter-based drug-delivery system that delivers urokinase locally. This system consists of a standard angioplasty balloon coated with a hydrogel polymer into which various agents can be absorbed. Inflation of the hydrogel balloon in an artery results in polymer contact with the arterial intimal surface. Subsequent hydrogel compression results in diffusion of a given agent from the polymer into the space between the balloon surface and arterial wall and directly into the vessel wall. Previous animal work from our laboratory has documented the efficacy of this system in the intramural delivery of horseradish peroxidase, heparin, and antisense oligonucleotides to c-myc.26-29
We hypothesized that use of a urokinase-coated hydrogel balloon in an artery with intracoronary thrombus would create a local environment of relatively high urokinase concentration during balloon inflation, resulting in enhanced thrombolysis. Moreover, intramural deposition of the urokinase with the hydrogel system could theoretically produce a localized reservoir of drug at the angioplasty site that could inhibit ongoing platelet deposition and thrombus formation. To test these hypotheses, we designed this study with four specific goals: first, to test in vitro the ability of hydrogel balloons to absorb urokinase, retain surface-bound drug during blood exposure, and transfer drug to the arterial wall during balloon inflation; second, to measure in vivo urokinase washoff from the hydrogel balloon surface in the intact circulation and intramural drug deposition during balloon angioplasty; third, to assess in vivo the effect of local urokinase delivery on platelet deposition after conventional balloon angioplasty; and fourth, to test in patients the ability of urokinase-coated balloons to treat intracoronary thrombus and abrupt thrombotic closure after conventional angioplasty of thrombus-containing coronary stenoses.

Methods

Equipment

The local drug-delivery system used in this study consists of a standard angioplasty catheter with a hydrogel compound (Hydro Plus, Mansfield/Boston Scientific Corp) coating a 2- to 4-cm polyethylene balloon. The hydrogel coating consists of a latticework of polyacryl acid chains that are adhered to the balloon surface. At intervals along their length, the polymers are covalently linked to one another. When the hydrogel contacts an aqueous environment, it absorbs water, and the lattice begins to swell and form a stable matrix of polymer and water. Any agents dissolved in the water will also be incorporated into this matrix. The thickness of the coating ranges from 5 μm when dry to 25 μm when fully saturated with water.

Loading of Urokinase Onto Hydrogel Balloons

All balloons used in this study were loaded with urokinase by immersing the inflated balloon at 2 atm pressure into a concentrated urokinase solution (50,000 U/mL), deflating the balloon while still in solution, and allowing the balloon to dry under ambient conditions.

For in vitro and in vivo pharmacokinetic studies, 125I-labeled urokinase (New England Nuclear) was used to measure the amount of drug. Urokinase was radiolabeled with 125I by the Bolton Hunter method and suspended in Abbokinase (50,000 U/mL) (Abbott Laboratories) with a final specific activity of 1 mCi/50,000 U. A known quantity of the radiolabeled urokinase solution was counted in a gamma counter for determination of the relation between 125I counts per minute and urokinase concentration. Counts per minute measured from balloon surfaces and arterial segments were subsequently converted to microliters of absorbed fluid and units of urokinase after subtraction of background counts.

In Vitro Pharmacokinetic Studies

We performed four in vitro pharmacokinetic protocols to determine the ability of hydrogel balloons to absorb urokinase, retain surface-bound drug during blood exposure, and transfer drug to the arterial wall during balloon dilatation. A total of 86 hydrogel and 12 nonhydrogel balloons were used in this part of the study.

Protocol 1

To determine the optimal loading method for maximizing the amount of urokinase absorbed by the balloon surface, we immersed twenty-four 3.5-mm hydrogel balloons into the radiolabeled urokinase solution using the following four techniques: immersion for 30 seconds (n=6), immersion twice for 30 seconds (n=6), immersion for 60 seconds (n=6), and immersion for 5 minutes (n=6). After urokinase loading, all balloons were excised from their balloon shafts and counted in a gamma counter for measurement of drug absorption.

Protocol 2

To determine the absolute amount of urokinase absorbed by hydrogel balloons of various sizes, we immersed 36 hydrogel balloons ranging in size from 2 to 8 mm into the radiolabeled urokinase solution for 60 seconds and then counted them in a gamma counter. Drug absorption on the following balloon sizes was assessed: 2 mm (n=6), 2.5 mm (n=6), 3.0 mm (n=6), 3.5 mm (n=6), 7 mm (n=6), and 8 mm (n=6).

Protocol 3

To determine the effect of blood exposure on urokinase retention by the hydrogel balloon surface, we initially loaded twelve 3.5-mm hydrogel balloons with urokinase by immersion into the radiolabeled urokinase solution for 60 seconds and then agitated them in test tubes containing 10 mL anticoagulated porcine blood for either 60 (n=6) or 120 (n=6) seconds. All 12 balloons were counted in a gamma counter after initial drug loading and then after blood exposure.

Drug retention by the hydrogel balloons was compared with twelve 3.5-mm nonhydrogel balloons (Mansfield/Boston Scientific) that were also immersed into the radiolabeled urokinase solution for 60 seconds and then agitated in blood for either 60 (n=6) or 120 (n=6) seconds exactly as described above for hydrogel balloons.

Protocol 4

To document urokinase transfer from the hydrogel surface to the arterial wall, we dilated 14 freshly harvested porcine peripheral or coronary arteries (4 carotid, 4 iliac, 6 coronary) in vitro with fourteen 3-mm (n=6) or 8-mm (n=8) hydrogel balloons that had been loaded with radiolabeled urokinase by immersion for 60 seconds. Before in vitro angioplasty, 7 of the 14 balloons were agitated in blood for 60 seconds. All balloon dilations were performed for 60 seconds with an inflation pressure of 6 atm and with a balloon-to-artery ratio of approximately 1.3:1. After in vitro angioplasty, the lumen of all dilated vessels was flushed with saline, and then the arterial segment was counted in a gamma counter. The number of urokinase counts in the arteries was compared with the number of counts on 12 equal-sized control balloons that were similarly loaded with urokinase and then counted in a gamma counter.

In Vivo Pharmacokinetic Studies

We performed two in vivo pharmacokinetic protocols to assess urokinase washoff from the hydrogel balloon surface in the intact circulation and the quantity of urokinase intramuscularly deposited during balloon angioplasty. Ten New Zealand rabbits (3 to 5 kg) were used in this study. Each animal was anesthetized with ketamine (50 mg/kg IM) and xylazine (5 mg/kg IM), and a 5F introducer was placed in the right carotid artery using standard surgical techniques. A total of 32 hydrogel balloons and 24 nonhydrogel balloons were used.

Protocol 1

To measure in vivo drug washoff in the intact circulation, we counted 24 125I-labeled, urokinase-loaded 3-mm hydrogel balloons in a gamma counter after initial drug loading (n=6) or after introduction into the rabbit circulation through the
carotid sheath for 30 (n=6), 60 (n=6), or 120 (n=6) seconds. Balloon counts were compared with twenty-four 3-mm non-hydrogel balloons measured similarly after drug loading (n=6) or in vivo exposure for 30 (n=6), 60 (n=6), or 120 (n=6) seconds. A total of six rabbits were studied with six balloons used in each rabbit. After removal of each balloon, the arterial sheath was flushed with saline.

**Protocol 2**

For measurement of intramural drug deposition during in vivo balloon angioplasty, four rabbits underwent balloon dilatation of both iliac arteries with radiolabeled urokinase-coated 3-mm hydrogel balloons. For each dilatation, a 0.014-inch angioplasty guidewire was introduced through the carotid sheath for 30 minutes, advanced through the carotid sheath over the guidewire to the femoral artery under fluoroscopic guidance. After guidewire placement, each balloon was advanced through the carotid sheath over the guidewire to the level of the iliac artery. Balloon position was chosen to achieve a balloon-to-artery ratio of 1:1. All inflations were performed for 90 seconds at 6 atm. After dilatation of both iliac vessels, the dilated vessels were surgically removed, gently flushed with saline, and counted in a gamma counter for measurement of intramural \(^{125}\text{I}\)-urokinase. Arterial counts were compared with six 3-mm hydrogel balloons that were counted immediately after drug loading.

**In Vivo Platelet Deposition Studies**

Seven 3- to 4-month-old (20 to 30 kg) male Yorkshire swine were used for determination of the effect of local urokinase delivery on platelet deposition after balloon angioplasty. Platelet deposition was measured as described by Johnstone et al\(^{30}\) using \(^{111}\text{In}\)-labeled platelets. \(^{111}\text{In}\)-labeled platelets were prepared by mixing 50 \(\mu\text{g}\) tropolone (Sigma Chemical Co) in normal saline solution with 0.5 MCl of \(^{111}\text{In}\)-chloride (New England Nuclear). Forty-three milliliters of whole blood was withdrawn into 7 mL ACD saline (Mallinckrodt) and centrifuged at 180g for 15 minutes. Platelet-rich plasma was removed and centrifuged at 10 minutes at 1600g. The pellet was resuspended in 4 mL platelet-poor plasma, incubated for 5 minutes and centrifuged at 1600g for 10 minutes. The platelet pellet was then resuspended with \(^{111}\text{In}\)-labeled platelets and 2 mL ACD saline solution for 20 minutes and centrifuged for 10 minutes at 160g. The pellet was resuspended in 4 mL platelet-poor plasma, incubated for 5 minutes, and centrifuged for 10 minutes at 160g. The labeled platelet pellet was resuspended in 5.5 mL platelet-poor plasma and centrifuged at 100g for 10 minutes to remove any macroaggregates. The supernatant containing \(^{111}\text{In}\)-labeled platelets was then removed and reinjected intravenously.

Approximately 2 hours after reinjection of labeled platelets, all animals were premedicated with tiletamine/zolazepam (100 mg IM) and atropine (2 mg IM) and then endotracheally intubated. Anesthesia was maintained with enflurane and ventilation provided by a volume respirator. Using standard surgical techniques, short 1-cm, 9F introducer sheaths (USCI) were inserted into both carotid and both femoral arteries approximately 10 cm distal to the aorta by direct arterial exposure.

Before angioplasty, intravascular ultrasound using a 20-MHz, 3.5F catheter (Mansfield/Boston Scientific) was performed on both carotid and iliac arteries to assess arterial diameter and also to localize the site of balloon inflation measured from the origin of the vessel from the aorta. Imaging was performed with a console adapted for 20-MHz operation and 360\(^\circ\) scans (Diasonics).

In all pigs, balloon angioplasty was subsequently performed on an iliac and carotid vessel using a urokinase-coated hydrogel balloon. All balloons were loaded with urokinase by immersion into a concentrated solution of Abbokinase (50 000 U/mL) for 60 seconds. Balloon dilatation using a same-sized hydrogel balloon that had been immersed into saline was performed in the contralateral iliac or carotid vessel to serve as a control. All inflations were performed for 5 minutes at 6 atm with a balloon-to-artery ratio of approximately 1.3:1, as assessed by intravascular ultrasound. Balloon sizes for the entire study group ranged from 7 to 8 mm.

One hour after angioplasty, all dilated arterial segments were 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 measure \(^{111}\text{In}\). Blood samples for determination of free and cell-bound \(^{111}\text{In}\) activity were also obtained at the time of angioplasty and vessel removal. The number of platelets deposited in the treated vessel was determined by multiplying the number of \(^{111}\text{In}\) counts per minute in the vessel by the number of platelets per milliliter of blood and dividing by the \(^{111}\text{In}\) counts per minute per milliliter of blood.

All animal studies, including both rabbit and porcine protocols, conformed to the principles of the American Physiological Society.

**Clinical Studies**

Fifteen consecutive patients with intracoronary thrombus seen on coronary angiography were treated with urokinase-coated balloons. All balloons were coated with urokinase by immersion into a concentrated Abbokinase solution (50 000 U/mL) for at least 60 seconds. Intracoronary thrombus was identified as a clearly localized, discrete intraluminal filling defect that was surrounded by contrast medium and was seen in multiple angiographic views. In 10 patients, urokinase-coated balloons were used after failure of conventional balloon angioplasty due to abrupt thrombotic closure or persistent intracoronary thrombus with slow antegrade flow. In an additional 5 patients, urokinase-coated balloons were used as primary therapy for treatment of thrombus-containing lesions.

**Statistics**

Mean and standard deviation for the amount of fluid and number of urokinase units absorbed by hydrogel and nonhydrogel balloons and for urokinase units delivered to the arterial wall were determined for in vitro and in vivo pharmacokinetic studies. Differences in urokinase loading with different loading techniques and washoff of urokinase from balloon surfaces were assessed using one-way ANOVA. Intergroup comparisons of urokinase loading and washoff between hydrogel and nonhydrogel balloons were determined using paired \(t\) tests.

Mean and standard deviation for \(^{111}\text{In}\)-labeled platelets were determined for in vivo platelet studies. Differences in platelet deposition between urokinase-treated and control vessels were analyzed using the Wilcoxon signed rank test.

For clinical studies, all angiograms were reviewed in a blinded fashion by two observers who independently evaluated the angiographic presence of intracoronary thrombus and the grade of TIMI coronary flow. Differences were resolved by consensus.

A probability value less than .05 was considered significant.

**Results**

**In Vitro Pharmacokinetic Studies**

**Protocol 1: Optimal Method for Balloon Loading**

Table 1 lists the amount of urokinase absorbed by 3.5-mm hydrogel balloons loaded with urokinase by balloon immersion for 30 seconds, immersion twice for 30 seconds, immersion for 60 seconds, and immersion for 5 minutes. Although there was a trend for longer periods of balloon immersion to result in more urokinase loading, these differences were not significant when assessed by ANOVA (\(P=.07\)). For the remaining in vitro, in vivo, and clinical protocols, all balloons were loaded with urokinase using the 60-second immersion technique.
TABLE 1. Urokinase Loading onto Hydrogel Balloons

<table>
<thead>
<tr>
<th>Technique</th>
<th>Absorbed Fluid, μL</th>
<th>Absorbed Urokinase, U</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-Second balloon immersion</td>
<td>8.5±1.5</td>
<td>425±90</td>
</tr>
<tr>
<td>Two 30-second balloon immersions</td>
<td>10.7±1.7</td>
<td>537±87</td>
</tr>
<tr>
<td>60-Second balloon immersion</td>
<td>11.0±2.4</td>
<td>551±119</td>
</tr>
<tr>
<td>120-Second balloon immersion</td>
<td>11.2±1.8</td>
<td>562±91</td>
</tr>
</tbody>
</table>

Six balloons were tested for each technique.

Protocol 2: Urokinase Absorbed by Balloons of Varying Sizes

Table 2 lists the amount of urokinase absorbed by balloons ranging in size from 2 to 8 mm. The total amount of drug absorbed increased as balloon size increased and was proportional to the balloon surface area and amount of hydrogel available for binding.

Protocol 3: Retention of Urokinase During Blood Exposure

Fig 1 illustrates the amount of urokinase on twelve 3.5-mm hydrogel balloons after initial balloon loading (383±62 U), after washoff in blood for 60 seconds (134±21 U), and after washoff in blood for 120 seconds (120±12 U). During 60 seconds of blood exposure, 65% of the urokinase washed off the balloon surface. However, an additional 60 seconds of blood exposure resulted in no significant drug loss from the balloon surface.

Fig 1 also illustrates the amount of urokinase on twelve 3.5-mm nonhydrogel balloons after initial loading (241±56 U), after washoff in blood for 60 seconds (79±66 U), and after washoff in blood for 120 seconds (32±8 U). Nonhydrogel balloons initially absorbed 63% of the urokinase absorbed by similar-sized hydrogel balloons. Similar to hydrogel balloons, approximately 67% of the drug washed off during the first 60 seconds of blood exposure. Unlike hydrogel balloons, however, further urokinase washoff occurred after 120 seconds of blood exposure with only 13% of drug on the balloon surface at that time (P=.05). After initial loading and the 120-second washoff, there was significantly more urokinase on hydrogel balloons than on nonhydrogel balloons (P<.01).

TABLE 2. Quantity of Urokinase Loaded Versus Balloon Size

<table>
<thead>
<tr>
<th>Balloon Size, mm</th>
<th>Absorbed Fluid, μL</th>
<th>Absorbed Urokinase, U</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>4.8±1.1</td>
<td>241±53</td>
</tr>
<tr>
<td>2.5</td>
<td>6.4±0.8</td>
<td>319±40</td>
</tr>
<tr>
<td>3.0</td>
<td>8.3±0.8</td>
<td>419±40</td>
</tr>
<tr>
<td>3.5</td>
<td>11.0±2.4</td>
<td>551±119</td>
</tr>
<tr>
<td>4.0</td>
<td>14.0±4.5</td>
<td>737±134</td>
</tr>
<tr>
<td>5.0</td>
<td>17.0±6.5</td>
<td>919±203</td>
</tr>
<tr>
<td>6.0</td>
<td>20.0±8.5</td>
<td>1101±284</td>
</tr>
<tr>
<td>7.0</td>
<td>23.0±10.5</td>
<td>1394±519</td>
</tr>
<tr>
<td>8.0</td>
<td>30.2±6.6</td>
<td>1509±328</td>
</tr>
</tbody>
</table>

Six balloons were tested for each size.

Protocol 4: Intramural Deposition of Urokinase During In Vitro Balloon Angioplasty

Table 3 lists the amount of urokinase and percentage of urokinase on the balloon surface that was delivered to the vessel wall after in vitro angioplasty of 14 arterial segments. In all 14 specimens, radiolabeled urokinase was detected. The amount of urokinase intramurally deposited was 398±70 U (26±5%) for unwashed 8-mm balloons, 203±82 U (14±5%) for washed 8-mm balloons, 56±45 U (13±11%) for unwashed 3-mm balloons, and 9±3 U (2±1%) for washed 3-mm balloons. Less urokinase was intramurally deposited with smaller (P<.01) and washed (P<.01) balloons.

TABLE 3. In Vitro Intramural Urokinase Deposition

<table>
<thead>
<tr>
<th>Vessel Dilated</th>
<th>Balloon Size, mm</th>
<th>Washoff for 60 Seconds</th>
<th>Quantity of Urokinase Deposited, U</th>
<th>Loaded Urokinase Deposited, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid</td>
<td>3</td>
<td>–</td>
<td>38</td>
<td>33</td>
</tr>
<tr>
<td>Carotid</td>
<td>6</td>
<td>–</td>
<td>503</td>
<td>33</td>
</tr>
<tr>
<td>Iliac</td>
<td>3</td>
<td>–</td>
<td>374</td>
<td>25</td>
</tr>
<tr>
<td>Iliac</td>
<td>6</td>
<td>–</td>
<td>349</td>
<td>23</td>
</tr>
<tr>
<td>Iliac</td>
<td>8</td>
<td>+</td>
<td>267</td>
<td>18</td>
</tr>
<tr>
<td>Iliac</td>
<td>8</td>
<td>+</td>
<td>110</td>
<td>7</td>
</tr>
<tr>
<td>Carotid</td>
<td>8</td>
<td>–</td>
<td>276</td>
<td>18</td>
</tr>
<tr>
<td>Carotid</td>
<td>8</td>
<td>+</td>
<td>160</td>
<td>11</td>
</tr>
<tr>
<td>RCA</td>
<td>3</td>
<td>–</td>
<td>38</td>
<td>9</td>
</tr>
<tr>
<td>LAD</td>
<td>3</td>
<td>–</td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td>CFX</td>
<td>3</td>
<td>–</td>
<td>108</td>
<td>26</td>
</tr>
<tr>
<td>RCA</td>
<td>3</td>
<td>+</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>LAD</td>
<td>3</td>
<td>+</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>CFX</td>
<td>3</td>
<td>+</td>
<td>12</td>
<td>3</td>
</tr>
</tbody>
</table>

Mean±SD 15±10

RCA indicates right coronary artery; LAD, left anterior descending artery; and CFX, circumflex artery.
In Vivo Pharmacokinetic Studies

Protocol 1: Urokinase Washoff in the Intact Circulation

Fig 2 illustrates the amount of radiolabeled urokinase on 3-mm hydrogel balloons after initial drug loading (511±127 U) and after exposure to the intact rabbit circulation for 30 (116±12 U), 60 (90±15 U), and 120 (77±6 U) seconds. Progressive drug loss occurred at the 30- and 60-second time intervals, with approximately 82% of drug lost after 1 minute of exposure. Similar to the in vitro studies, no significant additional drug loss occurred by 120 seconds.

Fig 2 also illustrates drug loss from nonhydrogel balloons, with 342±55 U after initial loading, 26±4 U at 30 seconds, 24±4 U at 60 seconds, and 22±4 U at 120 seconds. Approximately 92% of urokinase washed off within 30 seconds, with no additional drug loss at later time intervals. Similar to the in vitro studies, there was more urokinase on hydrogel balloons than nonhydrogel balloons at all four time intervals (P<.01).

Protocol 2: In Vivo Intramural Deposition of Urokinase

Table 4 lists intramural urokinase deposition after in vivo balloon angioplasty in seven iliac vessels with 3-mm balloons. Approximately 2±1 urokinase units, or 0.4±0.3% of urokinase on the balloon surface, was intramuscularly deposited. After in vivo angioplasty, hydrogel balloons still retained 13±1% of the loaded urokinase on their surface.

Platelet Deposition Studies

Fig 3 illustrates 111In-labeled platelet deposition in 13 iliac (n=6) and carotid (n=7) vessel pairs dilated with either urokinase-coated hydrogel balloons or saline-coated hydrogel balloons. A 14th vessel pair was not used because one of the vessels was transected during sheath insertion. For the entire group, local urokinase delivery decreased platelet deposition from 2.54±3.93×10^8 platelets in control vessels to 1.34±1.66×10^8 platelets in urokinase-treated vessels (P=.03). Ten of the 13 urokinase-treated vessels demonstrated less platelet deposition than control arteries.

Clinical Studies

Fifteen patients were treated with urokinase-coated balloons during balloon angioplasty. The study group consisted of 12 men and 3 women with an age range of 42 to 79 years. The clinical diagnosis was postinfarction angina in 7, unstable angina in 4, acute myocardial infarction in 3, and postinfarction cardiogenic shock in 1. Six patients had received systemic thrombolytic therapy 1 to 16 hours before intervention. Four patients had contraindications to systemic thrombolysis secondary to inguinal or retroperitoneal hematomas (n=2), large facial hematoma (n=1), and active hemoptysis (n=1). At the time of catheterization, all patients were treated with aspirin, intravenous heparin, intravenous nitroglycerin, and various combinations of calcium channel blockers and β-blockers.

Selective coronary angiography identified a “culprit” coronary stenosis located in the left anterior descending artery (n=8), right coronary artery (n=3), circumflex artery (n=3), left main (n=1), right coronary artery (n=1), left circumflex artery (n=1), and left main (n=1). The coronary lesion that was treated with the urokinase-coated balloon was the thrombus-related lesion in 10 patients (67%) and a restenosis in 5 patients (33%).

Fig 3. Bar graph shows the effect of local urokinase delivery on in vivo platelet deposition. 111In-labeled platelet deposition in 13 peripheral porcine arteries was measured 1 hour after dilation with urokinase-coated balloons. Contralateral vessels that underwent angioplasty with saline-coated hydrogel balloons served as controls. Local urokinase delivery decreased platelet deposition by 47% (urokinase-treated vessels, 1.34×10^8; control vessels, 2.4×10^8, P=.03).
artery (n=1), or a saphenous vein graft (n=3). Total occlusions were initially present in 5 patients, including 2 of the 3 patients with acute myocardial infarctions. Preintervention intracoronary thrombus was identified in 10 patients.

Ten patients initially underwent conventional balloon angioplasty of their culprit stenoses. After initial arterial access, all patients were treated with 10 000 U heparin. An ACT greater than 300 seconds was maintained in all patients. All balloon dilatations were performed with a balloon-to-artery ratio of 1:1, as gauged by a normal reference segment adjacent to the coronary stenosis. Conventional angioplasty involved a mean of 3.9±1.8 inflations (range, two to six) with a mean inflation time of 112±47 seconds per inflation (range, 60 to 180 seconds) at 9.3±1.7 atm (range, 8 to 12 atm). After the initial balloon inflation, all patients developed evidence of intraluminal filling defects, with subsequent abrupt closure (TIMI grade 0) in 7 patients or slow antegrade flow (TIMI grade 1 to 2) in the remaining 3. Angiographic coronary dissections were not identified in any patient. Repeat prolonged balloon inflations (range, 120 to 180 seconds) in all patients did not reverse abrupt closure, improve coronary flow, or eradicate thrombotic filling defects.

All patients who demonstrated abrupt closure or slow antegrade flow subsequently underwent balloon dilatation with a urokinase-coated balloon that had been immersed in a solution of 50 000 U/mL Abbotakinase. In each patient, the urokinase-coated balloon was the same size as the conventional angioplasty balloon. A total of 2.3±0.7 inflations (range, one to three) with a mean inflation time of 182±75 seconds per inflation (range, 60 to 300 seconds) at 9.1±2.2 atm (range, 5 to 12 atm) were performed. After the initial urokinase balloon dilatation, all patients demonstrated vessel patency with TIMI grade 3 in 9 patients and TIMI grade 2 in 1. In addition, as seen angiographically, there was complete dissolution of intraluminal clot in all patients. Angiographic evidence of distal embolization did not occur in any patient.

In an additional 5 patients, urokinase-coated balloons were used as the primary treatment of thrombus-containing stenoses. In 1 of these patients, abrupt closure with TIMI grade 0 flow developed despite pretreatment with 500 000 U intracoronary Abbbokinase over 1 hour. After dilation with a urokinase-coated balloon, successful lesion reduction with complete dissolution of intracoronary thrombus, TIMI grade 3 coronary flow, and no evidence of distal embolization occurred in all patients.

After local urokinase delivery, all patients were treated with intravenous heparin, aspirin and a combination of nitrates, calcium channel blockers, and β-blockers. No patient demonstrated clinical evidence of coronary reocclusion or increased elevation of baseline cardiac enzymes after angioplasty. One of the patients who initially presented with postinfarction cardiogenic shock died 11 days after angioplasty from progressive pump failure. The remaining patients were all discharged from the hospital.

Table 5 lists demographic and angiographic data for patients treated with urokinase-coated balloons for abrupt thrombotic closure, and Table 6 describes patients in whom urokinase-coated balloons were used as the primary therapy. Figs 4 and 5 show two angiographic examples of local urokinase delivery in patients with intracoronary thrombus.

**Discussion**

This study demonstrates that urokinase-coated hydrogel balloons are effective in the local inhibition of platelet deposition and the treatment of intracoronary thrombus and thrombus-containing stenoses during balloon angioplasty. In vitro and in vivo pharmacokinetic studies have shown that hydrogel balloons absorb and retain more urokinase than standard, nonhydrogel balloons and are capable of transferring urokinase from the hydrogel surface to the arterial wall during balloon inflation. In vivo animal studies have demonstrated that this technique significantly reduces early platelet deposition after balloon injury. Most importantly, clinical studies have shown that this technique was successful in
treated intracoronary thrombus and reversing abrupt thrombotic closure in patients unsuccessfully treated with conventional balloon angioplasty.

**Potential Mechanisms of Action**

The presumed mechanism of platelet deposition inhibition and enhanced intracoronary thrombolysis with urokinase-coated balloons is the delivery of a relatively small amount of urokinase from the hydrogel coating on the balloon surface directly to the angioplasty site. Hydrogel compression during balloon inflation facilitates diffusion of urokinase off the polymer into the space between the balloon surface and arterial wall and also directly into the arterial wall. Urokinase retained on the balloon surface, intramurally deposited in the arterial wall, and eluted from the hydrogel may act directly on circulating plasminogen within the vicinity of balloon inflation or on plasminogen trapped within thrombus to create plasmin.

Local urokinase delivery may limit platelet deposition by resulting in plasmin lysis of fibrinogen and fibrin forming at the angioplasty site and subsequently dissolving platelet aggregates. Delivered immediately at the time of angioplasty, the early thrombolytic effects exerted by the enzyme on platelet thrombi may interfere with the positive feedback mechanisms leading to additional platelet deposition, resulting cumulatively in less platelet deposition hours later after initial balloon injury.

In the presence of preexisting intracoronary thrombus, local delivery of urokinase with the hydrogel system may result in a relatively high urokinase concentration in thrombus "trapped" between the balloon surface and arterial wall. Previous in vitro studies with human plasma have demonstrated that a urokinase concentra-
tion of at least 100 U/mL is required to initiate significant clot lysis.31 Based on pharmacokinetic studies, this local concentration of enzyme in the space between the balloon surface and arterial wall can be achieved with the hydrogel system. Mechanical dilation of the thrombus may further accentuate thrombolysis by compressing the clot along the balloon surface and increasing the thrombus surface area available for urokinase action.

An important component of the mechanism underlying local urokinase therapy may involve the intramural deposition of the drug at the angioplasty site. Although in vivo rabbit studies demonstrated deposition of minute urokinase quantities, in vitro studies with larger hydrogel balloons produced much larger amounts of intramural drug. Larger balloons may result in a higher percentage of intramural deposition because of an increased balloon surface area available for drug transfer. Intramural deposition of urokinase may create a small reservoir of drug at the angioplasty site that slowly elutes from the wall into the bloodstream, resulting in a persistent, local thrombolytic effect. This localized reservoir of drug would bypass the normal hepatic extraction of urokinase that usually occurs within 15 minutes after a systemic intravenous urokinase bolus.20 Previous studies from our laboratory with horseradish peroxidase, heparin, and antisense oligonucleotides have demonstrated the intramural persistence of locally delivered agents for as long as 24 to 48 hours.26-29

**Technical Aspects of Urokinase Delivery**

Pharmacokinetic studies suggest that hydrogel balloons are maximally saturated with urokinase after a brief 60-second exposure of the polymer surface to a concentrated urokinase solution, with greater amounts of drug absorbed on larger balloons with more hydrogel polymer. This rapid preparation of the drug-delivery balloon facilitates use of the hydrogel system in emergency clinical situations in which local urokinase delivery may be needed to reverse abrupt closure or poor angiographic results from conventional angioplasty in the setting of intracoronary thrombus.

In addition to hydrogel absorption of urokinase, nonspecific urokinase binding occurs on both hydrogel and nonhydrogel balloons, presumably because urokinase is a "sticky" protein that simply adheres to the balloon surface.32 In vitro and in vivo studies have suggested that most of this nonspecific drug binding is washed off during blood exposure, with greater retention of hydrogel-bound urokinase. Washoff of both hydrogel-bound and nonspecifically bound urokinase may have been minimized in this study further by the trapping of enzyme within the balloon folds not exposed to the bloodstream and/or by rapid deployment of the balloon to the angioplasty site.

Several potential improvements in the hydrogel catheter system could theoretically result in increased efficacy of the technique. In vitro studies in our laboratory have demonstrated increased drug absorption with a thicker hydrogel coating, suggesting that more drug could be locally delivered with more thickly coated balloons.28 Second, additional animal studies from our laboratory have demonstrated that use of a protective sheath over the hydrogel balloon may minimize drug washoff.28 Third, use of a hydrogel-coated perfusion balloon would theoretically allow for longer balloon inflation and more time for local drug delivery. Finally,
use of alternative thrombolytic agents, particularly those that are more “clot specific” with increased fibrin binding, might improve the efficacy of local clot lysis.

Implications From Clinical Studies

Intracoronary thrombus is an extremely common problem observed in the cardiac catheterization laboratory. The incidence of intracoronary thrombus ranges from 6% to 15% in patients referred for percutaneous revascularization and from 40% to 90% in patients with unstable coronary syndromes.33-37 Conventional balloon angioplasty in the presence of intracoronary thrombus may result in abrupt closure rates as high as 73%, in distal embolization rates as high as 31%, and in an incidence of no-reflow phenomenon as high as 7%.38 These suboptimal results are related to the fact that angioplasty itself is a powerful thrombogenic stimulus because it exposes additional wall components to circulating platelets and may result in the release of platelet factor IV and fibrin-bound thrombin from intraluminal clots. A no-reflow phenomenon may also occur secondary to the release of potent microvascular vasonestrictors such as thromboxane and serotonin.39

Current pharmacological therapies for treating intra-coronary thrombus have focused on the administration of antiplatelet, antithrombin, and thrombolytic agents either systemically or locally.15-25 Multiple nonrandomized studies have demonstrated the efficacy of administering urokinase and other thrombolytic agents using a variety of regimens when thrombus is present or when it develops during percutaneous transluminal coronary angioplasty.18-25 More recently, enhanced thrombolysis has been demonstrated with the use of prolonged urokinase infusions (eg, 18 to 48 hours) through a subselective catheter.24,25

Local delivery of urokinase to the site of intracoronary thrombus with hydrogel balloons may have specific advantages over current pharmacological regimens. One obvious advantage of the hydrogel technique is that it may avoid the need for prolonged administration of antithrombin and thrombolytic agents that lengthen the patient’s hospitalization and catheterization procedure. A second advantage of this approach is that a much lower dose of urokinase can be administered to the patient but in high local concentrations directly to the thrombus, thereby preventing systemic thrombolysis and subsequent bleeding complications. This may be particularly useful in patients who have contraindications to systemic thrombolysis. A final advantage of the hydrogel system is that it uses mechanical modification of the thrombus during balloon inflation, which may further enhance thrombolysis by exposing more thrombus surface to urokinase. In addition, angioplasty itself reduces the stenosis that limits flow and promotes stasis.

Study Limitations

Although locally delivered urokinase improved angiographic findings in patients in this study, it is impossible to rule out the possibility that these results occurred simply secondary to the effects of conventional angioplasty. Multiple prolonged inflations may eventually result in the angiographic disappearance of thrombus. The angiographic identification of intracoronary thrombus likewise has its limitations.35,36 A randomized, controlled trial of local urokinase delivery, perhaps involving coronary angioscopy to identify thrombus, would be necessary to fully ascertain the benefit of this technique.

Conclusions

Local urokinase delivery with a hydrogel system is possible and appears to be efficacious in reducing early platelet deposition after experimental balloon angioplasty and in treating intracoronary thrombus and thrombotic abrupt closure in patients. The presumed mechanism of this technique involves trapping of thrombus in a local environment of high urokinase concentration, with enhanced thrombolysis. In addition, there may be a persistent thrombolytic effect at the angioplasty site from intramurally deposited drug. A randomized, controlled trial of this therapy may be useful in fully evaluating the efficacy of this technique.

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