Local Anticoagulation Without Systemic Effect Using a Polymer Heparin Delivery System

Tomohisa Okada, MD, Don H. Bark, PhD, and Marc R. Mayberg, MD

Polymeric drug delivery systems that allow the application of substances to a localized region for specified periods of time have been developed. A model for intravascular thrombosis in the rat common carotid artery was established using a combination of balloon catheter endothelial injury with 1-hour occlusion of the vessel. After endothelial injury in 11 Sprague-Dawley rats, the adventitial surface of the carotid artery was exposed to the polymer polyvinyl alcohol (PVA) containing heparin and was compared with exposure to PVA alone in the contralateral (control) vessel. Subsequent determinations of the coagulation parameters systemic prothrombin and partial thromboplastin times showed no systemic effect of heparin. All 11 control vessels demonstrated complete or partial thrombosis, whereas only one of 11 heparin/PVA-treated vessels showed a small thrombus. Morphometric analysis of the cross-sectional thrombus: lumen ratio in 10 rats showed a significant reduction (p<0.005) in thrombus size for treated vessels (4.1±9.6%) compared with control vessels (60.2±25.8%). Scanning electron microscopy verified the absence of thrombus in the treated vessels despite complete endothelial desquamation. In a second group of eight rats, endothelial injury without occlusion did not cause thrombosis in treated or control arteries. The coagulation parameters for this group of eight unoccluded rats were similarly unaffected by heparin/PVA treatment. Our observations suggest that a localized antithrombotic effect of heparin can be achieved without systemic anticoagulation using a polymeric drug delivery system. This technique may be applied to a variety of surgical and nonsurgical clinical conditions. (Stroke 1988;19:1470-1476)

Intravascular thrombosis is a critical complication in a variety of arterial and venous vascular disorders. Endothelial injury or stagnation of blood may initiate adherence of platelets to the vessel wall, with subsequent platelet aggregation and activation of the intrinsic coagulation system. Heparin prevents thrombus formation by several mechanisms involving both antiplatelet and anticoagulant actions and has been shown to effectively inhibit intravascular thrombus formation in a number of disorders. Systemic administration of heparin, however, is associated with a considerable risk of hemorrhage, and the use of heparin is frequently limited in various settings with increased potential risks for bleeding.

Polymers that release embedded substances at known rates for specified periods of time have been developed. Polyvinyl alcohol (PVA) is a water-soluble, nontoxic polymer with characterized release kinetics. We describe a new technique for the localized application of heparin to the adventitia of injured blood vessels using controlled release from PVA. By this means a localized antithrombotic effect of heparin can be achieved at a site of endothelial injury and altered blood flow without systemic anticoagulation.

Materials and Methods

Eleven male Sprague-Dawley rats weighing 350-450 g were anesthetized with 80 mg/kg i.p. sodium pentobarbital and were mechanically ventilated through a tracheostomy. The distal common, external, and internal carotid arteries were exposed bilaterally in the rat’s neck through a midline incision, and a 2-French balloon embolectomy catheter was introduced into both external carotid arteries. Advancing the inflated embolectomy catheter cephalad over 1 cm proximal to the carotid bifurcation produced consistent endothelial desquamation in this segment of the artery. Additional vessel wall injury was produced by passing an 0.8-mm wire along the desquamated luminal surface. Six hundred units of heparin sulfate (0.03 ml; 20,000 units/ml) was mixed with 0.06 ml PVA (Elvax, Du Pont

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SILASTIC SHELL
HEPARIN IN PVA
CAROTID ARTERY

FIGURE 1. Schematic representation of polymeric drug delivery system. Heparin incorporated into 16% polyvinyl alcohol (PVA) is applied to adventitia of rat common carotid artery and enclosed in Silastic shell to prevent diffusion into surrounding tissue.

SILASTIC SHELL
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Corp., Wilmington, Delaware; 16% wt:vol in water) to produce a viscous gel. Immediately after mixing, the heparin/PVA gel was applied around the adventitial surface of the de-endothelialized distal left (treated) common carotid artery and surrounded by a Silastic (Dow-Corning, Midland, Michigan) shell to prevent release into adjacent tissues (Figure 1). PVA without heparin (0.09 ml) was similarly applied to the right (control) common carotid artery.

Thirty minutes after application of the PVA, both common carotid arteries were occluded by microclips at the proximal and distal ends of the segment with injured endothelium. After 1 hour of occlusion, the systemic prothrombin time (PT) and partial thromboplastin time (PTT) were determined from arterial blood drawn from a femoral catheter. The microclips were then removed, and blood flow was established again in both carotid arteries for 5 minutes. Vessels were perfusion-fixed in situ at physiologic pressure (mean 80 mm Hg) with intracardiac 0.12 M phosphate buffer (pH 7.4) followed by 4% paraformaldehyde and 1% glutaraldehyde in buffer. The common carotid arteries were removed and placed in 1.5% glutaraldehyde/buffer overnight.

One pair of carotid arteries for scanning electron microscopy (SEM) was placed in buffered 1% osmium tetroxide, dehydrated in graded ethanol, and critical-point dried. The luminal surface was exposed after mounting, coated with gold, examined, and photographed with a JEOL scanning electron microscope (Peabody, Massachusetts).

For light microscopy, the remaining 10 pairs of vessels were embedded in ethyl methacrylate (JB-4, Sorvall, Wilmington, Delaware), sectioned at 3 μm thicknesses, mounted on glass slides, and stained with hematoxylin and eosin. Cross-sectional areas for intraluminal thrombus, vessel wall, and lumen were determined from 10 adjacent sections at the site of maximal thrombosis for each vessel using an automated image analysis system (Bioquant System IV, Nashville, Tennessee). Thrombus size was expressed as the percentage ratio of the cross-sectional area of thrombus to lumen, and treated and control vessels were compared statistically using Student’s paired two-tailed t test.

Additional experiments were performed to evaluate the systemic distribution of heparin from PVA after endothelial injury only (no carotid occlusion). A group of eight rats underwent balloon catheter endothelial desquamation and application of PVA as above, but the vessels were not occluded. In this model, there was no significant thrombosis in treated or control arteries. Systemic PT and PTT for these eight unoccluded rats were determined 60 minutes after application of PVA.

PT and PTT were also determined in a third group of five untreated rats receiving balloon catheter endothelial desquamation without application of heparin/PVA.

Results

Table 1 shows PT and PTT for the nine rats undergoing endothelial injury with temporary occlusion and heparin/PVA treatment, the eight unoccluded rats undergoing endothelial injury and heparin/PVA treatment, and the five untreated rats undergoing endothelial injury only. There is no significant difference in PT or PTT between the two treated groups (with or without temporary occlusion) and the untreated group after endothelial injury.

SEM of the luminal surface of control and heparin/PVA-treated desquamated rat common carotid arteries are shown in Figures 2 and 3.

Table 1. Coagulation Parameters in Rats After Application of Heparin in Polyvinyl Alcohol Polymer to Common Carotid Artery

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Prothrombin time (sec)</th>
<th>Partial thromboplastin time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial injury with temporary occlusion + heparin</td>
<td>9</td>
<td>12.2±1.2</td>
<td>22.0±0</td>
</tr>
<tr>
<td>Endothelial injury + heparin</td>
<td>8</td>
<td>12.4±0.6</td>
<td>21.7±2.2</td>
</tr>
<tr>
<td>Endothelial injury only</td>
<td>5</td>
<td>11.8±0.8</td>
<td>22.0±0</td>
</tr>
</tbody>
</table>

Data are mean±SD.
FIGURE 2. Low-power scanning electron micrograph of luminal surface of rat common carotid artery after endothelial damage and 1-hour occlusion. a: Control artery shows prominent thrombus that completely fills lumen. b: Heparin-polyvinyl alcohol-treated vessel shows no thrombus formation over region of endothelial removal. (Both photomicrographs ×73.)

vessel shows an extensive thrombus composed primarily of erythrocytes in a dense fibrin network that completely occludes the lumen. The treated vessel, on the other hand, demonstrates complete endothelial desquamation with exposed subendothelial collagen. The luminal surface of the treated vessel is coated with a monolayer of adherent platelets, but no fibrin formation or erythrocyte thrombus is present.

In light microscopic sections, severe endothelial injury was seen in control and treated vessels, with frequent disruption of the elastica and occasional damage to the media (Figure 4). At regions of

FIGURE 3. High-power scanning electron micrograph of luminal surface of rat common carotid artery after endothelial damage and 1-hour occlusion. a: Control vessel demonstrates thrombus composed of erythrocytes in dense fibrin mesh. b: Heparin-polyvinyl alcohol-treated luminal surface shows absence of normal endothelium, with flattened platelets (arrows) adherent to underlying exposed collagen. (Both photomicrographs ×2412.)
endothelial injury without thrombus in either class of vessel, there was a sparse monolayer of platelets and leukocytes adherent to the underlying vessel wall. Intraluminal thrombus was composed of erythrocytes associated with abundant fibrin strands. Except for occasional localized disruptions of the medial architecture, there were no alterations in smooth muscle cell morphology or vessel wall thickness in either class of vessel. In control vessels, thrombus formation was more extensive in those with more pronounced damage to the media. Inflammatory changes related to PVA application were not observed in either class of vessel.

Table 2 shows the distribution of thrombus formation for control and treated vessels, expressed as the thrombus:lumen ratio. A significant (>20%) intraluminal thrombus was present in all 10 control vessels, four of which were completely occluded. In contrast, significant thrombus was visible in only one of 10 treated vessels.

Figure 5 shows the average cross-sectional area for intraluminal thrombus, vessel wall, and lumen for control and treated vessels. Thrombus area was reduced from $251 \pm 119 \ \mu m^2$ in control vessels to $15 \pm 36 \ \mu m^2$ in treated vessels ($p<0.005$) although there were no significant differences in vessel wall or lumen area between classes of vessels. The reduction in intraluminal thrombus formation is reflected in the thrombus:lumen ratio, which was reduced from $60.2\pm25.8\%$ in control vessels to $4.1\pm9.6\%$ ($p<0.005$) in treated vessels (Table 2).
TABLE 2. Distribution of Thrombus: Lumen Ratios in Rat Common Carotid Arteries Treated With Polyvinyl Alcohol Polymer or Polymer Containing Heparin After Endothelial Damage and 1-Hour Occlusion

<table>
<thead>
<tr>
<th>Thrombus/lumen ratio (%)</th>
<th>Control (polymer only)</th>
<th>Treated (heparin/polymer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>20-40</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>40-60</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>60-80</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>80-100</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>60.2±25.8%</td>
<td>4.1±9.6%</td>
</tr>
</tbody>
</table>

Discussion

Thromboembolic disorders are associated with a wide variety of vascular, hematologic, and systemic conditions and affect nearly every organ system. The complex interactions between vessel wall and circulating blood elements determine the dynamics of hemostasis and thrombus formation.11 Endothelial damage with exposure of the underlying media stimulates platelet adhesion and aggregation1-11 although coagulation may not occur in large arteries with high blood flow rates.3 Stasis thrombosis, on the other hand, may occur in the absence of discernible endothelial injury.1,7 Velocity of blood flow, as well as shear rate and dispersion of aggregating platelets,12 presumably determine the local concentrations of platelets and clotting factors.3,12 Clinically significant thrombosis is frequently related to both endothelial damage and alterations in blood flow.13

In our study, a combination of endothelial damage and temporary cessation of blood flow reliably produced significant intravascular thrombosis in control rat common carotid arteries; endothelial damage without occlusion did not produce thrombosis. As we employed temporary occlusion to produce thrombosis, it is not clear whether the antithrombotic effect of heparin/PVA would be present in a state of flowing blood. Histologically, thrombi were composed primarily of erythrocytes in a fibrin network, resembling the "red" thrombus observed in experimental stasis thrombosis.3,7 Of interest, increased thrombus formation in this model was associated with damage to the media, as previously noted in other models.14 Medial constituents are considerably more thrombogenic than those in the subendothelium due to the presence in the media of specific collagen subtypes (I and III)15 and tissue factor,16 which preferentially stimulate platelet aggregation and the intrinsic clotting mechanism.

Thrombosis at the site of disrupted endothelium is a major complication of any vascular surgery. In several large-artery endarterectomy models,17-20 the formation of mural thrombus was most prominent in the first 4 hours, after which thrombogenicity was apparently reduced. Systemic heparin administered during the procedure significantly inhibited thrombus formation during the immediate postoperative period.13,17 Thirty minutes after endarterectomy, the luminal surface of arteries in animals receiving systemic heparin was coated with a monolayer of flattened platelets and fibrin, which apparently conferred an antithrombotic effect lasting beyond the period of anticoagulation.17 The significance of immediate thrombosis after endarterectomy in humans has been documented angiographically21-22 and clinically23 and has led to the recommendation that intraoperative anticoagulation should not be reversed with protamine.13,24 Animal models of microvascular anastomosis suggest that a similar period of increased thrombogenicity occurs in the immediate postoperative period,25 although the clinical significance of this observation is unknown.

Heparin compounds are sulfated glycosaminoglycans that inhibit coagulation at several stages in the clotting sequence.4 The most prominent anticoagulant effect is related to potentiation of antithrombin III,26 and heparin has antiplatelet effects at high doses.4,26 Heparin can be administered subcutaneously, intramuscularly, or intravenously, although the latter route provides the most consistent systemic anticoagulation.4,27 Intravenous or subcutaneous administration of heparin with careful monitoring of coagulation parameters has been shown to be effective for the prevention of deep-vein thrombosis or for the prophylaxis of pulmonary embolism,26 although heparin's merit in other thromboembolic disorders has not been proven. Although low-dose heparin therapy28 and low-molecular-weight heparin29 may provide prophylaxis against venous thrombosis, full systemic anticoagulation is required to prevent propagation or dissemination of existing intravascular thrombi.4,7 Variability in pro-

FIGURE 5. Histogram, comparison of mean±SD cross-sectional area for intraluminal thrombus, vessel wall, and lumen in control (shaded bars) and heparin/polyvinyl alcohol–treated (filled bars) rat common carotid arteries after endothelial damage and 1-hour occlusion. There is significant reduction in thrombus cross-sectional area for treated vessels, but no change in area of vessel wall or lumen.
tein binding, half-life, distribution, and coagulation testing procedures complicate the accurate administration of heparin, which must be undertaken in the hospital setting. Optimal anticoagulation is frequently difficult to achieve and often requires continuous infusion.

Hemorrhage is the primary complication associated with heparin anticoagulation although thrombocytopenia, hypersensitivity reactions, lipolysis, and rebound hypercoagulability occur less frequently. Hemorrhage during heparin therapy is directly related to the degree of anticoagulation and the duration of therapy. Recent surgery, thrombocytopenia, peptic ulcers, uremia, and concurrent antiplatelet agents all increase the risk of hemorrhage during systemic anticoagulation with heparin and represent relative contraindications to its use. From 5% to 33% of patients receiving full heparin anticoagulation suffer hemorrhagic complications, many of which are fatal or disabling.

Biodegradable polymers are ideally suited for the continuous, localized application of drugs. A number of such drug delivery systems have been developed, with zero-order release kinetics ranging from days to months. PVA is a water-soluble, nontoxic polysaccharide that has been used in humans for embolization procedures and volume expansion. The physical characteristics of PVA can be modified by liquid to solid depending on concentration. PVA release kinetics for large molecules are linear over 24–48 hours, during which time >80% of incorporated material is released. Using an application technique similar to that we employed, the diffusion of a neuronal tracer (horseradish peroxidase) was limited to <10 mm from the site of application in feline cerebral arteries. These observations suggest that PVA and other polymeric drug delivery vehicles may be useful for the sustained application of substances to a localized region.

In our experiment, the local application of heparin to the rat common carotid artery adventitia significantly reduced thrombus formation after endothe
tial injury and temporary occlusion of the vessel. The anticoagulant effect of heparin was limited to the site of application through the use of a continuous-release polymeric drug delivery system as demonstrated by normal systemic coagulation parameters during drug administration. Although highly protein-bound in the circulation, heparin is readily absorbed from the extravascular space, and presumably the drug released from PVA at the adventitia permeated the vessel wall from the outside. In this manner, locally high concentrations of heparin were achieved without significant systemic distribution, preventing thrombosis in the treated artery. The significance of this observation is severalfold. Selective local antithrombosis without systemic anticoagulation may be applicable to a number of surgical settings, including endarterectomy, large-vessel and microvascular anastomosis, cerebral and systemic venous procedures, arteriovenous shunts, and free-flap plastic surgery. Non-surgical applications may include deep-vein thrombosis, cardiac valvular disease, and inoperable arterial stenoses. Systemic heparin administration reduces myointimal proliferation after endo
thelial injury, and polymer delivery systems may be modified to provide long-term local release in the setting of atherosclerosis or the prevention of recurrent stenosis after endarterectomy. Finally, the concept of localized drug delivery using polymers may be expanded to a wide range of applications with other drugs and other tissue in both experimental and clinical settings.

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