Heparin Adheres to the Damaged Arterial Wall and Inhibits Its Thrombogenicity

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Heparin binds to thrombogenic extracellular matrices as well as to smooth muscle cells of the vascular wall in vitro. The inhibitory effects of heparin on thrombogenicity of the damaged arterial wall were examined in vivo using small mesenteric arteries of rats and a video recording system attached to a microscope. To induce thrombosis, we damaged the vessel wall over a short segment by compression and exposed the media to the blood stream. A platelet-rich thrombus enlarged gradually at the damaged site, occluded the vascular lumen for a short period, and then flowed away. Compression damage induced such thrombus formation several times. Heparin (500 units/ml) was given in three different ways: intravenous and intra-arterial administration (both 300 units/kg) and intraluminal application under stopped-flow conditions (<0.01 ml) for 1–2 minutes with subsequent draining out. Intravenous heparin significantly decreased both the total duration and the number of thrombotic occlusions, whereas intra-arterial heparin abolished thrombotic occlusion. Both routes of heparin administration similarly prolonged the blood coagulation time. Intraluminal application of heparin significantly inhibited subsequent thrombus formation after restoring the flow without changes in the blood coagulation time. After an intra-arterial administration or intraluminal application of fluorescein isothiocyanate–bound heparin, strong fluorescence was observed only at the damaged vascular segment. A heparin fraction with low affinity to antithrombin III or chondroitin sulfate A did not inhibit thrombosis. To clarify anticoagulant activity of vascular wall–bound heparin, damaged carotid arterial segments of rats were incubated (inside out) in platelet-poor plasma with thrombin, and fibrin clot formation around the segments with or without heparin binding was measured. Heparin-bound segments inhibited clot formation. We suggest that heparin inhibits thrombus formation on the damaged arterial wall in vivo not through its anticoagulant action on circulating blood but by its vascular binding and inactivation of the thrombogenic site, for which local inhibition of thrombin activity may be important. (Circulation Research 1992;71:577–584)

KEY WORDS: thrombosis • vascular damage • heparin • thrombogenic site

Arterial thrombosis is a major cause of myocardial and cerebral infarction. Considerable effort has been made to prevent thrombosis by inhibiting either platelet adhesion/aggregation or the blood coagulation system. However, we cannot prevent occlusive thrombus formation effectively in patients by these means. In most patients with myocardial or cerebral infarction, thrombotic occlusion of the artery is not due to changes in platelet aggregability or blood coagulability but is related to vascular wall properties interfacing with the flowing blood, such as rupture of atheromatomous plaques.1–3 To prevent this type of thrombosis, treatment of the thrombogenic site itself appears to be the method of choice.

Heparin inhibits the blood coagulation system by accelerating the action of antithrombin III (ATIII) and has long been used as an anticoagulant drug in clinical practice. In experimental thrombosis models, heparin inhibits thrombus formation on the damaged arterial wall when given intravenously in doses higher than that needed to inhibit the blood coagulation system.4,5 Heparin seems to inhibit arterial thrombosis more effectively when given directly into the arterial thrombogenic site such as in everted (i.e., inside out) femoral arterial segments6 or in mesenteric arterial segments damaged by compression (authors' unpublished observation). These results suggest that inhibition of the circulating blood coagulation system is not the major mechanism of the antithrombotic action of heparin in these models.

Heparin binds to various thrombogenic extracellular matrices of the vascular wall7,8 as well as to vascular smooth muscle cells9 in vitro. Heparin or a heparin-like substance, such as heparan sulfate bound to the surface of artificial biomaterials10,11 or to the endothelium,12 plays an important role in keeping the surface nonthrombogenic. We hypothesized that the heparin-induced inhibition of thrombus formation on the exposed subendothelial tissues was not derived from its systemic anticoagulant effect but rather from its local effect at the thrombogenic site. Therefore, we examined this hypothesis using our arterial thrombosis model of rats in vivo. We also examined whether such a local effect of heparin was derived from its antithrombin action or was due to its negative charge13 by applying a heparin fraction having low affinity to ATIII or chondroitin sulfate A, which had a strong negative charge but no antithrombin effects.14,15

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FIGURE 1. Schematic illustration of experimental model showing the method of application of drugs directly to the damaged arterial segment. Panel A: Intra-arterial administration of drugs was performed through a cannula inserted into the upstream side branch. Panel B: After stopping the blood flow by gently compressing the downstream site, drugs were applied intraluminally through a cannula inserted into the downstream side branch. Compression damage was done just after the application. Drugs were allowed contact with the damaged segment for 1–2 minutes and then drained out through the cannula.

Materials and Methods

Animal Models

Eighty-five male Wistar rats weighing 230–270 g were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg). The right jugular vein was cannulated with polyethylene tubing for the administration of additional pentobarbital to maintain a surgical plane of anesthesia as well as for drug administration. After making a small midabdominal incision, a part of the ileum was exteriorized and spread over the transparent warm (37°C) stage. Small mesenteric arteries (=300 µm i.d.) were dissected free from adherent fat and connective tissues under a dissecting microscope. In some animals, we cannulated a side branch of the mesenteric artery with polyethylene tubing either proximal or distal to the observation site for the intra-arterial administration of drugs (Figure 1). The preparation was mounted on a biological triocular microscope (Optiphot, Nikon, Tokyo) attached to a color video recording system (model HR-D75, Victor, Tokyo) or, in some cases, to a camera (model M53S, Nikon). The surface of the mesentery was superfused continuously with warm Tyrode’s solution at a rate of 1 ml/min. Magnification with the microscope was ×40 or ×100.

To induce thrombosis, we damaged a short segment of the artery by compression using a heat-blunted glass micropipette attached to a micromanipulator and exposed the damaged media or, in some cases, the adventitia (for deep vessel injury) as previously reported. A thrombus mainly consisting of platelets gradually enlarged at the damaged site and occluded the lumen completely. However, the thrombus spontaneously broke away within a few minutes. Such a thrombus formation was observed several times after compression damage for approximately 20–30 minutes. The duration of each thrombotic occlusion shortened gradually after compression damage; later, mural thrombus formation that did not enlarge enough to occlude the lumen completely was observed several times. Compression damage of the same segment was induced twice in a 30-minute interval, and the total duration and number of thrombotic luminal occlusions were calculated for the 30 minutes after each instance of compression damage. Reproducibility of thrombus formation after two successive instances of compression damage with a 30-minute interval was examined without any interventions in seven rats.

Drug Administration

Drugs used were heparin sodium salt (from porcine intestinal mucosa, Novo-Kodama, Tokyo, or Sigma Chemical Co., St. Louis, Mo.), chondroitin sulfate A (Sigma), thrombin (Mochida, Tokyo), and a heparin fraction having a low affinity to ATIII (low ATIII affinity heparin). Low ATIII affinity heparin was prepared by affinity chromatography using a Sepharose-antithrombin III column and had activity of 29 units/mg (activity of unfractionated heparin, 170 units/mg). Unfractionated heparin was diluted or dissolved in Tyrode’s solution at a concentration of 500 units/ml. Low ATIII affinity heparin and chondroitin sulfate A were dissolved in phosphate buffer and Tyrode’s solution at a concentration of 2.9 mg/ml for both, respectively. We used benzyl alcohol diluted in Tyrode’s solution (5 mg/ml, Wako, Osaka, Japan) as a vehicle for heparin (Novo-Kodama). Heparin was given in three different ways: 1) intravenous administration (300 units/kg) over 1 minute starting just after compression damage (n=7), 2) intra-arterial administration (300 units/kg) over 1 minute in divided bolus doses starting just after compression damage through the cannulated side branch upstream from the damaged segment (n=7, Figure 1A), and 3) intra-arterial application under stopped flow for 1–2 minutes (500 units/ml, <0.01 ml) with subsequent draining out; heparin was applied just before compression damage through a cannula inserted into the downstream side branch as shown in Figure 1B (n=7). Low ATIII affinity heparin (n=7) or chondroitin sulfate A (n=7) was administered intra-arterially at a dose of 1.76 mg/kg, which was equivalent to 300 units/kg unfractionated heparin on a weight basis. We administered all vehicles first and drugs second, and each rat received only one drug.

Heparin Binding and Histological Studies

We used heparin bound with fluorescein isothiocyanate (FITC) as a probe. To observe fluorescence we used a triocular biological microscope equipped with epifluorescent illumination (model BH-2, Olympus, Tokyo) and attached to a camera (model M53S, Nikon). FITC heparin (500 units/ml) with or without benzyl alcohol was administered intra-arterially (n=7) or intravenously (n=5) at a dose of 300 units/kg or applied intraluminally for 1 minute and drained out as shown in Figure 1 (n=4). Fluorescence on the wall of the mesenteric artery or on the thrombus was observed both in vivo and in vitro. For in vitro observation of fluorescence, mesenteric arteries were washed with 0.25 M sucrose solution, fixed with 70% methanol solution under a perfusion pressure of 80 mm Hg, cut out, and photographed directly with a cover glass on it. Conven-
tional tissue fixation, embedding, and sectioning caused loss of fluorescence.

For histological examination of the thrombus and vascular wall, mesenteric arteries were fixed with 10% formalin, and the transverse sections were subjected to hematoxylin and eosin staining.

**Measurement of Activated Blood Coagulation Time**

At the end of most experiments, we sampled blood by puncture of the inferior vena cava with a plastic syringe and 21-gauge needle and immediately measured the activated blood coagulation time (ACTest, Kendall McGaw Laboratories, Sabana Grande, Puerto Rico). We did not draw blood during the experiment to prevent its possible effects on thrombosis. We used different animals to measure the activated blood coagulation time at control (n=10).

**Anticoagulant Activity of Vascular Wall–Bound Heparin In Vitro**

In 13 rats, both carotid arteries of approximately 1.0–1.2 cm in length were cut out and everted (turned inside out). The intimal surface of both arterial segments was rubbed and compressed repeatedly with the edge of a spatula. This exposed the damaged media at the surface of the arterial preparation. One end of the segment was tied with nylon thread to ease its handling, and the total weight was measured after gentle blotting. The arterial segment was incubated either in 500 units/ml heparin solution (Novo-Kodama) or in its vehicle for 2 minutes at 37°C. We randomly assigned two segments from each rat to either solution. In six experiments, incubated segments were washed five times in different bottles containing 20 ml Tyrode’s solution each for a total of 10 minutes at room temperature, and in another five experiments, segments were washed 10 times for a total of 30 minutes. Platelet-poor plasma was obtained by centrifuging the autologous citrated blood at 2,000g for 20 minutes. Washed vascular segments were immersed in 0.2 ml platelet-poor plasma and incubated for 5 minutes at 37°C just after the addition of 0.02 ml of 10 units/ml thrombin solution. Clot formation around the arterial segments was examined by gently pulling up the nylon thread attached to the segment and weighing the preparation after gentle blotting. In another two rats, excised carotid arteries were subjected to the same procedure as stated above (incubation in 500 units/ml heparin solution for 2 minutes and subsequent washing five times for a total of 10 minutes) and then incubated for 5 minutes at 37°C in Tyrode’s solution instead of platelet-poor plasma. The concentration of heparin released during the incubation into Tyrode’s solution was determined by measuring anti-Xa activities in the presence of ATIII using a chromogenic substrate S-2222 (Kabi Diagnostica, Stockholm). We also examined the binding of heparin with the damaged carotid arteries using FITC heparin as stated above.

**Statistical Analysis**

We used paired t test analysis to compare the thrombosis data between vehicle and drug groups. Comparison of blood coagulation times among six experimental groups was done using analysis of variance followed by modified t test analysis using the Bonferroni correction. All values are expressed as mean±SEM.

**Results**

The total duration and the number of thrombotic occlusions did not change for two successive inductions of compression damage in the absence of any intervention: the total duration was 372±73 (mean±SEM) seconds after the first instance of compression damage versus 374±72 seconds after the second (n=7, p=NS), and the number of occlusions was 5.5±0.8 versus 6.1±0.8 (n=7, p=NS), respectively.

An intravenous administration of heparin (Novo-Kodama, 300 units/kg) had a significant inhibitory effect on thrombus formation as evidenced by a significant decrease in the total duration (from 403±21 seconds after vehicle to 230±51 seconds after heparin, n=7, p<0.01) and the number of thrombotic occlusions (5.9±0.3 after vehicle versus 3.3±0.9 after heparin, n=7, p<0.01, Figure 2). The same dose of heparin, however, disaggregated and preventing the developing thrombus completely when given intra-arterially through a cannula inserted into an upstream side branch (the total duration of thrombotic occlusions was 401±48 seconds after vehicle versus 0±0 seconds after heparin, n=7, p<0.01). The inhibition of thrombus formation by intra-arterial heparin lasted for the entire observation period of 60–90 minutes. After intra-arterial heparin administration, no discernible mural thrombus formation was observed, and most of the damaged wall seemed free of thrombus on observations in vivo. Histological study revealed a scattered
adhesion of platelets on most of the damaged segment (Figure 3). Small residual thrombi, which had been observed in vivo before intra-arterial heparin administration, are also seen in Figure 3. The blood coagulation time was markedly prolonged by these two routes of heparin administration to more than 180 seconds (Table 1).

The heparin preparation (Novo-Kodama) we used contained benzyl alcohol as a preservative. We found that intra-arterial benzyl alcohol disaggregated the developing thrombus and exposed the damaged vascular wall to the flowing blood, although thrombi reoccurred soon after the termination of its application. A heparin preparation that did not contain benzyl alcohol (porcine intestinal mucosa, Sigma) also inhibited thrombus formation significantly (the total duration of occlusions, from 446±163 seconds with vehicle to 16±19 seconds with heparin; n=4, p<0.01) and abolished thrombotic occlusion in three of four rats. However, it was unable to disaggregate the enlarging thrombus effectively, and nonocclusive thrombus formation (i.e., the formation of mural thrombi) was observed several times.

In preliminary studies, we found that a rapid intra-arterial injection of heparin in a single bolus did not inhibit thrombus formation effectively and thus administered it in divided doses over approximately 1 minute in this study. Initial doses of heparin (Novo) disaggregated the thrombus and exposed the damaged vascular wall to the blood stream. A subsequent application of heparin came in direct contact with the damaged vascular wall; thereafter, no thrombus formation was observed on the wall. For the effective inhibition of thrombus formation, this contact of heparin with the damaged vascular wall seemed very important.

Figure 4 shows the effects of heparin applied intra-arterially on the total duration and the number of thrombotic occlusions. This intra-arterial application significantly reduced the total duration of thrombotic occlusions from 401±56 to 51±27 seconds (n=7, p<0.01) without effects on blood coagulation time (Table 1). The number of thrombotic occlusions also decreased significantly after this intervention (from 6.3±1.3 at control to 1.3±0.7 after heparin, n=7, p<0.01). In two rats, thrombus formation was observed only approximately 20 minutes after compression damage and heparin application, suggesting waning of the inhibitory effects of heparin at this time.

An intra-arterial bolus injection of FITC heparin opacified the vascular lumen for several seconds. After the luminal fluorescence had disappeared, clear fluorescence remained only at the damaged segment. The intensity of fluorescence seemed to increase as additional FITC heparin was given as a bolus (Figure 5C). A part of the damaged segment covered with enlarging thrombus did not show any fluorescence unless the thrombus was disaggregated and the vascular wall was directly exposed to FITC heparin. Weak fluorescence was observed on the surface of the enlarging thrombus. The damaged segment showed strong fluorescence after the intra-arterial application and removal of FITC

### Table 1. Effects of Heparin, Heparin Fraction With Low Affinity to Antithrombin III, and Chondroitin Sulfate A on the Activated Blood Coagulation Time

<table>
<thead>
<tr>
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<th>Control (300 units/kg)</th>
<th>Intravenous heparin (300 units/kg)</th>
<th>Intra-arterial heparin (500 units/ml)</th>
<th>Intra-arterial low ATIII affinity heparin (1.76 mg/kg)</th>
<th>Intra-arterial chondroitin sulfate A (1.76 mg/kg)</th>
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<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>7</td>
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<tr>
<td>Activated coagulation time (seconds)</td>
<td>53±1</td>
<td>&gt;180*</td>
<td>&gt;180*</td>
<td>56±3</td>
<td>74±5†</td>
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Intraluminal, intraluminal application and subsequent draining out of drug; low ATIII affinity heparin, heparin fraction with low affinity to antithrombin III; n, number of rats. Values are mean±SEM. See text and Figure 1 for details.

*p<0.001 and †p<0.005 vs. control.
heparin as well. The fluorescence was observed for more than 30 minutes after both routes of heparin application. After intravenous administration of FITC heparin, only weak fluorescence appeared gradually at the damaged segment. Enlarging thrombi again prevented fluorescence accumulation at the vascular wall.

At the periphery of the damaged segment where smooth muscle cells were not damaged extensively, strong fluorescence was observed along the nuclei of circular smooth muscle cells within approximately 15 minutes after the intra-arterial administration of FITC heparin (Figures 5D and 5E), suggesting the uptake of FITC heparin by these cells.

Effects of intra-arterial administration of low ATIII affinity heparin and chondroitin sulfate A on thrombus formation are shown in Figure 6. Low ATIII affinity heparin only slightly decreased the total duration of thrombotic occlusions (from 352±27 seconds with vehicle to 231±54 seconds with low ATIII affinity heparin, n=7), but no significant difference from vehicle was observed. Chondroitin sulfate A had no effects on thrombosis. Activated blood coagulation time was prolonged slightly by low ATIII affinity heparin but did not change after the administration of chondroitin sulfate A (Table 1).

Incubation of the damaged carotid arterial segment in vitro in platelet-poor plasma with thrombin caused fibrin clot formation around the arterial segment as shown in Figures 7A and 7C. Preincubation of the segment in heparin solution prevented clot formation even after the repeated washing (five times for a total of 10 minutes) in Tyrode’s solution (Figures 7B and 7D). The damaged segments preincubated in vehicle had significantly larger clot formation (wet weight of clot, 77±9.8 mg; n=6) than did those segments preincubated in heparin solution (0.6±0.2 mg, n=6, p<0.01). Binding of heparin with the damaged carotid arterial segment was confirmed by observing strong fluorescence at the surface after incubation in FITC heparin solution and subsequent washing. The concentration of heparin released into the medium during the incubation was under the detectable range of less than 0.1 unit/ml (n=4). Such inhibition of clot formation was still observed after the heparin-treated segment was washed more thoroughly, 10 times for a total of 30 minutes (clot weight, 79±9.7 mg for vehicle versus 1.6±1.5 mg for heparin; n=5, p<0.01).
In our thrombosis model, thrombus developed not on an artificial surface (i.e., copper coil or glass micropipette), which has previously been used in arterial thrombosis models, but on the damaged arterial wall itself and was composed primarily of aggregating platelets. These properties of thrombus formation may mimic those in patients with myocardial infarction. Because the vascular wall was thin, we could precisely observe thrombus formation and subsequent breakdown through the vascular wall. Even the small mural thrombus was discernible. Without any interventions, the thrombus formation among two successive compressions was reproducible in both the total duration and the number of thrombotic occlusions. Thus, our thrombosis model seems reproducible and suitable to evaluate the effects of antithrombotic agents. Furthermore, we could examine the direct effects of drugs on the thrombogenicity of the vascular wall by applying drugs locally through intra-arterial cannulas.

Intravenous administration of heparin significantly inhibited thrombus formation after vascular damage, confirming previous reports. Direct intra-arterial administration of the same dose of heparin, however, was much more effective in inhibiting thrombosis and abolished any discernible mural thrombus formation on the damaged vascular wall, despite similar prolongation of blood coagulation times after intravenous and intra-arterial administration of heparin. These results suggest that the inhibition of the circulating blood coagulation is not solely responsible for the inhibition of thrombosis by heparin. Intra-arterial heparin inhibited thrombus formation more effectively when it was given after disaggregation of the thrombus and exposure of the damaged vascular wall to the blood stream and thus to heparin. Contact of the damaged vascular wall with heparin at a high concentration may have an important role in the antithrombotic process. An intravenous administration of heparin at very high doses (2,000–3,000 units/kg).

**FIGURE 6.** Bar graphs showing effects of intra-arterial administration of heparin fraction having low affinity to antithrombin III (low ATIII affinity heparin) and chondroitin sulfate A on thrombus formation. Date are mean±SEM for seven experiments. NS, not significant.

**Discussion**

The present study has clearly demonstrated that intra-arterial administration of heparin inhibits thrombotic luminal occlusion induced by vascular damage more efficiently than does intravenous administration of heparin, although the blood coagulation time was equally prolonged. An intraluminal application and subsequent removal of heparin also inhibited thrombus formation without effects on the blood coagulation time. Furthermore, we demonstrated that heparin adhered exclusively to the damaged vascular segment.

**FIGURE 7.** Photographs and photomicrographs (hematoxylin and eosin staining) showing clot formation around a damaged carotid arterial segment and its inhibition induced by preincubation in heparin solution. Everted arterial segments were preincubated either in vehicle (panels A and C) or in heparin solution (panels B and D), washed repeatedly in Tyrode's solution, and then incubated in platelet-poor plasma with thrombin; see text for detail. Note that no fibrin clot is seen around the arterial segment preincubated in the heparin solution. Horizontal bars, 1 mm in panel B and 100 μm in panel D.
also abolished any thrombus formation (data not shown).

To prevent the systemic anticoagulant effects of heparin and to examine its direct vascular effects, we applied heparin directly to the damaged vascular segment and subsequently removed the heparin. This significantly inhibited the thrombus formation without influencing coagulation time of the circulating blood. These findings suggest that heparin might adhere to the damaged vascular wall and thereby inhibit its thrombogenicity.

We examined the adherence of heparin to the vascular wall by using FITC heparin as a probe. After intra-arterial administration of FITC heparin, fluorescence was observed only at the damaged segment. The surface of the small organized thrombus also had some fluorescence. These results strongly suggest that heparin adheres exclusively to the exposed subendothelial tissues, although the precise mode and site of heparin binding is unclear in the present study. In vitro studies have already demonstrated the binding of heparin to various thrombogenic extracellular matrices and smooth muscle cells. The damaged vascular wall-bound heparin may inhibit thrombosis effectively by its potent antithrombin effects as well as by its strong negative charge, as does heparan sulfate on the surface of the endothelium and covalent-bound heparin on artificial biomaterials.

We found that low ATIII affinity heparin did not inhibit thrombosis as did unfractionated heparin, although both preparations had a similar negative charge. Chondroitin sulfate A, which had a strong negative charge and binding capacity with positively charged subendothelial tissues but no antithrombin effects, also did not inhibit thrombosis. These results indicate that the negative charge of heparin itself may not play an important role in inhibiting thrombus formation in the present study. Instead, inhibition of thrombin activity by heparin at the thrombogenic site may play a key role as already reported in studies with various arterial thrombosis models.

The inhibition of thrombin activity by surface-bound heparin was confirmed in vitro by demonstrating an inhibition of thrombin-induced clot formation around the damaged media of the carotid arterial segments incubated in platelet-poor plasma. The inhibitory effect was observed even after vigorous washing of the arterial segment, which was done to minimize the possible inhibitory effect of heparin released into platelet-poor plasma during incubation. The concentration of heparin released into the incubation medium was below the detectable range. However, we cannot exclude the possibility that very small amounts of heparin released continuously from the vessel prevent clot formation. Even if this is the case, we may still be able to conclude that vascular bound heparin remains active at least up to 30 minutes after its binding in vitro.

Apart from inhibiting thrombin-mediated platelet activation, heparin has several inhibitory effects on platelet–vascular wall interaction. In small arteries with a high shear rate, the von Willebrand factor plays an important role in platelet adhesion to subendothelial tissues. The von Willebrand factor is reported to have a heparin-binding domain, and heparin inhibits binding of the von Willebrand factor to platelets. These results suggest that heparin bound to the damaged vascular wall inhibits thrombus formation through an interaction with the von Willebrand factor. Heparin is also reported to inhibit adhesion of platelets to collagen, a major thrombogenic substance exposed after deep vessel injury. Further studies will be needed to explore the precise mechanism of the inhibition of thrombosis induced by heparin bound to the damaged vascular wall.

The damaged vascular wall is known to become nonthrombogenic during the natural time course after vascular damage. The nonthrombogenic damaged wall is not covered with either regenerated endothelial cells or with platelets, and changes in the property of the damaged vascular wall themselves are suggested to play roles in this lack of thrombogenicity. In our thrombosis model, the damaged arterial segment lost its thrombogenicity gradually over 30–60 minutes even without the administration of heparin. It will be interesting to examine whether endogenous heparin or heparin-like substances such as heparan sulfate play an important role in the natural inactivation of the thrombogenic site of the damaged vascular wall and whether exogenous heparin acts as a substitute for endogenous heparin in accelerating the inactivation process.

Within 15 minutes after the intra-arterial administration of FITC heparin, a strong fluorescence was demonstrated along the nuclei of smooth muscle cells. This indicates the uptake of heparin by the smooth muscle cells, as already reported in in vitro studies. The uptake of heparin by smooth muscle cells may prevent their proliferation and subsequent luminal narrowing after vascular damage.

This is the first report demonstrating that the properties of the thrombogenic site itself can be modified with a drug that adheres to the injury site in vivo. Such modification of the thrombogenic site itself may be a promising approach to prevent arterial thromboses such as myocardial and cerebral infarctions. Prevention of thrombosis and restenosis after vascular damage caused by balloon angioplasty is of great clinical importance.

Coronary restenosis after angioplasty will be an interesting application for this new antithrombotic therapy, because we can administer heparin directly to the damaged vascular wall through an intra-arterial catheter during angioplasty. Heparin treatment may not only prevent thrombotic luminal narrowing soon after the angioplasty, but it may also prevent the proliferation of vascular smooth muscle cells and subsequent long-term restenosis.

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