Local Delivery of r-Hirudin by a Double-Balloon Perfusion Catheter Prevents Mural Thrombosis and Minimizes Platelet Deposition After Angioplasty

Beat J. Meyer, MD; Antonio Fernández-Ortiz, MD; Alessandra Mailhac, MD; Erling Falk, MD; Lina Badimon, PhD; Anthony Don Michael, MD; James H. Chesebro, MD; Valentin Fuster, MD, PhD; Juan J. Badimon, PhD

Background. The major morbidity of percutaneous transluminal coronary angioplasty is acute thrombosis and restenosis of the dilated lesion. Platelet-thrombus deposition occurs within minutes after injury, is primarily mediated by thrombin, causes acute occlusion, and contributes to late restenosis. Experimentally, specific thrombin inhibitors have prevented mural thrombosis. However, local therapy may be more effective than systemic treatment. We tested the hypothesis that high local concentrations of an antithrombin drug at the site of arterial injury following balloon angioplasty inhibit platelet thrombus formation equally or better than conventional systemic treatment and at lower systemic anticoagulant levels.

Methods and Results. Balloon angioplasty of the carotid arteries of 29 pigs was performed using systemic intravenous treatment with heparin (100 U/kg, groups I and II), suboptimal r-hirudin (0.3 mg/kg, group III), and higher-dose r-hirudin (0.7 mg/kg, group IV), which is the lowest dose that completely inhibited arterial thrombosis in the pig. Immediately after balloon angioplasty of the first carotid, additional local therapy with placebo (group I) or r-hirudin (groups II, III, and IV; 0.3 mg/kg in 1 mL) was administered with distal perfusion through a new percutaneous double-balloon catheter. After 1 hour of local drug delivery, angioplasty of the contralateral carotid was performed. Reflow for 1 hour was permitted to both carotids to compare the short-term effect of local plus systemic treatment with systemic treatment on quantitative 111In-labeled platelet deposition and macroscopic mural thrombus formation on deeply injured carotid segments. Local drug delivery of placebo compared with systemic heparin treatment resulted in no change of platelet deposition (×104/cm², mean±SEM) in controls (group I, 91.0±23.5 versus 80.8±19.4), but local delivery of r-hirudin resulted in a significant reduction in group II (15.2±5 versus 71.3±14.5; P<.02) and group III (11.4±2.5 versus 80.5±11.4; P<.01) and was borderline in group IV (7.4±1.8 versus 14.1±7.4; P=.05), respectively. The incidence of macroscopic mural thrombus formation with local and systemic treatment was 86% and 75% in group I, 16% and 70% in group II, 14% and 71% in group III, and 0% and 16% in group IV, respectively.

Conclusions. Local therapy with the specific thrombin inhibitor r-hirudin significantly reduces short-term quantitative platelet deposition and macroscopic mural thrombus formation following balloon angioplasty compared with systemic treatment of conventional doses of heparin and hirudin and requires a significantly smaller amount of the recombinant drug. (Circulation. 1994;90:2474-2480.)

Keywords: angioplasty • r-hirudin • thrombosis • catheters • platelets

Sequelaes of coronary balloon angioplasty are acute thrombosis in 3% to 12% of patients and restenosis in 25% to 40% of patients.1,2 Balloon angioplasty disrupts the arterial wall with injury into the atherosclerotic plaque or media that releases tissue thromboplastin (tissue factor) and exposes highly thrombogenic deep arterial structures (smooth muscle cells [SMC], collagen types I and III) to flowing blood.3 Deep arterial injury activates the coagulation system with generation of large amounts of thrombin via the formation of the prothrombinase complex (factors II, Va, and Xa; Ca²⁺—all assembled on a phospholipid membrane such as platelets or SMC). Thrombin is a potent stimulus for platelet activation and results in a positive feedback for activation of factors V and VIII, conversion of fibrinogen to fibrin, and cross-linking of fibrin by activation of factor XIII.4 Heparin is a coenzyme of circulating antithrombin III (ATIII) and greatly accelerates the interaction of ATIII with thrombin but at the conventional dose does not completely prevent mural thrombus formation.5 Specific inhibition of thrombin in vivo eliminates macroscopic mural thrombus formation and limits platelet deposition to a single layer or less.6

Thrombin also contributes to SMC proliferation by stimulating platelet secretion of growth factors (especially platelet-derived growth factor [PDGF]) and directly acting on SMC. Thus, specific thrombin inhibition may be important for preventing restenosis.7 Failure of conventional pharmacological therapies to prevent thrombus and restenosis following coronary angioplasty in patients has led to the evaluation of local

Received April 27, 1994; revision accepted June 27, 1994.
From the Cardiovascular Biology Research Laboratory, Cardiac Unit, Massachusetts General Hospital, Boston, Mass.
*Dr Don Michael developed the double-balloon perfusion catheter discussed in this article and is a consultant with the Cordis Corp.
Correspondence to Juan J. Badimon, PhD, Cardiovascular Biology Research, Cardiovascular Institute, PO Box 1030, Mount Sinai School of Medicine, Annenberg Bldg, Rm 24-201, One Gustave L. Levy Place, New York, NY 10029.
© 1994 American Heart Association, Inc.
delivery of higher concentrations of drugs. Several devices are under investigation. High-pressure balloons have created arterial injury and pressure-related dissections. Therefore, we evaluated a double-balloon catheter that creates a protected space at the injury site and an autoperfusion lumen that allows prolonged balloon inflation and prolonged drug administration.

We hypothesized that local delivery of the specific antithrombin recombinant (r)-hirudin would reduce quantitative \(^{111}\text{In}\)-labeled platelet deposition and eliminate macroscopic mural thrombosis after deep arterial injury by carotid angioplasty in the pig. We evaluated locally administered, highly concentrated r-hirudin compared with three different systemic dosages of heparin and hirudin.

Methods

Experimental Design

Autologous platelets in 29 normal Yorkshire pigs (weight, 25 to 35 kg) were labeled with 296±22 μCi of \(^{111}\text{In}\)-tropolone. The animals were randomized into four groups: group I was the control group for local saline delivery, and groups II, III, and IV received local r-hirudin. Before balloon angioplasty and throughout the study period, animals received three different regimens of systemic anticoagulation (Fig 1). Groups 1 (n=5) and II (n=8) received systemic heparin (100 U/kg bolus plus 100 U·kg\(^{-1}\)·h\(^{-1}\) infusion). Group 3 (n=8) received low-dose hirudin (0.3 mg/kg bolus plus 0.3 mg·kg\(^{-1}\)·h\(^{-1}\) infusion). Group 4 (n=8) received the lowest effective systemic dose of r-hirudin (0.7 mg/kg bolus plus 0.7 mg·kg\(^{-1}\)·h\(^{-1}\) infusion). The level of systemic anticoagulation was chosen according to the current clinical use with activated partial thromboplastin time (aPTT) ratios of 1.5 to 2.5 for heparin (groups I and II). Suboptimal antithrombotic doses of hirudin provide aPTT ratios of 1.5 to 2.0 (group III). The higher dose of hirudin (aPTT >2.0, group IV) has effectively prevented macroscopic mural thrombosis and was selected as a standard for systemic antithrombotic drug efficacy in this model of carotid angioplasty. A continuous infusion of r-hirudin at a dosage of 0.3 and 0.7 mg·kg\(^{-1}\)·h\(^{-1}\) previously resulted in average plasma levels of 0.3 and 0.6 μg/mL, respectively.

After systemic anticoagulation, balloon angioplasty of one carotid artery was performed, and immediately treatment with local saline (group I) or local r-hirudin (groups II, III, and IV) in a concentration of 0.3 mg/kg in 1 mL resulted in a >1000-fold local drug concentration over systemic levels. After 1 hour of local drug delivery, angioplasty of the contralateral carotid was performed. Reflow for 1 hour was permitted to both carotids to compare the short-term effect of local plus systemic treatment with systemic treatment alone on quantitative \(^{111}\text{In}\)-labeled platelet deposition and macroscopic mural thrombus formation.

Drug Delivery System

The drug delivery system used in the study was a double-balloon perfusion catheter (Cordis). The catheter has a 4.5F quadruple-lumen shaft with two distal balloons made of polyethylene terephthalate with separate inflation lumens. The balloons were 5 mm in diameter, 10 mm long, and 43 mm apart. r-Hirudin was locally administered through a central instillation port to the injury site within the protected space created by the two balloons. During balloon inflation, autoperfusion of the distal artery occurred via the fourth lumen with proximal side holes in the catheter shaft at a perfusion flow rate of 55 mL/min (in vitro studies, conducted with a glycerol solution at 37°C and a proximal perfusion pressure of 80 mm Hg).

All procedures performed in this study conformed to the National Institutes of Health and American Heart Association guidelines for animal research and were approved by the Massachusetts General Hospital Animal Care Committee.

Experimental Procedures

The animals were sedated with ketamine (20 mg/kg Ketalar; Parke-Davis) and atropine (0.6 mg/kg) administered in combination by intramuscular injection. An intravenous line was established, and each animal received intravenous sodium pentobarbital (10 mg/kg Injection C, Anpro Pharmaceutical). The pigs were intubated and mechanically ventilated with a volume ventilator (Searle). The ECG and intra-arterial blood pressure were continuously monitored throughout the procedure. Both femoral arteries were catheterized with 8F introducer sheaths. After a bolus of heparin or hirudin, the infusions were administered through the left mammary vein delivered with a Harvard pump (Harvard Apparatus) at a rate of 10 mL/h. Heparin used in this study was heparin sodium derived from porcine intestine that contained 1000 USP U/mL (Elkins-Sinn). The r-hirudin was recombinant desulfatohirudin from yeast (CGP 39393, CIBA-GEIGY).

Carotid Angioplasty and Local Drug Delivery of r-Hirudin

The model of carotid arterial injury has been previously described. A 5F Cordis polyethylene balloon angioplasty catheter (8×20 mm) was advanced via the left femoral artery under fluoroscopic guidance into the common carotid artery and placed 6 cm distal to the bifurcation by a radiopaque ruler attached to the skin (between cervical vertebrae 1 and 3). The common carotid arterial segments were dilated by five 30-second inflations to 6 to 8 atm with 60-second intervals between inflations. After the final inflation, the angioplasty equipment was removed, and the double-balloon device was positioned through an 8F multipurpose guiding catheter between radiopaque markers to cover the injured carotid segment. The proximal balloon was inflated to a pressure of 1 to 3 atm for complete occlusion. Autoperfusion was checked by

---

**Fig 1.** Timetable of carotid angioplasty and local drug delivery (LD) of r-hirudin.
contrast injections through the guiding catheter. The carotid artery distal to the occlusive balloon was flushed with saline 0.9% via instillation port of the dual-balloon catheter, and the distal balloon was inflated to occlusion (1 to 3 atm). Then, placebo (group I) or 0.3 mg/kg r-hirudin in 1 mL (groups II, III, and IV) was administered into the compartment for 1 hour. Balloon occlusion was repeatedly checked with contrast injections (diatrizoate meglumine and diatrizoate sodium; Angioist, Berlex Laboratory Inc), and spot films and video recordings were taken during balloon inflations. Pressure calibration and monitoring were performed using two digital pressure manometers (Indeflator, ACS). The autoperfusion lumen was flushed every 5 minutes with 1 mL of 0.9% saline containing 50 U heparin/mL. After 1 hour of local drug delivery with the balloon, angioplasty of the contralateral carotid artery was performed via the right femoral artery as described above. After the final inflation, the angioplasty catheter and the double-balloon perfusion catheter were removed, and local r-hirudin was released systematically. Pigs were killed after 1 hour of perfusion to measure quantitative 111In-labeled platelet deposition as well as to evaluate the depth of injury and macroscopic mural thrombus formation.

Postmortem Procedures
Fifty milliliters of 0.5% Evans blue dye in 0.9% saline was injected into a femoral artery 15 minutes before death. The pigs were given an overdose of pentobarbital and perfused antegradely with 0.01 mol/L phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS into the aorta at a pressure of 100 mm Hg for 15 minutes to allow fixation of the arteries in situ. The carotids were dissected and cleaned of all periadventitial tissue. The dilated portion of the injured carotid arteries, and the segments representing the location of the occlusive balloons were easily identified by Evans blue staining and by measurements taken during fluoroscopy and at autopsy with the carotid bifurcation as the point of reference. The dilated portion of the fixed carotid artery was divided into three 10-mm segments. From the adjacent ends, two proximal and distal segments of similar size were taken, representing the area of the occlusive balloons and proximal and distal controls, respectively. All deep arterial injury was confined to the three segments in the dilated region except for two arteries where a tear extended proximally; in these animals an extra segment was analyzed. Segments were not analyzable if there was a mechanical problem, ie, rupture of the carotid artery during angioplasty resulting in a large perivascular hematoma, or if there was a leak of one of the balloons of the local delivery catheter.

Degree of Injury and Mural Thrombus
After isotope counting, each segment was cut open with a dissecting scissors and examined for the presence of macroscopic mural thrombus using a 2× magnifier. Then, all segments were processed for histology to evaluate the degree of vessel wall injury. From each arterial segment, three cross sections were stained with hematoxylin and eosin and Masson’s trichrome stain. Light microscopy by two observers (B.J.M., A.F.O.) documented the presence of a tear or a dissection into the media at the site of injury and the absence of an injury at the site of the occlusive balloons. A tear extending through the internal elastic lamina into the media was defined as severe/deep injury; endothelial denudation (identified by Evans blue staining) without a tear through the internal elastic lamina was considered mild subendothelial injury.

Quantitation of Platelet Deposition
Platelet deposition on the arterial segments was quantitated as previously described. Three blood samples were obtained at the time of death for determination of mean blood radioactivity in counts per minute (cpm) normalized by the weight of the collected blood (microbalance) and corrected for unbound 111In. Radioactivity in each arterial segment and in blood was measured in a gamma well counter (Packard Auto-Gamma 5650) with the spectrometer adjusted to include the photo peaks at 174, 247, and 421 keV (sum peak) of the 111In radionuclide. The number of platelets deposited on each segment was calculated from the 111In activity of the segment, the specific 111In activity in whole blood, and the platelet count of whole blood with the method previously described.6,8

Laboratory Tests
Blood was drawn from the femoral artery to determine basal platelet count, hematocrit, aPTT, fibrinogen, and heparin levels. Similar testing was performed in samples taken 10 minutes after starting the infusion of heparin and hirudin, just before and after carotid angioplasty, during and after local delivery, and immediately before death (Fig 1). Platelet count and hematocrit were measured with a cell counter (System 9000, Serono-Baker Diagnostics). Blood for aPTT and heparin levels was mixed 9:1 with a 3.2% trisodium citrate solution and then centrifuged to obtain platelet-poor plasma. aPTT levels were determined using a standard technique (ST4, American Bioproducts Co). Within 4 hours of blood collection, heparin levels were determined according to the level of anti–factor Xa activity using a commercially available kit (Hepactol, American Bioproducts Co). Fibrinogen concentration was determined after an excess of thrombin was added by measuring the clotting time (Fibri-Prest, American Bioproducts Co).

Statistical Analysis
Because of the large differences in platelet deposition between deep and subendothelial injuries, separate analysis was performed for these two types of injury. All values were recorded as the mean±SEM value unless otherwise stated. Comparisons of the extent of platelet deposition were performed by using the Mann-Whitney U and Kruskal-Wallis tests. Pearson’s χ2 was used to test for differences within groups in the incidence of mural thrombus. Mean values of aPTT, aPTT ratio, hematocrit, plasma fibrinogen, and platelets before and during the procedure were compared between groups.

Results
Study Design
The number of pigs and the segments with deep and subendothelial injury in each group are shown in the Table. Separate analysis was performed for these two types of injury because of the large differences in platelet deposition between deep and subendothelial injuries, as previously reported.6,16

Four animals with rupture and total occlusion after angioplasty of the carotid artery were excluded from the analysis as well as two animals with a defective local drug delivery device, ie, proximal balloon leak as documented by loss of pressure of the manometer and by angiographic contrast filling of the local delivery compartment.

In Platelet Deposition
When the number of platelets was adjusted for the area of deep injury, platelet deposition was not different in animals treated with local delivery of saline (controls) compared with systemic treatment with 100 U/kg heparin (Fig 2A). Platelet deposition in animals treated with locally administered r-hirudin (0.3 mg/kg in 1 mL) was significantly lower than in those with systemic treatment by 100 U/kg heparin (group II) and 0.3 mg/kg r-hirudin (group III) alone. Platelet deposition after...
local delivery of r-hirudin in pigs treated systemically with high-dose r-hirudin (0.7 mg/kg, group IV) was reduced but of borderline statistical significance (P=.05). As demonstrated by a previous study, this was the lowest systemic dose of r-hirudin that completely inhibited mural thrombosis after carotid angioplasty and was in fact as effective as the highest dose of 1.0 mg·kg⁻¹·h⁻¹ r-hirudin.6 The platelet deposition in carotid segments with subendothelial injury was low and not significantly different between locally and systemically treated segments in all treatment groups (Fig 2B). Platelet deposition in subendothelial injury is always significantly less than in severe injury, as we have previously shown.6,16

Mural Thrombus

In carotid segments with deep arterial injury, there was macroscopic white mural thrombus formation with systemic treatment with an incidence of 75% (n=8) in group I, 70% (n=11) in group II, 71% (n=12) in group III, and 16% (n=6) in group IV. Compared with systemic treatment, in the locally treated segment the incidence of mural thrombus was 86% (n=7, NS) in controls, was significantly lower in group II (16%, n=6, P<.01) and in group III (14%, n=7, P<.002), and was 0% (n=6, NS) in group IV. Fig 3 shows representative histological cross sections of carotid segments with deep injury treated with systemic anticoagulation (100 U·kg⁻¹·h⁻¹ heparin) and local r-hirudin, respectively.

Local Drug Delivery Catheter

Inflation pressure was easily maintained in the proximal and distal balloon of the local delivery catheter, and frequent contrast injections through the guiding catheter documented proper sealing of the balloon, except for the two experiments excluded from the analysis in which a defect of the proximal balloon occurred within the first 5 minutes of local drug delivery.

Histological examination of the carotid segments at the site of the double-balloon inflation for local drug delivery revealed subendothelial damage at the proximal and distal balloon sites in 74% and 90%, respectively. Laceration of the internal elastic lamina was found in 26% and 10%, respectively. However, a similar incident of medial injury was found in the corresponding segments of the contralateral carotid artery not treated with the local delivery balloon catheter. This indicates proximal or distal extension of the injury during balloon angioplasty and no additional deep injury due to the double balloon.

As Fig 4 shows, no difference in mean platelet deposition was found in the carotid segments adjacent to the balloon angioplasty area, representing the location of the two balloons and the control segments of the contralateral carotid artery.

Laboratory Data

Mean aPTT levels and ratios during systemic heparin and r-hirudin infusion as well as during local drug delivery (mean±SEM) are shown in Fig 5. With systemic anticoagulation, local thrombosis was reduced, whereas local heparin was associated with increased mural thrombus formation compared with systemic anticoagulation. A similar finding was observed with systemic anticoagulation and local heparin. The combination of local heparin and systemic anticoagulation resulted in significantly higher platelet deposition compared with systemic and local anticoagulation but significantly lower than systemic anticoagulation alone (P<.05).
administration are shown in Fig 5. The aPTT levels were achieved 10 minutes after starting the heparin and r-hirudin infusions and remained stable throughout the procedure. The mean aPTT level in the 0.3 mg/kg r-hirudin–treated group was significantly lower than in the 0.7 mg/kg r-hirudin– and 100 U/kg heparin–treated groups, as was the average aPTT ratio (1.7±0.1, 2.4±0.1, 2.6±0.1, and 2.4±0.1 [control], respectively).

There was no difference in platelet count, hematocrit, and fibrinogen levels regardless of the drug dosage, and the levels remained stable throughout the experiments.

**Discussion**

This study of deep arterial injury demonstrates that adjunctive local administration of highly concentrated r-hirudin reduces platelet deposition and macroscopic mural thrombus formation compared with systemic antithrombotic therapy. Local delivery of r-hirudin is more effective than a conventional dose of heparin (aPTT ratio >2) or a dose of hirudin (aPTT ratio ≥1.7) currently used in clinical trials. Adjunctive locally deliv-
thrombi that may have formed between arterial injury after balloon angioplasty and the beginning of local hirudin treatment may have led to dissolution of mural thrombi and account for the low incidence of mural thrombi in our study.

Initial systems designed for local drug delivery achieved significant drug concentrations in the vessel wall but were associated with arterial injury related to high-inflation pressures of the balloon catheter. A recent modification of the perforated dilatation catheter introduced a microporous design, minimizing streaming of the pores and reducing but not eliminating arterial wall damage.

Successful transfer of drugs, enzymes, and viral vectors using the passive diffusion technique has been reported with double-balloon systems. However, a potential limitation of this device is a short interaction time, limited by distal ischemia induced by total arterial occlusion. The local delivery device used in this study provided prolonged interaction times between a highly concentrated drug and the injury site due to an autoperfusion system and caused no significant injury with the low-pressure and slightly oversized balloons matched for the size of the carotid artery. A recent study with a perfusion catheter with a similar flow rate showed that prolonged autoperfusion angioplasty can be performed in patients without clinical evidence of myocardial damage of hemolysis. However, an important limitation of this device concerns vessels with significant side branches where appropriate seal with the double-balloon catheter may not be achieved.

**Conclusions**

Our findings in this short-term study indicate that local delivery of r-hirudin reduces quantitative platelet deposition and mural thrombus formation in the deeply injured porcine carotid artery after balloon angioplasty. The potential advantages of local r-hirudin were demonstrated at anticoagulant levels currently used in clinical trials. It remains to be determined whether there is a true advantage of local delivery of r-hirudin compared with higher doses of systemic therapy with r-hirudin. However, the risk of hemorrhage may be reduced by avoiding generalized impairment of hemostatic function after higher doses of systemic drug administration. The double-balloon autoperfusion catheter is a promising method for delivering high concentrations of drugs directly to the site of balloon injury for prolonged interaction times.

The present findings confirm that the specific thrombin inhibitor r-hirudin is a potent inhibitor of platelet-mediated arterial thrombosis and suggest that its local delivery may be of value for the prevention of arterial thrombosis after balloon angioplasty.

**Acknowledgments**

Dr Meyer is supported by a grant for young investigators from the Swiss National Foundation of Science. The authors thank Dr A. Padurean for his excellent assistance. The authors thank Cordis Corporation for providing a grant for this study and Jim Leone for his important input in the development of the balloon catheter.

**References**


Local delivery of r-hirudin by a double-balloon perfusion catheter prevents mural thrombosis and minimizes platelet deposition after angioplasty.

B J Meyer, A Fernández-Ortiz, A Mailhac, E Falk, L Badimon, A D Michael, J H Chesebro, V Fuster and J J Badimon

_Circulation_. 1994;90:2474-2480
doi: 10.1161/01.CIR.90.5.2474
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/90/5/2474

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/