A New Approach for Local Intravascular Drug Delivery
Iontophoretic Balloon

Antonio Fernández-Ortiz, MD, PhD; Beat J. Meyer, MD; Alessandra Mailhac, MD, PhD; Erling Falk, MD; Lina Badimon, PhD; John T. Fallon, MD, PhD; Valentín Fuster, MD, PhD; James H. Chesebro, MD; Juan J. Badimon, PhD

Background Catheter-based systems are being developed to deliver drugs directly into the vessel wall. Pressure-mediated trauma and lack of homogeneous delivery are key limitations of these approaches.

Methods and Results We studied a new catheter-based delivery system that uses electrical current to force the drug into the vessel wall. The in vivo feasibility of this approach has been assessed by delivering 125I-hirudin into porcine carotid arteries. Vascular levels of hirudin after active iontophoresis (4 mA/cm², 5 minutes) were 80-fold greater than those achieved by passive diffusion (without electricity). Tissue hirudin levels declined over time; by 1 hour after delivery, 80% of the drug had left the vessel wall, and by 3 hours later, the levels of hirudin within the wall were similar to those achieved by passive diffusion. Autoradiography revealed distribution of the drug throughout the entire circumference of the arterial wall within the intima, media, and adventitia. Iontophoresis-mediated vessel wall trauma was minimal (less than 10% endothelial denudation and medial smooth muscle cell damage). Balloon injury after local delivery changed neither kinetics nor distribution of the drug into the arterial wall.

Conclusions (1) High local concentrations of hirudin in the arterial wall may be achieved with the iontophoretic balloon catheter. (2) The drug is distributed throughout the entire vessel wall without significant damage. (3) The retention of hirudin in the arterial wall is time dependent. (4) This technique might be useful to deliver therapeutic agents before or after percutaneous vascular interventions. (Circulation. 1994;89:1518-1522.)

Key Words • restenosis • catheters • angioplasty

Restenosis, which occurs in 30% to 40% of cases, continues to be the “Achilles’ heel” of percutaneous transluminal coronary angioplasty. Restenosis is a complex process of injury-induced events triggered by vessel wall damage. Mechanisms contributing to restenosis include elastic recoil, smooth muscle cell migration and proliferation, extracellular matrix synthesis, vessel wall remodeling, and, possibly, thrombus incorporation and organization. Although newly developed devices such as stents may partially prevent elastic recoil, no other therapeutic interventions have been consistently successful in reducing the overall incidence of restenosis. Pharmacologic approaches to inhibit smooth muscle cell migration and proliferation have been effectively used at supraphysiological doses in animal research studies. However, such high concentrations may be impractical for clinical use because of the risk of systemic side effects and the lack of specific targeting of drugs given systemically. Therefore, there is considerable interest in the development of drug delivery systems that would allow high concentrations of pharmacologic agents to be delivered directly to the site of angioplasty without exposing the entire circulation to the medication. In the present study, we evaluated for the first time the in vivo feasibility of a newly developed delivery system based on the use of low levels of electrical current to force the drug into the arterial wall. We have chosen for this initial study a specific thrombin inhibitor, recombinant hirudin (r-hirudin), to be delivered into porcine carotid arteries, because of the pivotal role of thrombin in the pathogenesis of acute thrombosis after vascular injury.

Methods

Local Drug Delivery Catheter

The local drug delivery catheter used in the present study incorporated an iontophoretic mechanism to facilitate drug delivery. Iontophoresis is a form of drug delivery that uses electrical current to enhance the movement of charged molecules across or through tissue. The catheter system was developed by CorTrak Medical, Inc. It consists of a 7F catheter that includes a porous balloon with impermeable ends. The balloon used in this study was 5 mm in diameter, and its working length (permeable region) was 30 mm. An inflation pressure of 2 to 3 psi (0.1 to 0.2 atm) was used to expand the balloon, thus allowing contact of the porous membrane with the vessel wall. During the delivery procedure, the drug solution was recirculated through the balloon catheter at a flow rate of 1 to 2 mL/min with the use of a Masterflex peristaltic pump model 7013 (Cole-Parmer Instrument Co). The balloon includes an Ag-AgCl electrode that serves as the cathode. An adhesive electrode patch (10x16 cm) placed on the skin serves as the anode. A constant-current density of 4 mA/cm² was used to drive the charged drug molecules through the porous membrane into the arterial wall.

Experimental Procedure

All procedures performed in this study were approved by the appropriate institutional review committee and conformed

Received August 18, 1993; revision accepted December 15, 1993.

From the Cardiac Unit (A.F.-O., B.J.M., A.M., E.F., L.B., V.F., J.H.C., J.J.B.) and Department of Pathology (J.T.F.), Massachusetts General Hospital and Harvard Medical School, Boston, Mass.

Correspondence to Juan J. Badimon, PhD, Cardiovascular Biology Laboratory, Jackson Bldg, Rm 1328, Massachusetts General Hospital, Boston, MA 02114.
Experimental Design and Intramural Levels of Radiolabeled r-Hirudin

<table>
<thead>
<tr>
<th>Carotid Artery</th>
<th>Current, mA/cm²</th>
<th>Delivery Time, min</th>
<th>Survival Time, min</th>
<th>Balloon Angioplasty</th>
<th>r-Hirudin mg·g⁻¹·mm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Right</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>–</td>
<td>0.40</td>
</tr>
<tr>
<td>Left</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>–</td>
<td>0.01</td>
</tr>
<tr>
<td>2 Right</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>–</td>
<td>0.46</td>
</tr>
<tr>
<td>Left</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>–</td>
<td>0.36</td>
</tr>
<tr>
<td>3 Right</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>–</td>
<td>0.21</td>
</tr>
<tr>
<td>Left</td>
<td>4</td>
<td>5</td>
<td>60</td>
<td>–</td>
<td>0.09</td>
</tr>
<tr>
<td>4 Right</td>
<td>4</td>
<td>5</td>
<td>60</td>
<td>–</td>
<td>0.12</td>
</tr>
<tr>
<td>Left</td>
<td>4</td>
<td>5</td>
<td>120</td>
<td>–</td>
<td>0.07</td>
</tr>
<tr>
<td>5 Right</td>
<td>4</td>
<td>5</td>
<td>120</td>
<td>–</td>
<td>0.07</td>
</tr>
<tr>
<td>Left</td>
<td>4</td>
<td>5</td>
<td>180</td>
<td>–</td>
<td>0.02</td>
</tr>
<tr>
<td>6 Right</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>+</td>
<td>0.19</td>
</tr>
<tr>
<td>Left</td>
<td>4</td>
<td>5</td>
<td>60</td>
<td>+</td>
<td>0.11</td>
</tr>
<tr>
<td>7 Right</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>+</td>
<td>1.98</td>
</tr>
<tr>
<td>Left</td>
<td>4</td>
<td>5</td>
<td>60</td>
<td>+</td>
<td>0.38</td>
</tr>
<tr>
<td>8 Right</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>+</td>
<td>0.23</td>
</tr>
<tr>
<td>Left</td>
<td>4</td>
<td>5</td>
<td>60</td>
<td>+</td>
<td>0.16</td>
</tr>
<tr>
<td>9 Right</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>+</td>
<td>0.20</td>
</tr>
<tr>
<td>Left</td>
<td>4</td>
<td>5</td>
<td>60</td>
<td>+</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Radiolabeling of r-Hirudin

Recombinant desulfatohirudin (r-hirudin, CG39393, CIBAGEIGY) was labeled with ¹²⁵I using the chloramine-T method. In brief, 1 mCi of carrier-free ¹²⁵I in 50 μL of 1 mol/L sodium acetate (pH 4) and 30 μL of 0.1% chloramine-T were added to 0.1 mg of r-hirudin dissolved in 100 μL of distilled water. The reaction was stopped by the addition of 50 μL of sodium metabisulfite. Unreacted iodine was separated from the labeled r-hirudin by gel filtration on a prepacked Sephadex G-25 cartridge (PD10) from Pharmacia. While they were washed with Tris buffer saline, 201 mL fractions were collected and counted in a dose gamma calibrator (AtomLab 100). The major radioactive eluates, representing 89.2% of the original activity, were pooled (fractions 3 to 5) and added up to 10 mL of a solution of unlabeled r-hirudin in distilled water (final concentration, 5 mg/mL).

Protocol

Animals were sedated with intramuscular ketamine (20 mg/kg, Ketalar, Parke-Davis) followed by intravenous injection of sodium pentobarbital (10 mg/kg, Sodium Pentobarbital Injection C, Anpro Pharmaceutical). Adequate anesthesia was confirmed by the absence of a limb-withdrawal reflex. Animals were intubated and ventilated with a volume ventilator (Searle). The ECG was continuously recorded. Introducer sheaths (9F and 8F) were placed into the right and left femoral arteries, respectively. Thereafter, each animal received intravenous heparin for anticoagulation (100 IU·kg⁻¹·h⁻¹ bolus plus infusion). The iontophoretic balloon was advanced under fluoroscopic guidance through the 9F introducer over a 0.014-in. guidewire into either the right or left carotid artery. The balloon was fully inflated to a pressure of 2 to 3 psi by pumping the radiolabeled r-hirudin solution, and complete vessel occlusion was verified by contrast injection through a catheter inserted through the contralateral femoral artery. In total, 20 carotid arteries from 10 normal Yorkshire albino pigs (body weight, 24.3±1.3 kg) were treated by delivering the radiolabeled r-hirudin. In 2 arteries, electrical current was not applied at the time of delivery, so we could test passive diffusion of the drug through the system. In the remaining 18 arteries, a current density of 4 mA/cm² was used to deliver the drug during 5 minutes of balloon occlusion. Conventional balloon angioplasty was performed after delivery (lasting 5 minutes) in 8 arteries to assess the kinetics and distribution of the drug into injured vessel wall. To do so, we inflated an 8-mm-diameter balloon (balloon-to-vessel ratio, 1.5 to 2) to 8 to 9 atm during 30 seconds five times in the area previously treated by local delivery. Animals were euthanized with an overdose of sodium pentobarbital, and the carotid arteries were carefully dissected and removed. Experiments were planned to remove simultaneously both carotids from each animal. Two vessels were removed immediately after passive diffusion delivery, four immediately after iontophoresis, four immediately after iontophoresis followed by balloon angioplasty, three 1 hour after iontophoresis, four 1 hour after iontophoresis followed by balloon angioplasty, two 2 hours after iontophoresis, and one 3 hours after iontophoresis (Table). After we obtained weights, vessels were fixed in 4% paraformaldehyde.

So we could investigate iontophoresis-mediated vessel wall damage, both carotid arteries from an additional animal were similarly treated by delivering saline solution instead of radiolabeled r-hirudin. This animal was killed 48 hours after delivery. Evans blue (50 mL, 0.5%) was administered intravenously 15 minutes before death. The treated arteries were perfusion-fixed and processed for light and scanning electron microscopic examination.

Determination of Concentration and Distribution of r-Hirudin Within the Arterial Wall

The amount of r-hirudin delivered into the vessel wall was determined by counting ¹²⁵I activity in the treated arterial seg-
ment and in the drug solution before and after drug delivery. Iodine activity was measured in a gamma well counter (Packard Auto-Gamma 5650). Results were normalized by weight and length of the arterial segments. In 2 vessels, after radioactive counts, the concentration of r-hirudin was also determined by an enzyme-linked immunoassay (IMUBOND® Hirudin ELISA, American Diagnostica Inc) in the extracts from the treated arterial segments.

The drug distribution within the arterial wall was assessed by autoradiography in 2 noninjured and 2 balloon-injured arteries excised immediately and 1 hour after iontophoretic delivery. Specimens were fixed in 2.5% glutaraldehyde to prevent diffusion of r-hirudin during tissue processing. Paraffin sections were deparaffinized, rehydrated, washed, air dried, dipped in emulsion (Kodak NTB 1:1), dried, exposed for 4 weeks at 4°C in a light-tight plastic box, developed (Kodak D-19), fixed, washed, stained with hematoxylin and eosin, coverslipped, and examined by dark-field microscopy.

**Statistical Analysis**

All values are mean±SEM unless otherwise stated. Comparisons were performed by using the Mann-Whitney U test. Two-tailed P<0.05 was considered statistically significant.

**Results**

**Determination of r-Hirudin Penetration Into the Vessel Wall**

Intravascular levels of r-hirudin into noninjured carotid arteries, measured by iodine activity after iontophoresis with 4 mA/cm² continuous current for 5 minutes, were 80-fold greater than those achieved by passive diffusion (without electricity) (0.78±0.38 versus 0.01±0.06 μg·g tissue⁻¹·mm⁻¹, respectively). The arterial tissue levels of r-hirudin declined over time, reaching 0.14±0.04 μg·g tissue⁻¹·mm⁻¹ at 1 hour, 0.08±0.01 μg·g tissue⁻¹·mm⁻¹ at 2 hours, and 0.02±0.01 μg·g tissue⁻¹·mm⁻¹ at 3 hours after delivery (Fig 1). Balloon angioplasty performed after iontophoretic delivery did not change hirudin kinetics into the arterial wall. Intravascular levels of r-hirudin, measured by iodine activity, were 0.65±0.44 μg·g tissue⁻¹·mm⁻¹ immediately after delivery followed by balloon angioplasty and 0.19±0.06 μg·g tissue⁻¹·mm⁻¹ at 1 hour (NS compared with matching values in noninjured vessels).

The concentration of r-hirudin, as measured by ELISA, in the extracted arterial segment was 2.24 μg·g tissue⁻¹·mm⁻¹ immediately after delivery and 0.18 μg·g tissue⁻¹·mm⁻¹ at 1 hour after delivery. The amount of r-hirudin calculated by iodine activity in the same segments was 1.92 and 0.09 μg·g tissue⁻¹·mm⁻¹, respectively. Local delivery of r-hirudin into the arterial wall had no systemic anticoagulant effect; activated partial thromboplastin time ratio was 2.85±0.29 before local delivery (100 IU·kg⁻¹·h⁻¹ of heparin were used for systemic anticoagulation) and 2.77±0.32 after local delivery (NS).

**Distribution of r-Hirudin Within the Vessel Wall**

 Autoradiography of an artery excised immediately after r-hirudin delivery revealed many silver grains covering the entire arterial wall (Fig 2A), indicating that the drug was distributed throughout the intima, media, and adventitia. Although fewer autoradiographic grains were seen in the artery processed 1 hour after drug delivery (Fig 2B), the drug remained distributed throughout all vessel wall thicknesses. Balloon injury after local delivery did not change the distribution pattern of hirudin within the vessel wall, and the drug similarly was distributed through the entire vessel wall at the sites of arterial injury (Fig 2D and 2E).

**Iontophoresis-Mediated Vessel Wall Damage**

Evans blue stained less than 10% of the intimal surface where the iontophoretic delivery was performed. Scanning electron microscopy showed focal endothelial cell loss (less than 10% of intimal surface) at the site of iontophoretic delivery. Arterial cross sections taken from the treated arterial segments at 4-mm intervals revealed an intact internal elastic lamina, focal medial smooth muscle cell loss, an absence of inflammatory infiltrates, and a normal adventitia (Fig 3).

**Discussion**

This is the first demonstration of the in vivo use of the iontophoretic balloon delivery system to locally introduce r-hirudin into an arterial wall. The use of an electrical field enhanced the movement of r-hirudin into the arterial wall by 80-fold compared with passive diffusion delivery, ie, when electrical current was not applied to the system. The fact that r-hirudin was detected by ELISA in the extracts from treated arteries indicates that iontophoresis changes neither the structure of the r-hirudin molecule nor, probably, its functional properties.

Retention of r-hirudin in the vessel wall was time dependent; by 1 hour after delivery, approximately 80% of the drug had left the wall, and by 3 hours later, the levels of r-hirudin in the artery were similar to those achieved by passive diffusion. Balloon angioplasty performed after iontophoretic delivery did not change the kinetics of r-hirudin disappearance from the arterial wall. The reasons that r-hirudin rapidly disappears from the arterial wall are probably related to its pharmacokinetic characteristics. r-Hirudin, administered intravenously, is distributed into the extracellular space and has an average half-life of 1 to 2 hours with renal excretion being its predominant route of clearance.
Eventually, retention of small molecules, such as r-hirudin, might be improved by binding to larger biomolecules. For example, it has been reported that dextran-bound hirudin distributed in plasma exhibited a prolonged half-life of 7 hours. The kinetics of r-hirudin in an atherosclerotic vessel wall remains unknown.

A common problem in the investigation of local drug delivery systems is arterial damage caused by the deliv-
ery systems themselves. Such injury may result in significant neointimal proliferation.\textsuperscript{10,11} The degree of acute vessel wall damage associated with the use of the iontophoretic balloon system, assessed 48 hours after delivery, was minimal (less than 10% endothelial denudation and only focal smooth muscle cell damage) and less than that observed with either perforated balloon systems\textsuperscript{12,13} or polymer-coated stents.\textsuperscript{14} In addition, with iontophoresis, r-hirudin was distributed throughout the entire circumference of the artery and penetrated the intima, media, and adventitia.

The need for a relatively long duration of vessel occlusion to achieve high local concentrations of a drug within the vessel wall makes it desirable to use a reperfusion lumen catheter to avoid distal ischemia during delivery. However, shorter delivery times achieved by the use of multiple inflations and drug-delivering periods (ie, two or three inflations lasting 1 to 2 minutes each) might be investigated to allow percutaneous delivery within a clinically suitable duration of occlusion time to approach coronary atherosclerotic lesions.

This technology may have clinical relevance for the local treatment of arteries undergoing catheter-based interventions, such as angioplasty, atherectomy, rotablation, or stenting. We have chosen for this initial study r-hirudin because of its potent antithrombin activity and because thrombin is believed to play a key role in the pathogenesis of acute thrombosis after vascular injury. The effect of locally delivered r-hirudin and the strategy for preventing thrombus formation and intimal hyperplasia after vessel wall injury have not been addressed in this initial study and should be investigated in future studies. Moreover, other therapeutic agents or strategies (ie, antiproliferative agents or gene transfer therapy) also might be promising when approached by the iontophoretic balloon system.

**Acknowledgments**

Dr A. Fernández-Ortiz is supported by a fellowship from the Fondo de Investigación Sanitaria (F.I.S., Spain). This study was supported in part by a grant from CorTrak Medical, Inc, and funds from the Mr and Mrs D.Y. Fu Foundation. The authors gratefully thank Janet Robbins, Anne Skalicky, Adrian Paredes, Philip Seifert, Star Sevillano, and Dr Inmaculada Soria (CorTrak Medical, Inc) for their technical assistance. r-Hirudin CG39393 was a generous gift of CIBA-GEIGY Co.

**References**

A new approach for local intravascular drug delivery. Iontophoretic balloon.
A Fernández-Ortiz, B J Meyer, A Mailhac, E Falk, L Badimon, J T Fallon, V Fuster, J H Chesebro and J J Badimon

_Circulation._ 1994;89:1518-1522
doi: 10.1161/01.CIR.89.4.1518

_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/89/4/1518

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/