

Oral Heparin Prevents Neointimal Hyperplasia After Arterial Injury

Inhibitory Potential Depends on Type of Vascular Injury

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Background—In animal models, heparin delivered as a continuous intravenous infusion or via frequent (BID) subcutaneous dosing inhibits neointimal hyperplasia after balloon injury or stent implantation. However, human trials of subcutaneous heparin after percutaneous intervention have proven ineffective against restenosis. It may be that these failures are due to unfavorable heparin pharmacokinetics. Recently, the drug delivery agent sodium *N*-[8-(2-hydroxybenzoyl)amino] caprylate (SNAC) has been found to facilitate the gastric absorption of heparin.

Methods and Results—To investigate the effects of orally delivered heparin on neointimal hyperplasia after varying forms of arterial injury, 57 New Zealand White rabbits underwent iliac artery balloon dilatation. In half of the rabbits, endovascular stents were implanted and heparin was delivered through a variety of methods. Arteries were harvested at 14 days. Neointimal area was assessed with computer-aided morphometry. After balloon injury, both intravenous (0.3 mg/kg per hour) and oral heparin (90 mg/kg BID) effectively inhibited neointimal hyperplasia (0.11 ± 0.02 and 0.09 ± 0.07 mm², respectively, versus 0.16 ± 0.06 mm² in control; $P < 0.05$). After stent implantation, intravenous administration of heparin (0.3 mg/kg per hour) effectively inhibited neointimal growth (0.35 ± 0.05 mm² versus 0.51 ± 0.09 mm² in control; $P < 0.05$), but oral heparin at 90 mg/kg BID and 180 mg/kg BID (0.48 ± 0.04 and 0.49 ± 0.08 mm², respectively; $P = \text{NS}$ versus control) did not. A dose of 120 mg/kg TID, however, was effective (0.40 ± 0.10 mm²; $P < 0.05$ versus control).

Conclusions—These data suggest that oral heparin may be an effective therapy against restenosis after percutaneous intervention. Stented arteries required higher and more frequent dosing for efficacy. These data suggest that differences in the type of vascular injury must be considered in the design of drug delivery. (*Circulation*. 2001;104:3121-3124.)

Key Words: heparin ■ stents ■ restenosis

Heparin is the archetypical modulator of vascular repair after vascular injury in a variety of animal models. However, human trials of subcutaneous heparin after percutaneous intervention have proven ineffective in preventing restenosis.¹ An explanation of this paradox is suggested by data from prior animal studies, which suggest that the efficacy of heparin against neointimal growth critically depends on the type of vascular injury² and the duration and frequency of heparin administration.³ These data suggest that the type of vascular injury (stent versus balloon) and the pharmacokinetic profile of anti-inflammatory agents must be taken into account when designing strategies against neointimal growth after vascular injury. Furthermore, these data suggest that human studies of heparin after percutaneous intervention may have suffered from insufficient dosing intervals and durations of administration.

Heparin is poorly absorbed from and rapidly degraded within the intestinal tract and, therefore, it requires either intravenous or frequent subcutaneous administration, making administration on an outpatient basis problematic. Recently, the drug delivery agent sodium *N*-[8-(2-hydroxybenzoyl)amino] caprylate (SNAC) has been found to allow gastric absorption of heparin.^{4,5} The current study was designed to examine the efficacy of oral heparin against neointimal growth after vascular injury.

We now report an inhibitory effect of oral heparin on neointimal growth following vascular injury after balloon denudation, with or without stenting, in a rabbit iliac artery model. Stented arteries required a more frequent and higher drug dose to inhibit neointimal growth compared with balloon-injured arteries alone. The availability of an oral form of heparin may allow frequent administration and, therefore, more effective suppression of restenosis in humans. In addition, these data suggest that

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the type of vascular injury imposed on the artery (ie, stent versus balloon) must be taken into account when designing pharmacological strategies against restenosis.

Methods

Surgical Procedure

New Zealand White rabbits (Covance Products, Denver, Pa), weighing 3 to 4 kg, were housed individually in steel mesh cages and fed rabbit chow and water ad libitum. All animal care and procedures were in accordance with the guidelines of the American Association for Accreditation of Laboratory Animal Care and the National Institutes of Health. Under anesthesia with intramuscular ketamine (Aveco Co) 35 mg/kg and xylazine (Miles Inc) 15 mg/kg, both femoral arteries were exposed and ligated. A femoral arteriotomy was performed, and a 3-French balloon catheter (Baxter HealthCare Corp) was passed retrograde into the abdominal aorta and withdrawn in the inflated state 3 times to denude the iliac artery endothelium bilaterally. Two major series of experiments were performed.

In the first, balloon injury alone was performed (n=25 animals and 43 arteries). In the second (n=32 animals and 51 arteries), a 7-mm, corrugated-ring, stainless steel endovascular stent mounted on a 3-mm angioplasty balloon (Advanced Cardiovascular Systems/Guidant) was passed retrograde via arteriotomy into each iliac artery and expanded with 15 s of inflation to a pressure of 8 atm after endothelial denudation. Standard anticoagulant heparin (100 U/kg; Elkin-Sinn) was injected as a single intravenous bolus before deployment of all of the stents. To reduce the incidence of subacute stent thrombosis, all animals received aspirin (Sigma) via drinking water (0.07 mg/mL) to achieve an approximate dose of 5 mg/kg per day; aspirin administration started 1 day before the procedure and lasted for the duration of the experiment.

Sodium heparin USP (Hepar Industries) was delivered intravenously from subcutaneously implanted osmotic minipumps (Alza Corp) through a catheter within the femoral vein at 0.3 mg/kg per hour (n=8 animals in the balloon-injured group, n=4 animals in the stent group). Using 150 mg/kg SNAC to facilitate absorption, heparin (Hepar Industries) was delivered via oral gavage at 90 mg/kg BID (1 mL/kg animal body weight) in the balloon-injured group (n=4 animals). In the stented group, heparin was delivered via oral gavage at either 90 mg/kg BID (n=3 animals), 180 mg/kg BID (n=5 animals), or 120 mg/kg TID (n=5 animals). In 5 animals, SNAC alone at 150 mg/kg BID was given to balloon-injured animals to assess the effect of sham dosing.

Tissue Processing

Animals were killed 14 days after surgery. Anesthesia was administered as above, the caudal vena cava was opened, and pressure perfusion was performed with Ringer's lactate solution (300 cc) through a left ventricular puncture, followed by 0.4% paraformaldehyde for 10 minutes at a pressure of 100 mm Hg. The iliac arteries were excised and placed in a solution of 0.4% paraformaldehyde. Specimens were embedded in methyl methacrylate mixed with *n*-butyl methacrylate (Sigma). Five-micron sections were cut using a tungsten-carbide knife (Delaware Diamond Knives). Stented specimens were oriented to the proximal and distal ends, and sections were taken at 3 points along the stent, including at each end and the middle, to reduce sampling error.

Dose Determination

In a pilot study of 4 rabbits, heparin levels 2 days after the initiation of intravenous drug delivery via osmotic minipump (0.03 mg/kg per hour) were determined to be 0.005 ± 0.003 mg/mL. A short-term time-course study was then performed on 2 anesthetized rabbits in which gastric contents were removed via orogastric lavage. Oral heparin was delivered (with 150 mg/kg SNAC) through an orogastric tube at 36 mg/kg and 90 mg/kg. Plasma heparin levels were determined at baseline and 15, 30, 60, 120, 180, and 240 minutes after administration. Blood was sampled through the femoral vein using an 18-g intravenous catheter into a 3.8% sodium citrate

Intimal Areas After Balloon Injury or Stenting

Treatment	Intimal Area, mm ²	
	Balloon Injury	Stent
Control	0.16±0.02	0.51±0.09
Sham	0.16±0.06	...
Heparin		
0.3 mg/kg IV	0.11±0.05*	0.35±0.05*
90 mg/kg PO BID	0.09±0.07*	0.48±0.04
180 mg/kg PO BID	...	0.49±0.08
120 mg/kg PO TID	...	0.40±0.10*

Values are mean±SD.

* $P < 0.05$.

Vacuater. Heparin levels were measured using the Heptest and Heptest Hi reagent kits (Sigma) and an Amelung KC1 Clot Timer. This assay measures the inhibition of clotting by heparin in the presence of a known amount of Factor Xa. Coefficients of absorption and elimination were calculated to allow estimation of plasma concentrations at varying doses and dose intervals. An initial dose of 90 mg/kg BID was chosen to achieve an average plasma concentration of 0.004 mg/mL.

Histology and Statistical Analysis

Tissue and cells structures were identified in histological sections by staining with Verhoeff's tissue elastin stain. Neointimal and medial cross-sectional areas were measured by computer-assisted digital planimetry. For stented arteries, values from proximal, mid, and distal sections were averaged. All data are presented as mean±SD. Statistical comparisons were performed with a 1-way ANOVA using the least-significant difference methods for multiple comparisons versus control. Values of $P < 0.05$ were considered significant.

Results

Animals underwent 2 types of vascular injury: simple balloon endothelial denudation or the deep, chronic injury associated with balloon denudation followed by stent implantation. Heparin was delivered via 2 routes: a standard intravenous route (which has been shown in the past to effectively inhibit neointimal hyperplasia in both models of injury) or through a novel oral route.

Balloon Injury

At 14 days after balloon injury, heparin effectively suppressed neointimal growth, whether delivered intravenously or via oral gavage at 90 mg/kg PO BID (Table and Figures 1 and 2) compared with control. The oral drug delivery agent SNAC 150 mg/kg PO BID delivered alone had no effect compared with control.

Stent Injury

In contrast, after stent-induced injury, intravenous heparin effectively suppressed neointimal growth, but oral heparin at 90 mg/kg PO BID (twice the intravenous dose) had no effect when compared with controls. However, the same total large dose (360 mg/kg per day) was effective when delivered in 3 rather than 2 divided doses through the day as 120 mg/kg PO TID (Table and Figures 3 and 4).

Discussion

Our data demonstrate that heparin delivered via the oral route is an effective agent against neointimal growth after either simple balloon injury or the deeper chronic injury associated with stent

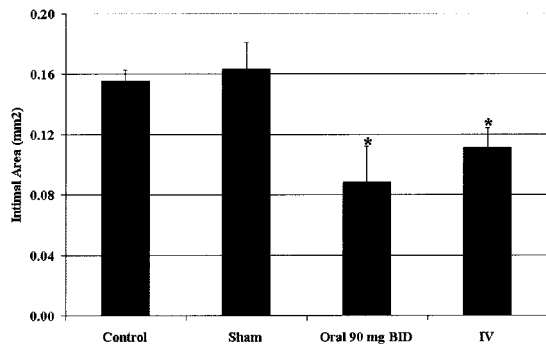


Figure 1. Bar graph demonstrating suppression of neointimal growth in balloon-injured arteries by different treatment groups. * $P < 0.01$.

implantation. In addition, stented arteries required both a larger dose and more frequent delivery for efficacy. These data suggest that oral heparin may be an effective agent against restenosis in humans and suggests that there are important differences between the injury associated with balloon endothelial denudation and that associated with stent implantation.

Prior Data of Heparin in Restenosis

Animal Models

Heparin is a potent inhibitor of smooth muscle cell proliferation *in vitro*⁶ and of neointimal growth in a variety of animal models of vascular injury.^{7–10} Potential mechanisms, clearly independent of its anticoagulant activity,¹¹ include inhibition of nuclear transcription factors,¹² modulation of growth factor activity or receptor binding,¹³ regulation of extracellular matrix production,¹⁴ direct inhibition of smooth muscle cell proliferation and migration,⁹ and perhaps most importantly, an anti-inflammatory effect.^{15–19}

Several studies have suggested that heparin's efficacy critically depends on the type of injury imposed on the artery and on heparin's pharmacodynamic profile. After balloon injury in a rabbit iliac artery model, heparin delivered via continuous intravenous infusion or via frequent (BID) subcutaneous dosing inhibits neointimal growth, but heparin delivered less frequently is not effective and may exacerbate

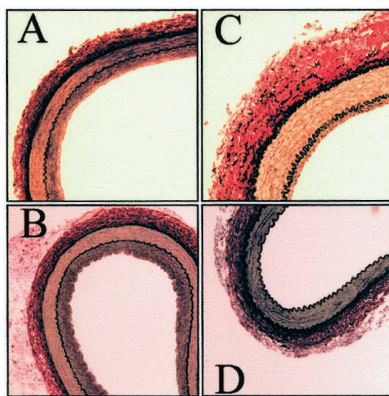


Figure 2. Photomicrographs (magnification $\times 40$) of sectioned balloon-injured rabbit iliac arteries. A, Control; B, sham dosing; C, intravenous heparin at 0.3 mg/kg per hour; and D, oral heparin at 90 mg/kg BID.

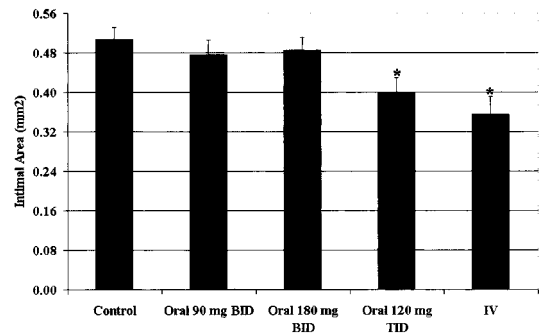


Figure 3. Bar graph demonstrating suppression of neointimal growth in stented arteries by different treatment groups. * $P < 0.02$.

neointimal growth.³ In another experiment using the rabbit iliac artery model, Rogers et al² showed that stented arteries require prolonged administration (14 days) of heparin to inhibit neointimal hyperplasia fully. In contrast, a short 3-day course of heparin inhibited the neointimal hyperplasia after balloon injury alone in a manner equivalent to drug administration for the 14-day course of the experiment.

Examination of the inflammatory response following vascular injury offers a possible explanation for these observations. After balloon injury in a rabbit iliac artery model, leukocyte recruitment is restricted to early and transient neutrophil infiltration.¹⁹ In contrast, after stenting, the early neutrophil infiltration is markedly more intense and is followed by a long-term accumulation of macrophages within the neointima.² Heparin, delivered intravenously, reduces the burden of infiltrative leukocytes with a concomitant suppression of smooth muscle cell proliferation and neointimal growth in both balloon-injured and stented arteries.^{2,19}

The data presented in the current study are consistent with these prior findings in that stented arteries, with their larger and more

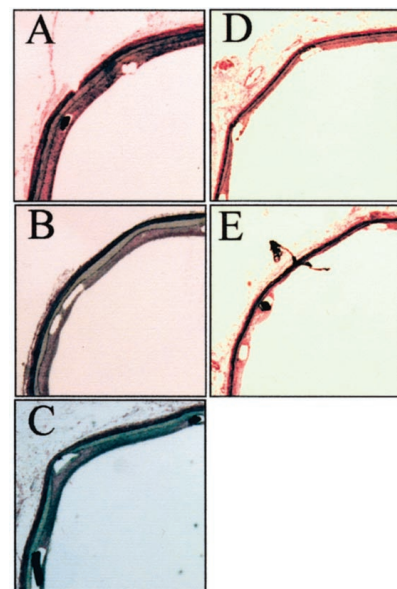


Figure 4. Photomicrographs (magnification $\times 20$) of sectioned and stained (Verhoeff's stain) stented rabbit iliac arteries. A, Control; B, oral heparin at 90 mg/kg BID; C, oral heparin at 180 mg/kg BID; D, intravenous heparin at 0.3 mg/kg per hour; and E, oral heparin at 120 mg/kg TID.

chronic burden of inflammatory cells, require a larger and more frequent dose of heparin compared with balloon-injured arteries.

Human Studies

Both unfractionated and low-molecular-weight heparin have been studied in patients undergoing balloon angioplasty. Ellis et al¹ randomized 416 patients to either 18 to 24 hours of unfractionated heparin or dextrose after balloon angioplasty and found no difference in late (180±81 days) angiographic follow-up. On the basis of longer half-life and greater bioavailability, low-molecular-weight heparins have been studied in a variety of situations involving patients after balloon angioplasty, and they have not been found to reduce angiographic restenosis rates.^{20–22} The prior animal data demonstrating the need for more frequent heparin dosing to achieve efficacy against neointimal growth after vascular injury suggests that these human studies may have suffered from improper dosing.

Oral Heparin

SNAC is a synthetic compound with a molecular weight of 310 Da. Although its exact mechanism of action is not clearly understood, it has been postulated that SNAC interacts noncovalently with heparin to facilitate gastric absorption.^{4,5} In animal models, oral heparin is an effective anticoagulant, elevating activated partial thromboplastin times (aPTTs) and effectively preventing deep venous thrombosis in a rat model.^{23,24} In humans, oral heparin effectively raises aPTTs in a dose-dependent fashion.²⁵ The preparation was well tolerated, without significant side effects or toxicity. We now extend these observations to include efficacy of oral heparin against neointimal growth after vascular injury in an animal model.

Conclusions

The present study demonstrates the efficacy of oral heparin to prevent restenosis after balloon-induced injury and the more chronic deep injury associated with stent implantation in a rabbit iliac artery model. In addition, the present study builds on prior data demonstrating differences in the vascular biological response to injury between balloon injury and stent implantation. The more robust and chronic inflammatory response to stenting requires larger and more frequent dosing than that required after simple balloon endothelial denudation.

The advent of a safe and effective method for oral delivery of heparin offers the promise of more frequent and prolonged delivery of heparin to patients and, therefore, a more favorable pharmacokinetic and pharmacodynamic profile than that of subcutaneous or intravenously injected heparin in the prevention of restenosis. The use of these compounds in phase III clinical trials for veno-occlusive disease may enable rapid translation of these preclinical data to human trial validation.

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