Background—Previous studies have shown that repeated systemic administration of human recombinant hepatocyte growth factor (hrHGF) in mg/kg levels modulates the wound-healing process in various diseases. Recently, HGF has been characterized as one of the most potent endothelial-cell–specific growth factors. We tested our hypothesis that local delivery of hrHGF, even at low μg/kg levels (≥2 orders of magnitude lower than systemically administered doses), might attenuate neointimal hyperplasia in response to vascular injury via accelerated reendothelialization.

Methods and Results—The iliac artery was denuded in 16 New Zealand White rabbits (3 kg), followed by administration, via a drug delivery catheter, of either hrHGF (10 μg; n = 11) or control vehicle (n = 5) over 20 minutes. In pilot studies using this device, the drug permeated into the medial tissues, where it persisted for ≥24 hours. Four weeks after the local delivery of hrHGF, computer-assisted morphometric analysis revealed significant reduction in the intimal area (hrHGF, 0.37 ± 0.21 versus control, 0.68 ± 0.16 mm²; mean ± SD; P < 0.05) but no change in the medial area (hrHGF, 1.03 ± 0.21 versus control, 1.10 ± 0.52 mm²). Scanning electron microscopy revealed extensive endothelialization with regular and confluent endothelial cell layer regeneration in the hrHGF-treated vessels.

Conclusions—Accelerated endothelialization after local delivery of hrHGF, a novel and potent endothelial cell mitogen, effectively attenuates neointimal proliferation even after single low-dose administration. This observation could have potential therapeutic implications in the prevention of restenosis after angioplasty. (Circulation. 2000;101:2546-2549.)

Key Words: angioplasty ■ catheters ■ endothelium ■ growth substances ■ restenosis

Despite extensive efforts, including the development of a variety of adjunctive therapies and mechanical techniques, 30% to 50% of patients undergoing percutaneous transluminal coronary angioplasty develop restenosis within 3 to 6 months of the procedure. A major cause of the restenosis is neointimal hyperplasia in response to the arterial injury. This process predominantly involves vascular smooth muscle cell proliferation, on which accelerated reendothelialization has been shown to have an inhibitory effect. As a potential method for the prevention of restenosis, local administration of pharmacological or biological agents directly to the injured site in the vessel has been attracting increasing interest. Local delivery may have the potential advantages of allowing a sufficient concentration of the agent to be accumulated at the site of injury while minimizing the likelihood of systemic adverse effects.

Hepatocyte growth factor (HGF), which is a disulfide-linked heterodimeric molecule composed of a 69-kD kringle–containing α-chain and a 34-kD β-chain, is a novel therapeutic growth factor with unique multipotent properties (promotes angiogenesis, wound healing, and cell survival). Previous studies have shown that repeated systemic administration of human recombinant (hr) HGF in mg/kg levels modulated the wound-healing process in liver, kidney, and lung diseases. Recently, HGF has been characterized as being among the most potent endothelial-cell–specific growth factors, contributing to vascular protection or repair. Therefore, in the present study, we tested our hypothesis that the local delivery of hrHGF, even at low, μg/kg levels (≥2 orders of magnitude lower than systemically administered doses), may attenuate neointimal hyperplasia in response to vascular injury via accelerated reendothelialization.

Methods

All the procedures followed were in accordance with institutional guidelines, which conformed to the “Position of the American Heart Association on Research Animal Use.”

Model

New Zealand White rabbits weighing 3.0 to 3.5 kg were used for this study. Anesthesia was induced by injection of ketamine (50 mg/kg IM), after premedication with xylazine (10 mg/kg IM). The femoral arteries were exposed by an incision below the inguinal ligament.
Heparin sulfate (1000 U IV) was administered to prevent thrombosis. After local administration of 2 mL 1% lidocaine, a 3.6F angioplasty catheter (Tokai Medical Products Inc) was introduced under fluoroscopic guidance in an over-the-wire system into the right iliac artery. This catheter possesses the triple functions of balloon inflation, local drug delivery, and perfusion. The 3.0-mm-diameter, 20-mm-long balloon was inflated with contrast medium at the right iliac artery immediately distal to the aortic bifurcation (a reliable and reproducible landmark for the removal of the vessels at a later date). The balloon, inflated at 6 atm, was then retracted 20 mm from the bifurcation and deflated. This procedure was repeated 3 times within the same segment to ensure complete endothelial denudation.

Local Delivery of hrHGF
After balloon injury, local drug delivery was achieved via the multifunctional angioplasty catheter. The use of this device has been described in detail. Briefly, the drug-delivery port was positioned at the injured site, and the balloon was inflated at a low pressure (2 atm) to allow drug accumulation. Then the guidewire was removed to a site proximal to the perfusion port for distal perfusion. In pilot studies using this device, the drug permeated into the medial tissues, where it persisted for ≥24 hours. In addition, this device could deliver the drug homogeneously at the target site. Via an infusion pump (STC-521, Terumo), the rabbits received local administration of 10 μg hrHGF (lot GJ04, Funakoshi Co) dissolved in 10 mL saline or vehicle solution without HGF (control) over 20 minutes to the injured site in the iliac artery. A similar protocol was followed for the local delivery of 1 μg hrHGF. In comparison with the previous studies of the application on 100 μg of vascular endothelial growth factor (VEGF) to a balloon-injured rat carotid artery, we examined the effects of much lower doses (1 to 10 μg) in the present study in rabbits.

Postmortem Procedures
Twenty-eight or 14 days after the procedure, the rabbits were euthanized by injection of a fatal dose of pentobarbital. For pressure perfusion fixation, a midabdominal incision was made and the lower abdominal aorta was isolated, flushed with saline, and fixed with 10% buffered formalin at 80 mm Hg over 15 minutes. After ≥24 hours of postfixation, the arterial segments were dehydrated and embedded in paraffin.

Data Analysis
For histology, 5-μm sections were cut and stained with van Gieson’s elastin stain. Morphometric analysis was performed on the arterial cross sections at 28 days, imaged on a Macintosh computer using a National Institutes of Health image software package. The endoluminal border, the circumference bounded by the internal elastic lamina, and the external elastic lamina were manually traced, and the luminal, intimal, and medial areas, respectively, were then calculated. The ratio of intimal to medial area was also calculated. We compared these parameters among the control (n=4), 10 μg hrHGF–treated (n=11), and 1 μg hrHGF–treated (n=5) vessels.

For the ultrastructural analysis, the specimens at 14 days from the control (n=4) and 10 μg hrHGF–treated (n=4) vessels were examined by scanning electron microscopy (S-4000; Hitachi). The degree of endothelialization of the luminal surface of the neointima (endothelial cell regeneration score) was quantified by planimetric analysis and expressed as a percentage of the total luminal surface. Endothelial cells were defined on the basis of the criteria provided by Schwartz et al.

Both the intraobserver and interobserver variabilities were <5% in the present study.

Statistics
All data were expressed as mean±SD. Comparisons between 2 groups were made by Student’s t test. Comparisons among ≥3 groups were carried out by ANOVA. When a significant difference among the groups was indicated by the initial analysis, individual paired comparisons were made by the Student-Newman-Keuls methods. A value of P<0.05 was considered to denote significance.

Results
The Table summarizes the group morphometric data. At 28 days, local delivery of 10 μg hrHGF induced significant reduction in the intimal area, but no change in the medial area was observed. This resulted in a significant decrease in the ratio of intimal to medial area in the HGF group compared with the control group, indicating attenuation by HGF of neointimal proliferation after balloon injury. Representative photomicrographs of histological cross sections from the injured arterial segments are shown in Figure 1.

Scanning electron microscopy revealed extensive endothelialization at 14 days, with the formation of a regular and confluent endothelial cell layer in 10 μg hrHGF–treated vessels as shown in Figure 2. In contrast, endothelialization was not observed in the control vessels, as shown in Figure 3. The endothelial cell regeneration score at 14 days was significantly higher in the HGF group than in the control group (90±6% versus 20±10%, P<0.001), indicating acceleration of endothelialization by HGF.

Significant attenuation of neointimal proliferation was also obtained after local delivery of 1 μg hrHGF. In comparison with the control group, a decrease in the intimal area but no change in the medial area was observed at 28 days (Table).

Discussion
The most significant finding of this study is that local delivery of hrHGF effectively attenuated neointimal prolifer-
HGF exerts its organ-regenerating, wound healing, angiogenic, and cytoprotective effects via mediation of the c-Met transmembrane tyrosine kinase receptor. On the basis of these multipotent effects, the use of HGF as a therapeutic tool is attracting increasing attention. In various models for acute and chronic disease of the liver, kidney, and lung, the therapeutic effects of repeated systemic administration of hrHGF in mg/kg levels have been demonstrated. However, it had not yet been definitely determined whether HGF can also modulate the healing response in arteries after balloon injuries.

Neointimal hyperplasia develops in response to arterial injury in experimental models. Despite its complex nature, endothelial cells are known to play an important role in the initial stages of this response. Reendothelialization (restitution of endothelial surface coverage) is associated with reduction in thrombus formation and smooth muscle cell proliferation and thereby suppresses neointimal proliferation. HGF is one of the most potent mitogens among the growth factors specific for endothelial cells, including VEGF. In addition, HGF, but not VEGF, is known to prevent endothelial cell death (apoptosis), which has been documented in lesions after balloon injury. Thus, also taking into consideration the recent finding that hypoxia downregulates HGF expression, whereas it upregulates VEGF expression in vitro, we focused on HGF, a novel vascular modulator with characteristics different from those of VEGF, in the present study.

Despite the preliminary report, systemic administration of hrHGF did not prevent intimal hyperplasia in a balloon-injured rat carotid artery model. In addition, particularly for expensive agents such as hrHGF, the efficiency of transfer to the target tissue is of practical importance. Therefore, we sought to determine the effects of local delivery of hrHGF to injured vessel sites. Local delivery may have the therapeutic advantages of greater tissue concentrations with a reduced likelihood of systemic toxicity. The catheter device used in this study enables relatively long duration of delivery (>20 minutes), long residence of the drug in the target tissue (approximately the first 24 hours), and homogeneity of drug transfer.

Unlike HGF gene therapy, active hrHGF is present at the time of injury, possibly targeting early events important for restenosis in the present study. We found that the local delivery of 10 μg of hrHGF significantly reduced intimal proliferation, as shown in Figure 1 and the Table. Similar results were obtained after local delivery of only 1 μg hrHGF. Acceleration of reendothelialization by HGF, which was demonstrated by the findings of scanning electron microscopy (Figures 2 and 3), is a possible mechanism for the attenuated response to balloon injury. These HGF effects on endothelialization may also be associated with restoration of nitric oxide, which directly inhibits smooth muscle cell growth and maintains vascular physiology.

The present findings indicate that HGF is a potential therapeutic tool for the prevention of neointimal hyperplasia after balloon injury. In addition to catheter-based drug delivery, coating stents with hrHGF might be of value, because stenting has largely replaced regular angioplasty and because neointimal proliferation is the primary factor accounting for in-stent restenosis. Further studies would be desirable for clinical implications in humans with mechanisms of restenosis, which may be somewhat different from that in animal models.

In conclusion, considering its biological actions as one of the most potent endothelial cell–specific growth factors, local delivery of low doses of HGF is a logical and efficient strategy for the treatment of balloon injuries of arteries.

**Acknowledgments**

This study supported by the Uehara Memorial Foundation, Osaka Heart Club, and Japan Cardiovascular Research Foundation (Dr Yasuda). We thank Dr Yasuhide Nakayama and Kanna Okuda for their assistance in the electron microscopic studies.
References

Single Low-Dose Administration of Human Recombinant Hepatocyte Growth Factor Attenuates Intimal Hyperplasia in a Balloon-Injured Rabbit Iliac Artery Model
Satoshi Yasuda, Teruo Noguchi, Masahiro Gohda, Takashi Arai, Nobumasa Tsutsui, Takehisa Matsuda and Hiroshi Nonogi

Circulation. 2000;101:2546-2549
doi: 10.1161/01.CIR.101.21.2546
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 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/101/21/2546

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/