

Bull Yamaguchi Med Sch 48(1-2):13-19, 2001

The Effect of Hepatocyte Growth Factor (HGF) on Promotion of Endothelial Healing of Balloon-injured Carotid Artery

Masakazu Harada, Hiroaki Takenaka, Nobuya Zempo, Kensuke Esato, Tomokazu Nagano *, Mutsuo Taiji *, and Hiroshi Noguchi *.

The First Department of Surgery, Yamaguchi University School of Medicine, Ube, Yamaguchi Japan; and * Discovery Research Laboratories II Sumitomo Pharmaceuticals Research Center 3-1-98 Kasugade-Naka, Konohana-ku, Osaka 554-0022, Japan
Correspondence: Masakazu Harada, M.D. First Department of Surgery, Yamaguchi University School of Medicine, Minamikogushi I - I - I, Ube, Yamaguchi 755-8505, Japan
(Received August 8, 2001, revised November 6, 2001)

Key words: hepatocyte growth factor (HGF), balloon injury, endothelial healing

Abstract

Purpose: This animal study was designed to examine the effect of hepatocyte growth factor on suppression of intimal hyperplasia resulting from reendothelialization after balloon injury in the rat carotid artery.

Methods: Fourteen Sprague-Dawley rats were subjected to balloon injury of carotid artery. Rats were divided into three groups: a control group (n=6) in which saline was infused intravenously once immediately after injury; treatment groups in which 0.1 mg/body HGF (0.1-mg HGF group; n=5) or 0.2 mg/body HGF (0.2-mg HGF group; n=3) was infused in the same way. Rats were killed 14 days after surgery, and the carotid artery was harvested.

Results: Intima/media (I/M) ratio was 0.98 ± 0.15 in the control group, 0.73 ± 0.17 in the 0.1-mg HGF group and 0.65 ± 0.05 in the 0.2-mg HGF group. Reendothelialization extended 3.4 ± 0.74 mm in the control group, 5.5 ± 0.5 mm in the 0.1-mg HGF group and 5.6 ± 2.1 mm in the 0.2-mg HGF group at the proximal site. There were no significant differences between groups in I/M ratio or reendothelialization.

Conclusion: HGF is not effective on intimal healing in this model in terms of endothelial cell ingrowth and suppression of intimal thickening.

Introduction

Most strategies for reducing restenosis of the artery after balloon angioplasty are based on treatment with biological reagents. The major cause of intimal hyperplasia after intimal injury is proliferation and migration of medial smooth muscle cells (SMCs) from the non-denuded artery during the chronic

phase.¹⁻²⁾ Endothelial cells (ECs) are another important component affecting restenosis. Once ECs cover the intraluminal surface of the denuded artery, they produce several SMC growth inhibitors, such as prostaglandin (PGI₂) and nitric oxide (NO), inhibiting proliferation and migration of SMCs.³⁾ Thus an agent that promotes ingrowth of ECs and inhibits proliferation and migration of SMCs would be invaluable in the prevention of obstructive

tive intimal hyperplasia after balloon injury in the artery.

Hepatocyte growth factor (HGF) was originally isolated from the sera of rats after partial hepatectomy.⁴⁾ The molecular weight of HGF is 82-85 KD, and it consists of two subunits, a 69-KD α -chain and a 34-KD β -chain.⁵⁾ HGF is synthesized by fibroblasts and vascular SMCs. The HGF receptor C-met is expressed on the membrane of vascular ECs.⁶⁾ HGF is a multipotential cytokine that acts on ECs in vitro to stimulate migration, protease production, proliferation, and differentiation into capillary-like tubes.^{7,8)} However, HGF does not promote the proliferation and migration of vascular SMCs. Because of its unique ability to act as a mitogen and a motogen for ECs, HGF shows the suppression of intimal hyperplasia after EC injury.⁹⁾

We hypothesized that HGF would prevent obstructive intimal hyperplasia in the carotid artery after balloon injury. This study was performed to evaluate the effect of HGF on endothelial healing in a rat balloon-injured carotid artery model.

Materials and Methods

All surgical procedures were performed by one trained operator, and all were carried out under the approval of the Ethics Committee on Animal Experiment in Yamaguchi University School of Medicine and carried out under the control of the Guideline for Animal Experiment in Yamaguchi University School of Medicine and The Law (No. 105) and Notification (No. 6) of the Government. Fourteen male, 3-to-4-month-old Sprague-Dawley rats, weighing 510-730 g, were used in this study. Balloon injury was induced in these rats based on a technique established by Reidy et al.¹⁰⁾ In short, all rats were anesthetized intraperitoneal injection of pentobarbital sodium solution of 60 mg/kg. The bifurcation of the left carotid artery was exposed through a midline skin incision in the anterior neck, and the left external carotid artery was temporarily ligated. A 2F Fogarty balloon catheter was introduced via arteriotomy in the left external carotid artery and advanced into the common carotid

artery. The balloon was filled with saline and drawn toward the arteriotomy site three times to produce a deendothelializing injury.

Treatment Groups

The rats were divided into three groups. HGF or vehicle was infused immediately via the jugular vein once after the balloon injury procedure. A control group of 6 rats was given 1.0 ml saline vehicle intravenously. The remaining rats were divided into two groups. One group, comprised of 5 rats, was treated with an intravenous injec-

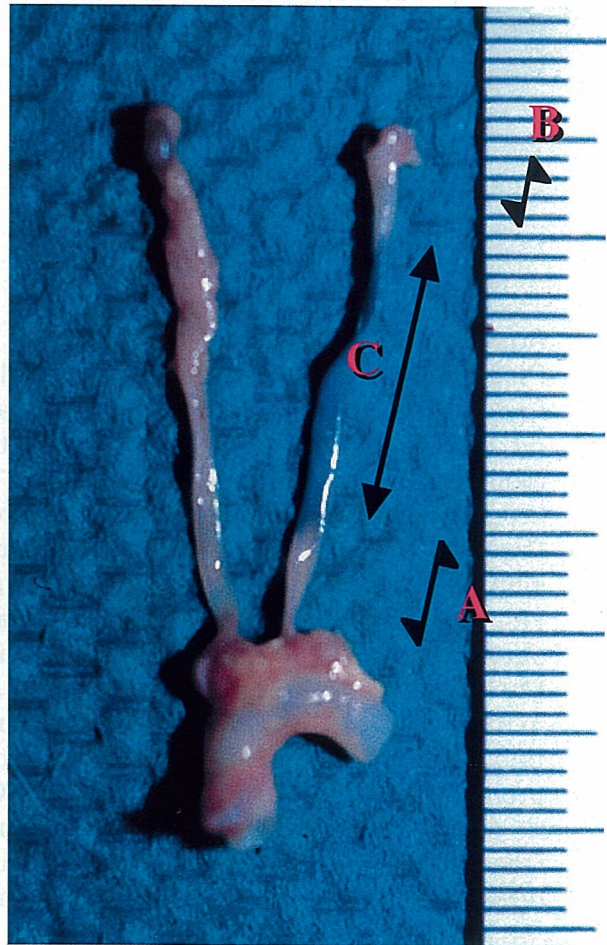


Fig. 1 Photograph shows the harvested balloon-injured left carotid artery of a rat.

(A) extent of endothelial ingrowth from the proximal site (B) extent of endothelial ingrowth from the distal site (C) extent of the Evans Blue stained area, which is the denuded area (no ECs)

tion of 0.1 mg/body HGF (0.1-mg HGF group), and the other group, comprised of 3 rats, was treated with 0.2 mg/body HGF in the same manner (0.2-mg HGF group). The dosages and prescribed period were selected in part based on previous evidence that 0.05-0.25 mg/kg HGF administered as a daily intravenous bolus injection prevents acute renal failure after HgCl₂ administration.¹¹⁾

Reagents

Recombinant human HGF (rhHGF) was purified from a culture medium of CHO cells transfected with an expression vector containing deletion-type human HGF cDNA¹²⁾ and the purity of HGF exceeded 98%.

Specimen Harvest

All rats were killed on postoperative day (POD) 14, and, 30 minutes before anesthetizing, they received an intravenous injection of 40 mg/kg Evans Blue stain via the tail vein. A cannula was inserted into the abdominal aorta to perfuse saline at a pressure of 100 mmHg until the effluent ran clear via bilat-

eral jugular venous vents. This was followed by 5 minutes of fixation with 4% paraformaldehyde. The entire left common carotid artery was harvested. The harvested segment of carotid artery was pinned to a corkboard and photographed in preparation for planimetric analysis of endothelial regrowth. Endothelialized areas were identified by the Evans Blue dye. Maximal endothelial ingrowth from the carotid bifurcation and the aortic arch was measured with a computerized sketching program (Mac Scope, version 2.55, Mitani Corporation, Japan) (Fig. 1). Measured values were averaged for each group of animals and used as representative values.

Evaluation of Intimal Thickness

Harvested specimens were embedded in paraffin and stained with Masson's trichrome stain. The extent of intimal hyperplasia was assessed on the stained specimens based on the cross-sectional area of the intima in relation to that of the media, i. e., and the I/M ratio. The intimal area was defined as the area within the internal elastic lamina minus the lumen; the medial area was defined as the area within the internal elastic lam-

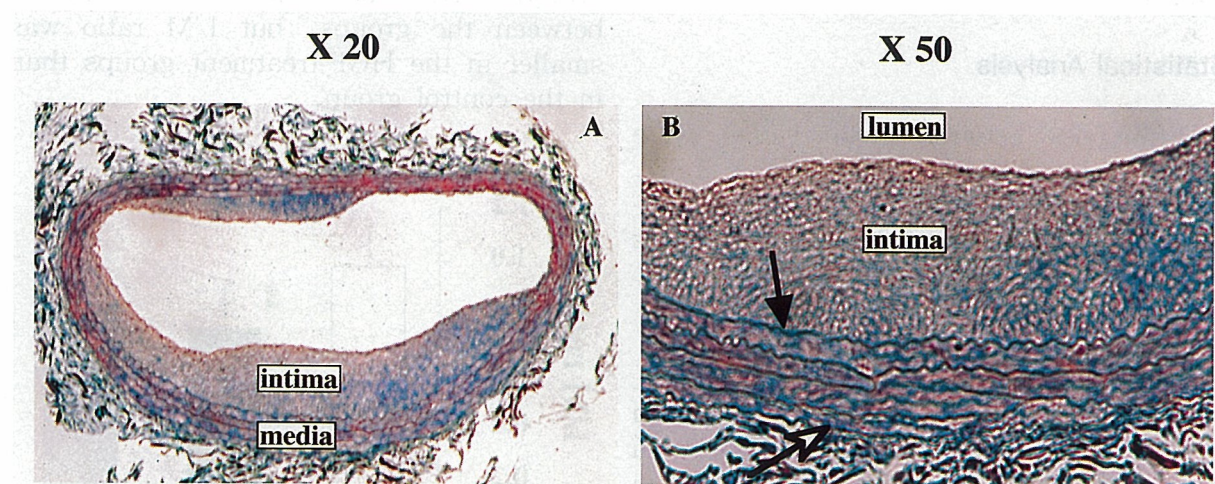


Fig. 2 Neointimal thickness was assessed with the intima area to media area (I / M) ratio measured from cross sections of Masson's trichrome stained specimens at the proximal and distal site of common carotid artery. The intimal area was defined as the area within the internal elastic lamina minus the lumen. The medial area was defined as the area between the internal (black arrow) and external (white arrow) elastic lamina.

A: Masson's trichrome stain $\times 20$ in the original photo B: Masson's trichrome stain $\times 50$ in the original photo.

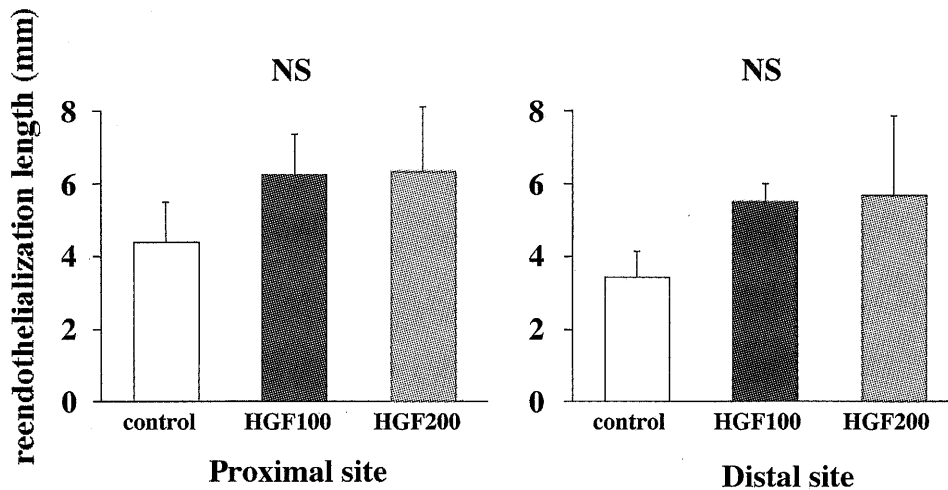


Fig. 3 This graph shows the extent of distal and proximal reendothelialization expressed as the maximal length of area that was not stained with Evans Blue in each group on POD 14. There were no significant differences between groups at either proximal or distal sites. HGF 100: 0.1 mg-HGF group, HGF 200: 0.2 mg-HGF group

ina minus the lumen; the media area was defined as the area between the internal and external elastic lamina. The intima and media at the proximal and distal site of common carotid artery were measured with the computerized sketching program (Fig. 2). These numerical values were averaged for each group and used as representative values.

Statistical Analysis

Differences between mean values were assessed by ANOVA and Fisher's LSD. Values are shown as means \pm the standard error (SEM). A P value of less than 0.05 was considered statistically significant.

Results

Reendothelialization on POD 14 is shown in Fig. 3. The extent of reendothelialization from the proximal site was 4.4 ± 1.22 mm in the control group, 6.25 ± 1.1 mm in the 0.1-mg HGF group, and 6.33 ± 1.76 mm in the 0.2-mg HGF group; the differences between the groups were not significant. The extent of reendothelialization from the distal site was 3.4 ± 0.74 mm in the control group, 5.5 ± 0.5 mm in the 0.1-mg HGF group, and 5.6 ± 2.1 mm in the 0.2-mg HGF group; these differences were not significant. It

was notable, however, that the extent was greater in the HGF-treatment groups than in the control group for both proximal and distal sites.

I/M ratios on POD 14 are shown in Fig. 4. The I/M ratio was 0.98 ± 0.15 in the control group, 0.73 ± 0.17 in the 0.1-mg HGF group, and 0.65 ± 0.05 in the 0.2-mg HGF group. There were no statistical differences between the groups, but I/M ratio was smaller in the HGF-treatment groups than in the control group.

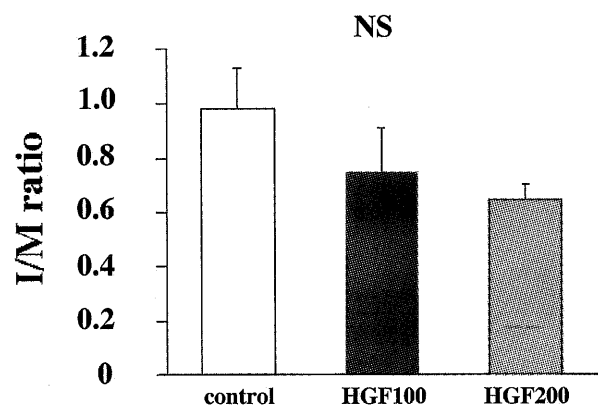


Fig. 4 This graph shows the I/M ratio in each group on POD 14. There were no significant differences among three groups. HGF 100: 0.1 mg-HGF group, HGF 200: 0.2 mg-HGF group

Discussion

In rat balloon-injured carotid artery model, HGF did not significantly inhibit the development of intimal hyperplasia or promote EC ingrowth. Intimal hyperplasia is the most common cause of occlusive disease after percutaneous transluminal angioplasty. It has been hypothesized that vascular endothelial injury and the subsequent exposure of sub-endothelial tissue to plasma constituents cause intimal thickness. In the initial stage of intimal thickness, many factors in addition to platelets, including macrophages derived from monocytes, granulocytes, T-cells adherent to denuded subendothelial tissue, and endothelium near the denuded subendothelial tissue, induce the migration and proliferation of SMCs. SMC proliferation, migration, and synthesis of connective tissue proteins, under the influence of various mitogenic factors, are now considered as major processes in the development of neointima.¹³⁾ SMC are stimulated by proliferative factors, such as platelet derived growth factor (PDGF), fibroblast growth factor (FGF), and transforming growth factor (TGF- β), all of which are released from platelets, macrophages, and ECs for paracrine, and from SMCs for autocrine³⁾. The proliferation of SMCs has been found to be suppressed by antiproliferative factors such as NO, PGI₂, and C-type natriuretic peptide released from ECs¹⁴⁾, and reendothelialization occurs 3~4 weeks after anastomosis¹⁵⁾. Thus, early promotion and facilitation of EC proliferation should control the proliferation and migration of SMCs in the acute phase, thereby preventing intimal hyperplasia.

HGF, vascular endothelial growth factor (VEGF), and bFGF are known to strongly stimulate the proliferation of ECs. The proliferative effect of HGF on ECs is 1.48 times stronger than that of bFGF, whereas that of VEGF is only 1.25 times stronger.¹⁶⁾ Furthermore, bFGF stimulates the proliferation and migration of SMCs, likely causing intimal hyperplasia; but HGF does not promote the proliferation and migration of SMCs.¹⁶⁾ Therefore, HGF seems to be the

most ideal growth factor to be used for the prevention of intimal hyperplasia.^{17,18)}

Morishita et al.¹⁶⁾ suggested that under conditions of EC dysfunction, such as hypertension, hyperglycemia, and hyperlipidemia, local HGF production is reduced by TGF- β and angiotensin II in ECs and vascular SMCs. HGF itself regulates local HGF production by auto-looped positive feedback and works in an autocrine-paracrine manner. A break by TGF- β and angiotensin II in this autocrine-loop that maintains ECs growth may result in abnormal growth of vascular SMCs. Thus, at the time of EC dysfunction, such as in the balloon-injury model, one form of treatment is to supplement HGF by systemic and local prescription. As for reendothelialization length, HGF treated groups was longer than the control group on POD 14. Rapid regeneration of ECs associated with the HGF treatment suggests the effectiveness of HGF in suppressing intimal hyperplasia. The lower I/M ratio on POD 14 in the HGF-treated animals supports the belief that proliferation and migration of SMCs are inhibited by rapid regrowth of ECs. Thus, systemic prescribed HGF was effective in this model, which demonstrates that HGF supplement often could be a treatment option for EC dysfunction. The half-life of HGF concentration in rats, however, has been reported as 3.8 minutes¹⁹⁾, 60% of which is metabolized in the liver; therefore, if HGF was to be administered as a bolus injection, a high dose would be necessary in order to prevent the suppression of intimal thickening. Thus, we thought that it is necessary to change other method of medication and dose of HGF.

Conclusion

HGF was not effective on facilitating intimal healing in terms of endothelial cell ingrowth and suppression of intimal thickening in a rat model of balloon-injured carotid artery.

References

- 1) Forrester, J. S., Fishbein, M., Helfant,

- R. and Fagin, J.: A paradigm for restenosis based on cell biology: clues for the development of new preventive therapies. *J. Am. Coll. Cardiol.*, **17**:758-759, 1991.
- 2) Huber, K. C., Garrat, K. N., Schwartz, R. S. and Edwards, W. D.: Pathology of postinterventional coronary restenosis. In: Schwartz RS, ed. *Coronary Restenosis. Cambridge, UK: Blackwell Scientific Publications*, p.192-204, 1993.
 - 3) Schwartz, S. M., Campbell, G. R. and Campbell, J. H.: Replication of smooth muscle cells in vascular disease. *Cir. Res.*, **58**:427-444, 1986.
 - 4) Nakamura, T., Nawa, K. and Ishihara, A.: Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats. *Biochem. Biophys. Res. Commun.*, **122**:1450-1459, 1984.
 - 5) Nakamura, T., Teramoto, H. and Ichihara, A.: Purification and characterization of a growth factor from rats platelets for mature parenchymal hepatocytes in primary cultures. *Proc. Natl. Acad. Sci. USA*, **83**:6489-6493, 1986.
 - 6) Bottaro, D. P., Rubin, J. S., Falletto, D. L., Chan, A. M. L., Kmiecik, E., Vande Woude, G. F. and Aaronson, S. A.: Identification of the hepatocyte growth factor receptor as the c-met protooncogene product. *Science*, **254**: 1382-1385, 1991.
 - 7) Weidner, K. M., Arakaki, N., Hartmann, G., Vandekerckhove, J., Weingart, S., Rieder, H., Christa, F., Hirohito, T., Tadashi, H., Yasushi, D. and Walter, B.: Evidence for the identity of human scatter factor and human hepatocyte growth factor. *Proc. Natl. Acad. Sci. USA*, **88**: 7001-7005, 1991.
 - 8) Montesano, R., Matsumoto, K., Nakamura, T. and Orti, L.: Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. *Cell*, **67**:901-908, 1991.
 - 9) Nakamura, T.: Structure and function of hepatocyte growth factor. *Prog. Growth Factor Res.*, **3**:67-86, 1991.
 - 10) Reidy, M. A., Clowes, A. W. and Schwartz, S. M.: Endothelial regeneration, ? : inhibition of endothelial regrowth in arteries of rat and rabbit. *Lab. Invest.*, **49**:569-575, 1983.
 - 11) Kawaida, K., Matsumoto, K., Shimazu, H. and Nakamura, T.: Hepatocyte growth factor prevents acute renal failure and accelerates renal regeneration in mice. *Proc. Natl. Acad. Sci. USA*, **91**:4357-4361, 1994
 - 12) Seki, T., Ihara, I., Sugimura, A., Shimonishi, M., Nishizawa, T., Asai, O., Hagiya, M., Nakamura, T. and Shimizu, S.: Isolation and expression of cDNA for different forms of hepatocyte growth factor from human leukocyte. *Biochem. Biophys. Res. Commun.*, **172**:321-327, 1990.
 - 13) O-hara, M., Esato, K. and Mohri, H.: Effect of pancreatopeptidase E (Elastase) on the suppression of intimal fibrous proliferation after arterial reconstruction in high cholesterol fed rabbits. *J. Cardiovasc. Surg.*, **29**:268-276, 1988.
 - 14) Tare, M., Parkington, H. C., Coleman, H. A., Neild, T. O. and Dusting, G. J.: Hyperpolarization and relaxation of atrial smooth muscle caused by nitric oxide derived from the endothelium. *Nature*, **346**:69-71, 1990.
 - 15) Aijima, U.: A pathological study on the repair processes at anastomotic site of atherosclerotic aorta in cholesterol-fed rabbits (In Japanese). *J. Jap. Coll. Angiol.*, **29**:295-304, 1989.
 - 16) Morishita, R., Aoki, M., Nakamura, S., Matsushita, H., Tomita, N., Hayasi, S., Moriguchi, A., Matsumoto, K., Nakamura, T., Higaki, J. and Ogihara, T.: Potential role of a novel vascular modulator, hepatocyte growth factor (HGF), in cardiovascular disease: characterization and regulation of local HGF system. *J. Atheroscler. Thromb.*, **4**:12-19, 1997.
 - 17) Nakamura, Y., Morishita, R., Nakamura, S., Aoki, M., Moriguchi, A., Matsumoto, K., Nakamura, T., Higaki, J. and Ogihara, T.: A vascular modulator, hepatocyte growth factor, is associated with systolic blood pressure. *Hypertension*, **28**:409-413, 1996.

- 18) Nakamura, Y., Morishita, R., Higaki, J., Kida, I., Aoki, M., Moriguchi, A., Yamada, K., Hayashi, S., Toshikage, Yo., Nakano, H., Matsumoto, K., Nakamura, T. and Ogihara, T.: Hepatocyte growth factor is a novel member of the endothelium-specific growth factors: additive stimulatory effect of hepatocyte growth factor with basic fibroblast growth factor but not with vascular endothelial growth factor. *Hypertension*, **14**:1067-1072, 1996.
- 19) R. Appasamy, Tanabe, M., Murase, M., R. Zarnegar., R. Venkataramanan., David H. V. T. and Michalopoulos, G. K.: Hepatocyte growth factor, blood clearance, organ uptake, and biliary excretion in normal and partially hepatectomized rats. *Lab. Invest.*, **68**:270-276, 1993.