Impaired endothelial dysfunction in diabetes mellitus rats was restored by oral administration of prostaglandin I₂ analogue

K Matsumoto¹, R Morishita^{1,2}, N Tomita¹, A Moriguchi¹,
K Yamasaki¹, M Aoki¹, K Matsumoto³, T Nakamura³, J Higaki¹
and T Ogihara¹

¹Department of Geriatric Medicine, Graduate School of Medicine, Osaka University, 2-2 Yamada-oka, Suita 565-0871, Japan

²Division of Gene Therapy Science, Graduate School of Medicine, Osaka University, Suita 565-0871, Japan

³Division of Biochemistry, Biochemical Research Centre, Graduate School of Medicine, Osaka University, Suita 565-0871, Japan

(Requests for offprints should be addressed to R Morishita; Email: morishit@geriat.med.osaka-u.ac.jp)

Abstract

We have previously reported that a decrease in hepatocyte growth factor (HGF), which has many protective functions against endothelial damage by high D-glucose, might be a trigger of endothelial injury. However, the regulation of vascular HGF in diabetes mellitus (DM) has not been clarified *in vivo*, although vascular disease is frequently observed in DM patients. In addition, our previous report revealed that a prostaglandin I₂ (PGI₂) analogue prevented endothelial cell death through the induction of vascular HGF production in cultured human epithelial cells. Thus, in this study, we examined the effects of a PGI₂ analogue in the regulation of the local HGF system using DM rats.

A PGI₂ analogue (beraprost sodium; 300 and 600 µg/kg per day) or vehicle was administered to 16-week-old DM rats induced by administration of streptozotocin for 28 days. Endothelial function was evaluated by the vasodilator response to acetylcholine, and the expression of vascular HGF mRNA was measured by Northern blotting. Of importance, expression of HGF mRNA was significantly decreased in the blood vessels of DM rats as compared with non-DM ($P < 0.01$). In addition, the *in vitro* vasodilator

response of the abdominal aorta to acetylcholine was markedly impaired in DM rats. Importantly, the vasodilator response was restored by PGI₂ treatment in a dose-dependent manner ($P < 0.01$), whereas N(omega)-nitro-L-arginine methyl ester inhibited the restoration of endothelial function. Of particular interest, vascular HGF mRNA and protein were significantly increased in the blood vessels of DM rats treated with PGI₂ as compared with vehicle. Similarly, an increase in HGF protein was also confirmed by immunohistochemical analysis. In addition, the specific HGF receptor, c-met, was also increased by PGI₂ treatment.

Overall, this study demonstrated that treatment with a PGI₂ analogue restored endothelial dysfunction in DM rats, accompanied by the induction of vascular HGF and c-met expression. Increased local vascular HGF production by a PGI₂ analogue may prevent endothelial injury, potentially resulting in the improvement of endothelial dysfunction.

Journal of Endocrinology (2002) **175**, 217–223

Introduction

Endothelial cells are known to secrete various vasoactive substances. Recently, it has been hypothesized that endothelial cells may also modulate vascular growth, because they secrete many anti-proliferative factors such as nitric oxide (NO) and vascular natriuretic peptides (Itoh *et al.* 1990, Tare *et al.* 1990, Suga *et al.* 1992). Therefore, it is apparent that dysfunction of endothelial cells may promote abnormal vascular growth such as in atherosclerosis and arteriosclerosis (Dzau & Gibbons 1991, Dzau 1993). Our previous studies demonstrated that hepatocyte growth factor (HGF) exclusively stimulated the growth of endothelial cells without the replication of vascular

smooth muscle cells (VSMCs) (Nakamura *et al.* 1996a,b). Moreover, the presence of a local HGF system (HGF and its receptor, c-met) in endothelial cells and VSMCs was also demonstrated *in vitro* as well as *in vivo* (Nakamura *et al.* 1995). Thus, it is important to know the physiological role of HGF in endothelial regulation, as the loss of anti-proliferative substances from endothelial cells might be related to the development and progression of atherosclerosis/arteriosclerosis in diabetes mellitus (DM) and hypertension.

On the other hand, prostaglandin (PG) I₂ has been reported to have a cytoprotective action on endothelial cells and to inhibit VSMC growth (Nilsson & Olsson 1984, Kainoh *et al.* 1992, Koh *et al.* 1993). PGI₂ is well known

to stimulate cAMP, which plays a pivotal role in the regulation of glucose metabolism, platelet aggregation and VSMC relaxation. Thus, PGI₂ analogues are widely used for the treatment of peripheral arterial disease observed in DM (Numano *et al.* 1992, Uchikawa *et al.* 1992, Fujitani *et al.* 1995). DM is characterized by the premature development of microvascular and macrovascular disease (Kannel & McGee 1979, Graier *et al.* 1993, Hsueh & Anderson 1993), and hyperglycaemia is an independent risk factor for the development of cardiovascular disease (Henry & Genuth 1996). Interestingly, high glucose treatment caused endothelial cell death through a decrease in local HGF production (Morishita *et al.* 1997a). As addition of recombinant HGF prevented the endothelial cell death induced by high glucose (Morishita *et al.* 1997a), HGF seems to play a pivotal role in the regulation of endothelial cells in DM. Interestingly, our previous report documented that PGI₂ stimulated local vascular HGF production using *in vitro* cultured VSMCs and endothelial cells (Morishita *et al.* 1997b). However, none of the reports described the *in vivo* effects of PGI₂ on local HGF production. Therefore, in this study, we examined the effects of a stable oral PGI₂ analogue (beraprost sodium) on local HGF production in DM rats, to clarify the potential role of HGF in endothelial injury induced by high D-glucose.

Materials and Methods

Experimental design

DM was induced in male Wistar-Kyoto (WKY) rats (16-weeks-old; Charles River Breeding Laboratories, Tokyo, Japan) by a single i.p. injection of streptozotocin (35 mg/kg in 50 mM citric acid buffer, pH 4.5). Then, 10 days later, blood was obtained periorbitally after 8 h of fasting. Only rats having a blood glucose concentration above 300 mg/dl were kept in the protocol and randomized for experiments. The rats were maintained and bred under conventional conditions with food and tap water provided freely, except on days of blood sampling, and a 12 h light:12 h darkness photoperiod. These rats were divided into three groups and treated for 28 days as follows: vehicle (distilled water) or beraprost sodium (300 or 600 µg/kg per day) (each *n*=13). The drugs were donated by Yamanouchi Pharmaceutical Company (Tokyo, Japan). The animals were randomly allocated to each group, and drugs were administered by gavage. After treatment, the rats were killed by decapitation and blood was collected. Systolic blood pressure (BP) was measured in conscious rats using the tail-cuff method with a sphygmomanometer (Softron Co., Ltd, Tokyo, Japan). All rats were free to drink water and eat standard laboratory rat chow (containing 11.3 mEq Na⁺/100 g, 32.6 mEq K⁺/100 g, and 24.6% protein by weight; Oriental Kobo Co.,

Osaka, Japan). Throughout the experiment, rats were housed in metabolic cages under light- and temperature-controlled conditions.

Evaluation of vasodilator properties in response to acetylcholine

Freshly harvested abdominal aortas were cleaned of fat and connective tissues, cut into helical strips, and mounted in 30 ml organ baths, containing Krebs-Henseleit buffer (120 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 5.5 mM glucose, pH 7.4) maintained at 37 °C and oxygenated with 95% O₂, 5% CO₂ (Hayashi *et al.* 2000). Vessels were equilibrated for 60 min, with changes of bathing fluid every 15 min. Isometric tension studies were performed using a Grass model 7D polygraph. Optimal resting tension was determined in baseline studies, and the response to vasoactive drugs was then determined. Cumulative dose-response curves to phenylephrine (PE; 10⁻⁹ to 10⁻⁴ M) were established. The vessels were then submaximally precontracted with PE (typically 3 × 10⁻⁶ M), and endothelial function was evaluated by means of vascular relaxation to acetylcholine (10⁻⁹ to 10⁻⁴ M). NO mediation of acetylcholine responses was confirmed by blocking acetylcholine-induced relaxation by N(omega)-nitro-L-arginine methyl ester (L-NAME) (1 mM), a specific competitive inhibitor of NO synthase. Contractile responses were measured from the polygraph chart and expressed as a percentage of the maximal contraction or, for relaxation, as a percentage of the precontracted tension.

Measurement of tissue and serum HGF concentration

Vascular HGF concentration was determined by enzyme immunoassay (EIA) using anti-rat HGF antibodies (HGF EIA kit: Tokushumeneki Institute, Tokyo, Japan) (Yamada *et al.* 1995). The aortas were promptly removed without excess fat after perfusion from the apex of the heart with saline, frozen in liquid nitrogen, and stored at -70 °C until use. On the day of extraction, the tissue was thawed at 4 °C, and homogenized with a polytron in assay solution. Each specimen was centrifuged at 20 000 g for 30 min at 4 °C, to remove the lysates. Tissue HGF concentration was determined by EIA using anti-rat HGF antibody (Yamada *et al.* 1995).

RNA analysis

The blood vessels were promptly removed, immediately frozen in liquid nitrogen and stored at -80 °C prior to RNA extraction. Total RNA was extracted from three individual blood vessels with guanidine thiocyanate by ultracentrifugation through a dense cushion of CsCl in a standard manner. For Northern blot analysis, 20 µg total RNA were subjected to electrophoresis on 1.5%

agarose-formaldehyde denaturing gel and transferred to a nitrocellulose membrane (Amersham International). The filter was baked, prehybridized, and hybridized to full-length cDNA for the rat HGF probe labelled with ^{32}P . Then the filter was washed and exposed to the films, and analysed by densitometry (Shimazu, Kyoto, Japan).

Immunohistochemical analyses

The aortas were also stained for immunoreactive HGF and c-met proteins. Tissue specimens (6 μm thick) from the mid-section of the blood vessels were sectioned at 60 μm intervals along the vessel in a proximal to distal fashion. Sections were stained using an enzyme immunohistochemical kit (Histostain-SP kit; Zymed Lab., Inc., South San Francisco, CA, USA) with rabbit polyclonal antibody against rat HGF (1:1000 dilution; Tokushu Meneki Institute (Tsuda *et al.* 1992)) and rat c-met (1:1000 dilution; Tokushu Meneki Institute (Nakamura *et al.* 2000)), which was tested for cross-reactivity with rat HGF (Nakamura *et al.* 2000).

Statistical analysis

All values are expressed as means $\pm$ S.E.M. ANOVA with a subsequent Bonferroni/Dunnett test was employed to determine the significance of differences in multiple comparisons. Values of $P < 0.05$ were considered statistically significant.

Results

As previously reported (Morishita *et al.* 1997a, Hayashi *et al.* 1999), HGF enhances endothelial regeneration without VSMC replication and protects against endothelial damage caused by high glucose, hypoxia or angiotensin II (Ang II), probably through potent mitogenic and anti-apoptotic actions on endothelial cells. In addition, our previous studies demonstrated that high D-glucose down-regulated vascular HGF expression in VSMCs and endothelial cells (Morishita *et al.* 1997a). Therefore, we further examined the effect of high D-glucose on local vascular HGF production in DM rats. In the WKY rats, HGF mRNA could be readily detected in the blood vessels by Northern blotting (Fig. 1). In contrast, a significant reduction of vascular HGF mRNA was observed in DM rats as compared with non-DM rats, as assessed by Northern blot analysis (Fig. 1). Similarly, vascular HGF protein was significantly decreased in DM rats as compared with non-DM rats (Fig. 2, $P < 0.01$). On the other hand, we found that cAMP stimulants such as PGI_2 stimulated local vascular HGF production in cultured VSMCs (Morishita *et al.* 1997b). Thus, rats were treated with a low (300 $\mu\text{g/kg}$) or high (600 $\mu\text{g/kg}$) concentration

Figure 1 Vascular HGF mRNA in aortas of diabetic rats. DM=diabetic rats, DM+ PGI_2 (300 $\mu\text{g/kg/day}$)=DM rats treated with a low dose of beraprost sodium (300 $\mu\text{g/kg}$ per day), DM+ PGI_2 (600 $\mu\text{g/kg/day}$)=DM rats treated with a high dose of beraprost sodium (600 $\mu\text{g/kg}$ per day), Endothelial cells=human aortic endothelial cells (positive control), WKY=non-diabetic WKY rats. GAPDH=glyceraldehyde 3-phosphate dehydrogenase. HGF mRNA expression was identical to the precise size (2.1 kb).

Figure 2 Vascular HGF concentration in aortas of diabetic rats as assessed by EIA. non DM=non-diabetic rats, DM=diabetic rats, untreated=DM rats treated with vehicle, PGI_2 (300)=DM rats treated with a low dose of beraprost sodium (300 $\mu\text{g/kg}$ per day), PGI_2 (600)=DM rats treated with a high dose of beraprost sodium (600 $\mu\text{g/kg}$ per day). $n=8$ per group (means $\pm$ S.E.M.).

of a stable PGI_2 analogue (beraprost sodium) or vehicle for 28 days. Of importance, vascular HGF protein was also significantly increased in DM rats treated with beraprost sodium as compared with vehicle (Fig. 2, $P < 0.01$). Consistent with the increase in HGF protein, administration of beraprost sodium significantly attenuated

Figure 3 (A) Expression of HGF protein in aortas of DM rats as assessed by immunohistochemical staining. Vehicle=DM rats treated with vehicle, PGI₂=DM rats treated with a high dose of beraprost sodium (600 µg/kg per day). (B) Expression of c-met protein in aortas of DM rats as assessed by immunohistochemical staining. Vehicle=DM rats treated with vehicle, PGI₂=DM rats treated with a high dose of beraprost sodium (600 µg/kg per day)

the decrease in vascular HGF mRNA induced by DM (vehicle 100%, +PGI₂ 37.2 ± 8.2%, $P < 0.01$).

We also examined staining for HGF and its specific receptor, c-met, in the aorta of DM rats. In non-DM rats, positive staining for HGF protein could be detected in VSMCs in the media and endothelial cells in the endothelium. In contrast, a marked reduction of vascular HGF protein was observed in DM rats as compared with non-DM rats as assessed by immunohistochemical analysis (Fig. 3A). Similarly, as shown in Fig. 3B, in non-DM rats, positive staining for c-met was observed in the endothelium, while little staining for c-met was detected in the blood vessels of DM rats. Interestingly, positive staining for vascular HGF protein and c-met was markedly increased in DM rats treated with beraprost sodium as compared with vehicle (Figs 2 and 3). Positive staining for HGF protein could be detected mainly in both endothelial cells and VSMCs in DM rats treated with beraprost sodium, whereas positive immunostaining for c-met could be

detected mainly in endothelial cells in DM rats treated with beraprost sodium (Fig. 3B).

Finally, we examined the effects of high glucose on vasodilator response. As shown in Fig. 4, blood vessels from DM rats treated with vehicle demonstrated impairment of the vasodilator response to acetylcholine administration as compared with untreated non-DM rats ($P < 0.01$). In contrast, administration of acetylcholine into pre-contracted vessels from DM rats treated with beraprost sodium resulted in significant dilation as compared with vessels treated with vehicle, in a dose-dependent manner (Fig. 4A and B, $P < 0.01$). The endothelium-dependent dilation in arteries treated with beraprost sodium was also supported by the observation that the increase in dilation was completely abolished by administration of L-NAME (Fig. 4B). In contrast, there was no significant change in systolic BP or body weight in rats treated with low and high concentrations of beraprost sodium (data not shown). Similarly, there was no significant difference in

Figure 4 (A) Representative vasodilator response of aortas of diabetic rats treated with PGI₂ analogue in response to acetylcholine (Ach). non-DM=non-diabetic rats, DM=diabetic rats treated with vehicle, PGI₂=diabetic rats treated with a high dose of beraprost sodium (600 µg/kg per day). (B) Effect of administration of PGI₂ analogue on the vasodilator response of aortas of diabetic rats to Ach. $n=8$ per group, means $\pm$ S.E.M. (◆) Non-DM, (■) DM treated with vehicle, (□) L-NAME treatment of DM with vehicle, (▲) diabetic rats treated with a low dose of beraprost sodium (300 µg/kg per day), (●) diabetic rats treated with a high dose of beraprost sodium (600 µg/kg per day), (△) L-NAME treatment of DM treated with a low dose of beraprost sodium (300 µg/kg per day), (○) L-NAME treatment of DM treated with a high dose of beraprost sodium (600 µg/kg per day). ** $P<0.01$ vs DM, # $P<0.01$ vs high.

blood glucose level among all groups (vehicle 612 ± 31 , beraprost sodium 300 µg/kg per day 604 ± 31 mg/dl, not significant).

Discussion

HGF is a mesenchyme-derived pleiotropic factor which regulates cell growth, cell motility, and morphogenesis of various types of cells, and is thus considered a humoral mediator of epithelial-mesenchymal interactions responsible for morphogenic tissue interactions during embryonic development and organogenesis (Matsumoto & Nakamura 1991, Nakamura 1991, Boros & Miller 1995). Moreover, we found that HGF fulfils the characteristics of an endothelium-specific growth factor, as HGF can stimulate the growth of endothelial cells without VSMC replication (Nakamura *et al.* 1996a,b). Therefore, we hypothesized that HGF may also have an important role in the regulation of endothelial cells. Indeed, we have previously reported that endothelial cell death induced by high D-glucose is accompanied by decreased local HGF production in vascular cells through transforming growth factor- β (TGF- β) activation (Morishita *et al.* 1997a). This phenomenon should be important, since dysfunction of endothelial cells causing loss of multiple endothelium-derived substances (PGI₂, NO, C-type natriuretic peptide) results in the progression of arteriosclerotic vascular changes in DM (Kannel & McGee 1979, Graier *et al.* 1993, Hsueh & Andersen 1993).

From this viewpoint, HGF would be more interesting, as HGF can abrogate the cell death of endothelial cells mediated by various conditions such as high D-glucose through the inhibition of apoptosis (Morishita *et al.* 1997b, Hayashi *et al.* 2000). Recently, we revealed that local HGF expression in vascular cells was up-regulated by cAMP, and down-regulated by Ang II and TGF- β (Morishita *et al.* 1997b, Hayashi *et al.* 2000). In addition, local HGF production in blood vessels was decreased in association with increased glucose concentration (Nakamura *et al.* 1998). Since multiple VSMC-derived substances have profound influences on the maintenance of endothelium (Dzau & Gibbons 1991, Dzau 1993), secretion of endothelial protectants from vascular cells is very important in the control of endothelial function. Expectedly, the present study demonstrated a significant decrease in vascular HGF mRNA and protein in DM rats. Probably, a decrease in local HGF production in VSMCs by high D-glucose may affect endothelial cell growth in a paracrine manner.

Thus, we studied the effects of a PGI₂ analogue, a drug well known to improve peripheral arterial disease in patients with DM (Numano *et al.* 1992, Uchikawa *et al.* 1992, Fujitani *et al.* 1995), on the vasodilator response of blood vessels in DM rats, to examine the contribution of an increase in vascular HGF to maintenance of the

endothelium. Of importance, PGE, a PGI₂ analogue, and cilostazol, which induce cAMP accumulation, stimulated vascular HGF expression in human cultured VSMCs and endothelial cells (Morishita *et al.* 1997b). Nevertheless, no report has documented the regulation of vascular HGF by these agents *in vivo*. The present study demonstrated that a stable PGI₂ analogue, beraprost sodium, increased local HGF expression in blood vessels in DM rats. Given that high D-glucose initiates apoptosis of endothelial cells (Baumgartner-Parzer *et al.* 1995), increased HGF production by beraprost sodium may attenuate apoptosis or injury of endothelial cells. This increase in local HGF production by a PGI₂ analogue may be due to an increase in cAMP, since the promoter region of HGF gene contains a cAMP responsive element (Okajima *et al.* 1993). Increased local HGF production from vascular cells by a PGI₂ analogue may improve endothelial dysfunction induced by high glucose, in addition to the direct vasodilative effects on VSMCs. This hypothesis is supported by the observation that overexpression of HGF gene accelerated endothelial regeneration and improved endothelial dysfunction in a rat balloon injury model (Hayashi *et al.* 2000). Thus, this hypothesis was supported by the observation that there was no significant difference in BP and plasma glucose level between rats treated with vehicle and beraprost sodium. Reversal of endothelial dysfunction by a PGI₂ analogue seems to be independent from haemodynamic effects. Unfortunately, the present study did not address the extent of regrowth of endothelial cells, since the current methodology to test the endothelial condition is quite limited. However, the increased vascular HGF production by these agents may contribute to the usefulness of PGI₂ analogues in the treatment of peripheral arterial disease such as arteriosclerosis obliterans observed in DM, although further studies are needed. Our previous clinical data demonstrated a significant negative correlation between serum HGF and glycosylated haemoglobin concentrations in DM patients without complications, probably due to the exposure of chronic high glucose (Nakamura *et al.* 1998). Decrease in a local and circulating endothelial stimulant, HGF, in DM may be a trigger of endothelial dysfunction, potentially resulting in the development and/or progression of vascular changes.

Overall, we demonstrated that treatment with a PGI₂ analogue improved endothelial dysfunction induced by high D-glucose, probably through activation of local HGF production. Increased local vascular HGF production by PGs may prevent endothelial injury, potentially resulting in the improvement of peripheral arterial disease.

Acknowledgements

We wish to thank Rie Kosai and Keiko Yamaguchi for their excellent technical assistance. This work was partially

supported by grants from the Japan Health Sciences Foundation, a Grant-in-Aid from The Ministry of Public Health and Welfare, a Grant-in-Aid for the Development of Innovative Technology, a Grant-in-Aid from Japan Promotion of Science, and through Special Coordination Funds from the Ministry of Education, Culture, Sports, Science and Technology, the Japanese Government.

References

- Baumgartner-Parzer SM, Wagner L, Pettermann M, Grillari J, Gessi A & Waldhausl W 1995 High-glucose-triggered apoptosis in cultured endothelial cells. *Diabetes* **44** 1323–1327.
- Boros P & Miller CM. 1995 Hepatocyte growth factor: a multifunctional cytokine. *Lancet* **345** 293–295.
- Dzau VJ 1993 The role of mechanical and humoral factors in growth regulation of vascular smooth muscle cells and cardiac myocytes. *Current Opinion in Nephrology and Hypertension* **2** 27–32.
- Dzau VJ & Gibbons GH 1991 Endothelium and growth factors in vascular remodeling of hypertension. *Hypertension* **18** (Suppl III) 115–121.
- Fujitani K, Kambayashi J, Murata K, Yano Y, Shiozaki K, Yukawa M, Sakon M, Murata T, Kawasaki T, Shiba E & Mori T 1995 Clinical evaluation of combined administration of oral prostacyclin analogue beraprost and phosphodiesterase inhibitor cilostazol. *Pharmacological Research* **31** 121–125.
- Graier WF, Wascher TC, Lackner L, Toplak H, Krejs GJ & Kukovetz WR 1993 Exposure to elevated D-glucose concentrations modulates vascular endothelial cell vasodilatory response. *Diabetes* **42** 1497–1505.
- Hayashi S, Morishita R, Nakamura S, Yamamoto K, Moriguchi A, Nagano T, Taizi M, Noguchi H, Matsumoto K, Nakamura T, Higaki J & Ogihara T 1999 Potential role of hepatocyte growth factor, a novel angiogenic growth factor, in peripheral arterial disease: down-regulation of HGF in response to hypoxia in vascular cells. *Circulation* **100** II301–II308.
- Hayashi K, Nakamura S, Morishita R, Moriguchi A, Aoki M, Matsumoto K, Nakamura T, Kaneda Y, Sakai N & Ogihara T 2000 *In vivo* transfer of human hepatocyte growth factor gene accelerates re-endothelialization and inhibits neointimal formation after balloon injury in rat model. *Gene Therapy* **7** 1664–1671.
- Henry RR & Genuth S 1996 Forum one: current recommendations about intensification of metabolic control in non-insulin-dependent diabetes mellitus. *Annals of Internal Medicine* **124** 175–177.
- Hsueh WA & Anderson PW 1993 Systemic hypertension and the renin-angiotensin system in diabetic vascular complications. *American Journal of Cardiology* **72** 14H–21H.
- Itoh H, Pratt RE & Dzau VJ 1990 Atrial natriuretic polypeptide inhibits hypertrophy of vascular smooth muscle cells. *Journal of Clinical Investigation* **86** 1690–1697.
- Kainoh M, Nishio S & Nakadate T 1992 Cytoprotective action of beraprost sodium against peroxide-induced damage in vascular endothelial cells. *Pharmacology* **45** 61–70.
- Kannel WB & McGee DL 1979 Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. *Diabetes Care* **241** 2035–2038.
- Koh E, Morimoto S, Jiang B, Inoue T, Nabata T, Kitano S, Yasuda O, Fukuo K & Ogihara T 1993 Effects of beraprost sodium, a stable analogue of prostacyclin, on hyperplasia, hypertrophy and glycosaminoglycan synthesis of rat aortic smooth muscle cells. *Artery* **20** 242–252.
- Matsumoto K & Nakamura T 1991 Roles of HGF as a pleiotropic factor in organ regeneration. In *Hepatocyte Growth Factor-Scatter Factor (HGF-SF) and C-Met Receptor*, pp 225–249. Eds ID Goldberg & EM Rosen. Basel: Birkhauser Verlag.

- Morishita R, Nakamura S, Nakamura Y, Aoki M, Moriguchi A, Kida I, Yo Y, Matsumoto K, Nakamura T, Higaki J & Ogihara T 1997a Potential role of endothelium-specific growth factor, hepatocyte growth factor, in endothelial damage in diabetes mellitus. *Diabetes* **46** 138–142.
- Morishita R, Higaki J, Hayashi S, Yo Y, Aoki M, Nakamura S, Moriguchi A, Matsushita H, Matsumoto K, Nakamura T & Ogihara T 1997b Role of hepatocyte growth factor in endothelial regulation: prevention of high D-glucose-induced endothelial cell death by prostaglandins and phosphodiesterase type 3 inhibitor. *Diabetologia* **40** 1053–1061.
- Nakamura S, Morishita R, Moriguchi A, Yo Y, Nakamura Y, Hayashi S, Matsumoto K, Nakamura T, Higaki J & Ogihara T 1998 Hepatocyte growth factor as a potential index of complication in diabetes mellitus. *Journal of Hypertension* **16** 2019–2026.
- Nakamura T 1991 Structure and function of hepatocyte growth factor. *Progress in Growth Factor Research* **3** 67–86.
- Nakamura T, Mizuno S, Matsumoto K, Sawa Y, Matsuda H & Nakamura T 2000 Myocardial protection from ischemia/reperfusion injury by endogenous and exogenous HGF. *Journal of Clinical Investigation* **106** 1511–1519.
- Nakamura Y, Morishita R, Higaki J, Kida I, Aoki M, Moriguchi A, Yamada K, Hayashi S, Yo Y, Matsumoto K, Nakamura T & Ogihara T 1995 Expression of local hepatocyte growth factor system in vascular tissues. *Biochemical and Biophysical Research Communications* **215** 483–488.
- Nakamura Y, Morishita R, Nakamura S, Aoki M, Moriguchi A, Matsumoto K, Nakamura T, Higaki J & Ogihara T 1996a A vascular modulator, hepatocyte growth factor, is associated with systolic blood pressure. *Hypertension* **28** 409–413.
- Nakamura Y, Morishita R, Higaki J, Kida I, Aoki M, Moriguchi A, Yamada K, Hayashi S, Yo Y, Nakano H, Matsumoto K, Nakamura T & Ogihara T 1996b Hepatocyte growth factor (HGF) is a novel member of endothelium-specific growth factors: additive stimulatory effect of HGF with basic fibroblast growth factor, but not vascular endothelial growth factor. *Journal of Hypertension* **14** 1067–1072.
- Nilson J & Olsson AG 1984 Prostaglandin E₁ inhibits DNA synthesis in arterial smooth muscle cells stimulated with platelet-derived growth factor. *Atherosclerosis* **53** 77.
- Numano F, Kishi Y & Ashikaga T 1992 Clinical studies of phosphodiesterase inhibitors for cardiovascular disease. *Advances in Second Messenger and Phosphoprotein Research* **25** 395–407.
- Okajima A, Miyazawa K & Kitamura N 1993 Characterization of the promoter region of the rat hepatocyte-growth-factor/scatter-factor gene. *European Journal of Biochemistry* **213** 113–119.
- Suga S, Nakao K, Itoh H, Komatsu Y, Ogawa Y, Hama N & Imura H 1992 Endothelial production of C-type natriuretic peptide and its marked augmentation by transforming growth factor- β . Possible existence of 'vascular natriuretic peptide system'. *Journal of Clinical Investigation* **90** 1145–1149.
- Tare M, Parkington HC, Coleman HA, Neild TO & Dusting GJ 1990 Hyperpolarization and relaxation of atrial smooth muscle caused by nitric oxide derived from the endothelium. *Nature* **346** 69–71.
- Tsuda H, Iwase T, Matsumoto K, Ito M, Hirono I, Nishida Y, Yamamoto M, Tatematsu M, Matsumoto K & Nakamura T 1992 Immunohistochemical localization of hepatocyte growth factor protein in pancreas islet A-cells of man and rats. *Japanese Journal of Cancer Research* **83** 1262–1266.
- Uchikawa T, Murakami T & Furukawa H 1992 Effects of the anti-platelet agent cilostazol on peripheral vascular disease in patients with diabetes mellitus. *Arzneimittelforschung* **42** 322–324.
- Yamada A, Matsumoto K, Iwanari H, Sekiguchi K, Kawata S, Matsuzawa Y & Nakamura T 1995 Rapid and sensitive enzyme-linked immunosorbent assay for measurement of HGF in rat and human tissues. *Biomedical Research* **16** 105–114.

Received in final form 22 May 2002

Accepted 10 June 2002