LOW MOLECULAR WEIGHT HEPARIN PREVENTS CARDIOVASCULAR REMODELING INDUCED BY THE LONG-TERM INHIBITION OF NITRIC OXIDE SYNTHASE WITH N-NITRO-L-ARGININE METHYL ESTER IN RAT HEARTS

Hitoshi Hasegawa[1)], Takashi Saito[1)], Yoshimasa Fujiwara[2)], Fukiko Kurokawa[1)], Toshimitu Kosaka[1)], Hiroyuki Watanabe[1)], Kenji Iino[3)], Masaru Ishida[1)], Takashi Koyama[1)], Kouichi Kawamura[4)], Hirotake Masuda[4)], Mamoru Miura[5)], and Hiroshi Ito[1)]

(Received 27 July 2004, Accepted 10 August 2004)

[1)]*Division of Cardiovascular Medicine, Department of Internal Medicine, Akita University School of Medicine, Akita 010-8543, Japan,*
[2)]*Fujiwara Memorial Hospital, Akita 010-0201, Japan*
[3)]*Japanese Red Cross Akita Hospital, Akita 010-1495, Japan*
[4)]*Division of Celluar and Organ Pathology, Department of Pathology and Immunology, Akita University School of Medicine, Akita 010-8543, Japan*
[5)]*Akita Medical Center, Akita 010-0874, Japan*

Abstract: To evaluate the effect of low molecular weight heparin (LMWH) on cardiovascular remodeling induced by long term inhibition of nitric oxide synthase with N-nitro-L-arginine methyl ester (L-NAME), 40 male Sprague-Dawley rats were randomly divided into four groups: the control group ($n=10$) that received 0.9% salt solution; the L-NAME group ($n=10$) that received 30 mg/kg/day of L-NAME, the LMWH3000 group ($n=10$) that received 30 mg/kg/day of L-NAME and 4 mg/kg/day of LMWH (molecular weight (M.W.) of 3000) and the LMWH6000 group ($n=10$) that received 30 mg/kg/day of L-NAME and 4 mg/kg/day of LMWH (M.W. 6000). All agents, including saline, were administered intraperitoneally by implantation of osmotic mini pumps. Systolic blood pressure was measured by the tail-cuff method on treatment days 1, 7, 14, and 28, and histological examination of harvested cardiac tissue and coronary arteries was performed on day 28. Systolic blood pressure was greater in the L-NAME, LMWH3000 and LMWH6000 groups when compared with the control group. There were no significant differences in activated coagulation time (ACT) when comparing the four groups. Medial smooth muscle cell proliferation and hypertrophy and increases in perivascular and myocardial fibrosis were observed in the L-NAME group compared with the control group. Further, these changes were inhibited by the coadministration of LMWH3000 or LWMH6000. This study demonstrates that LMWH prevents cardiovascular remodeling induced by L-NAME in rat hearts independent of its anticoagulant activity.

Key words: nitric oxide, low molecular weight heparin, cardiovascular remodeling, L-NAME

Introduction

Nitric oxide (NO) inhibits key steps in the atherogenic process, such as platelet adhesion and aggregation[1,2], adhesion molecule and chemokine expression, inflammatory cell infiltration[3], and smooth muscle cell migration and proliferation[4-7]. Further, long-term inhibition of NO synthesis using N-nitro-L-arginine methyl ester (L-NAME) results in cardiovascular remodeling (e.g., perivascular fibrosis and medial smooth muscle cell proliferation and hypertrophy) in rats[8,9]. Thus, deficits in NO contribute to a state of accelerated atherogenesis, and the L-NAME model offers unique opportunities to explore the atheroprotective properties of various agents. Various heparin fractions are effective in preventing smooth muscle cell hyperplasia after experimental arterial injury[10-16], and this antiproliferative effect appears to be independent of molecular size and anticoagulant effect[17-19]. Low molecular weight heparin (LMWH) have a longer half-life and higher bioavailability and may exert a greater atheroprotective effect than other fractions of heparin[20,21]. Because LMWH is completely absorbed and has relatively weak protein binding capacity, it can be administered subcutaneously in once or twice daily dosing without the need for daily monitoring[22]. Thus, LMWH may represent a viable option as an outpatient strategy to prevent cardiovascular remodeling in the clinical setting.

The purpose of the present study was to evaluate the effect of LMWH on the cardiovascular remodeling that is induced by long-term inhibition of nitric oxide synthase with L-NAME.

Methods

All animal protocols were approved by the Animal Research Committee of Akita University. Further, this investigation conforms to the Guidelines for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Experimental animal preparation

Forty male Sprague-Dawley rats were housed singly in a pyrogen-free facility and fed untreated rat chow. Animals were divided into four different groups: the control group ($n=10$) received 0.9% salt solution; the L-NAME group ($n=10$) received 30 mg/kg/day of L-NAME; the LMWH3000 group ($n=10$) received 30 mg/kg/day of L-NAME and 4 mg/kg/day of LMWH (LMWH, molecular weight (M.W.) of 3000); and the LMWH6000 group ($n=10$) received 30 mg/kg/day of L-NAME and 4 mg/kg/day of LMWH (M.W. 6000). All agents, including saline, were administered intraperitoneally via implantation of osmotic mini pumps.

Arterial systolic blood pressure was measured at the tail artery by the cuff sphygmomanometer method (APOLLO 179, IITC Co. Ltd., Woodland Hills, CA, USA) at five time points during the experimental period (days 0, 1, 7, 14 and 28).

Histopathological examination

On day 28, the abdominal aorta of each rat was cannulated with a polyethylene tube following anesthesia with intraperitoneal sodium pentobarbital (25 mg/kg). Blood samples were collected for assessment of activated coagulation time (ACT), and the heart was perfused with physiological saline and fixed with 3% glutaraldehyde solution at 100 mmHg in a retrograde fashion. Hearts were cut into three portions aligned perpendicular to the long axis (apex, middle and base of left ventricle) for histopathological examination, and left ventricular slices (5 μm thick) were created and mounted on glass slides for Elastica-Masson staining. Computer-assisted planimetry (COSMOSONE 1S,

Table 1 Changes in systolic blood pressure

Day	Groups			
	Control	L-NAME	LMWH3000	LMWH6000
0	109.4±1.3	110.4±3.2	113.0±5.4	108.6±0.9
1	110.2±2.2	136.4±2.8*	131.0±8.8*	128.7±2.8*
7	107.8±1.3	136.2±4.6*	134.0±3.5*	129.1±2.5*
14	107.3±1.8	135.8±6.4*	132.6±3.0*	131.3±4.7*
28	108.1±2.6	136.3±5.2*	132.0±3.1*	134.5±5.8*

Values are expressed as means±standard errors of the mean.
*$p<0.001$ versus the control group (control).

Nikon, Tokyo, Japan) was used to determine the area surrounded by the internal elastic lamina (IEL area), the area within the external elastic lamina (total vascular area: TVA) and the area of perivascular fibrosis (PVF) of each of the five coronary arteries in each slide. Medial layer area (MLA) was calculated as TVA-(IEL area). MLA and PVF values were normalized by dividing by TVA. In each heart, approximately fifteen coronary artery sections were examined. Myocardial fibrosis was determined by quantitative morphometry by scanning at 400x magnification. Percent ratio of fibrotic area (%FR) was calculated in the left ventricular cross-sections as the area of fibrosis divided by the myocardial area in the entire visual field of the section.

Morphometry and cell enumeration were performed by a single observer who was blinded to all treatment protocols.

Drugs and chemicals

All chemicals, including L-NAME, were purchased from Sigma Chemical Co. (Tokyo, Japan).

Statistical analyses

Values are expressed as mean±SEM. Multiple measurements ANOVA was used for statistical analyses. A P value of less than 0.05 was considered statistically significant.

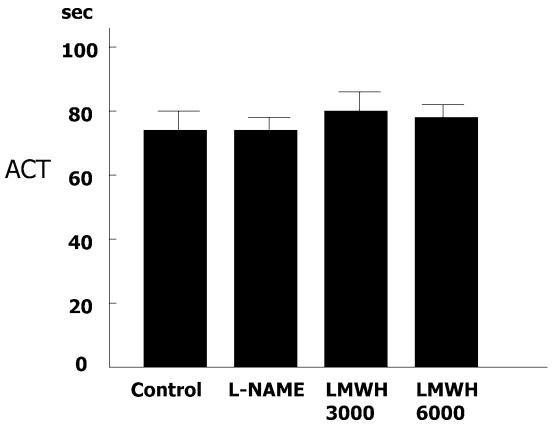

Fig. 1 Activated coagulation time (ACT) data are expressed as mean±SEM. There was no significant difference in ACT when comparing all animal groups.

Results

Body weight

During the 4-week treatment period, body weight increased in all animal groups. However, body weight did not differ significantly among the groups either before or after treatment.

Systolic blood pressure

Systolic blood pressure was greater in the L-NAME, LMWH6000, and LMWH3000 groups when compared with the control group (Table 1).

Activated coagulation time (ACT)

There was no significant difference in ACT when

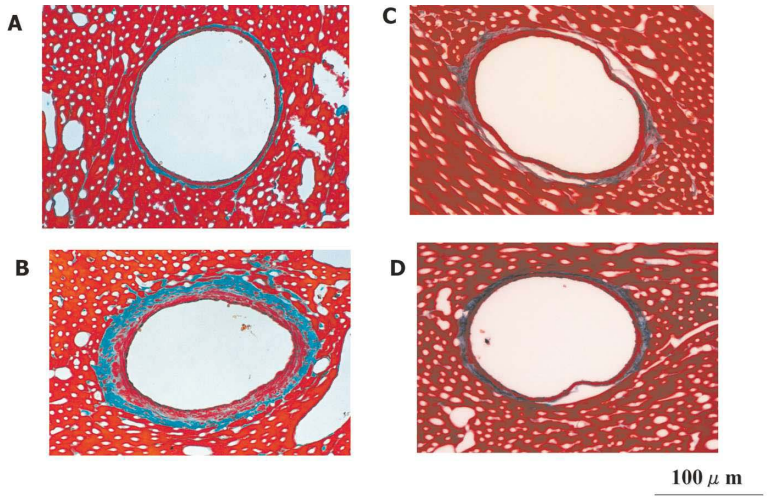

Fig. 2 Micrographs of coronary arteries with Elastica-Masson staining in the control (A), L-NAME (B), LMWH3000 (C) and LMWH6000 (D) groups at four weeks. Medial smooth muscle cell proliferation and hypertrophy and increases in perivascular and myocardial fibrosis were observed in the L-NAME group compared with the control group. Further, these changes were inhibited by the coadministration of either LMWH3000 or LMWH6000

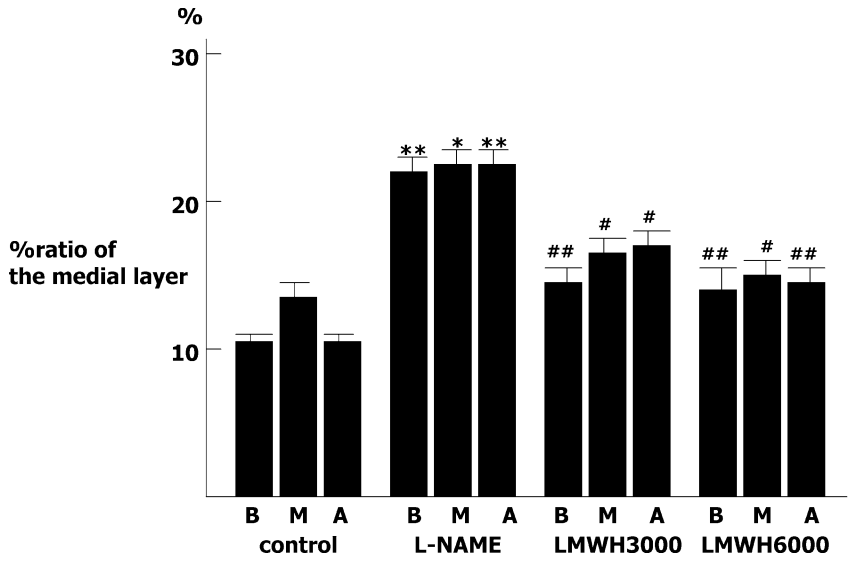

Fig. 3 Percent ratio of medial layer of the coronary artery. See text for detail. Data are expressed as mean±SEM. B: Base, M: Middle, A: Apex. $^{*}p<0.05$ compared to control, $^{**}p<0.01$ compared to control, $^{\#}p<0.05$ compared to L-NAME, $^{\#\#}p<0.01$ compared to L-NAME.

comparing the four groups (Fig. 1).

Histopathological examination

Micrographs of the coronary arteries obtained in each group are shown in Fig. 2. Medial smooth muscle cell proliferation and hypertrophy and increases in perivascular and myocardial fibrosis were observed in the L-NAME group compared

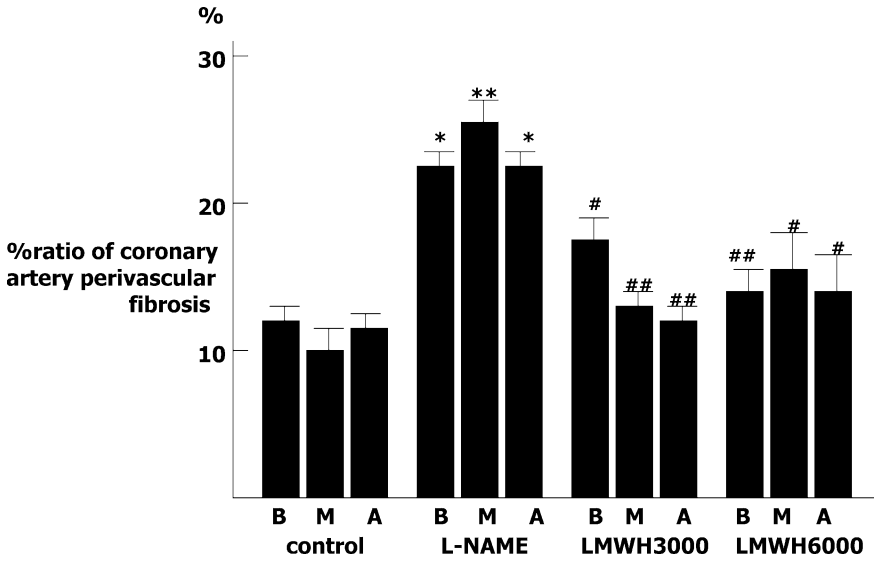

Fig. 4 Percent ratio of coronary artery perivascular fibrosis. See text for detail. Data are expressed as mean±SEM. B : Base, M : Middle, A : Apex. $^{*}p<0.05$ compared to control, $^{**}p<0.01$ compared to control, $^{\#}p<0.05$ compared to L-NAME, $^{\#\#}p<0.01$ compared to L-NAME

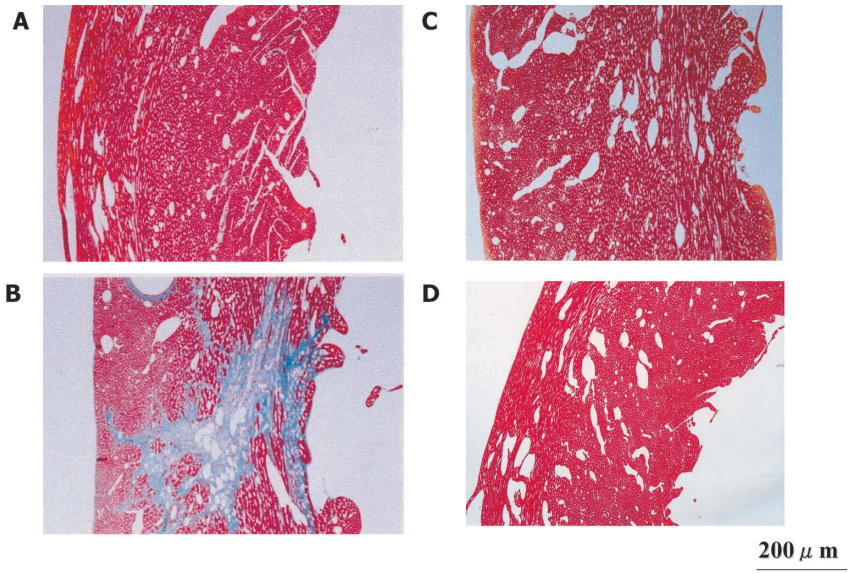

Fig. 5 Micrographs of myocardium with Elastica-Masson staining in the control (A), L-NAME (B), LMWH3000 (C), and LMWH6000 (D) groups at four weeks. Marked myocardial fibrosis is observed in the L-NAME group when compared with control. Further, these changes were inhibited by the coadministration of LMWH3000 or LMWH6000.

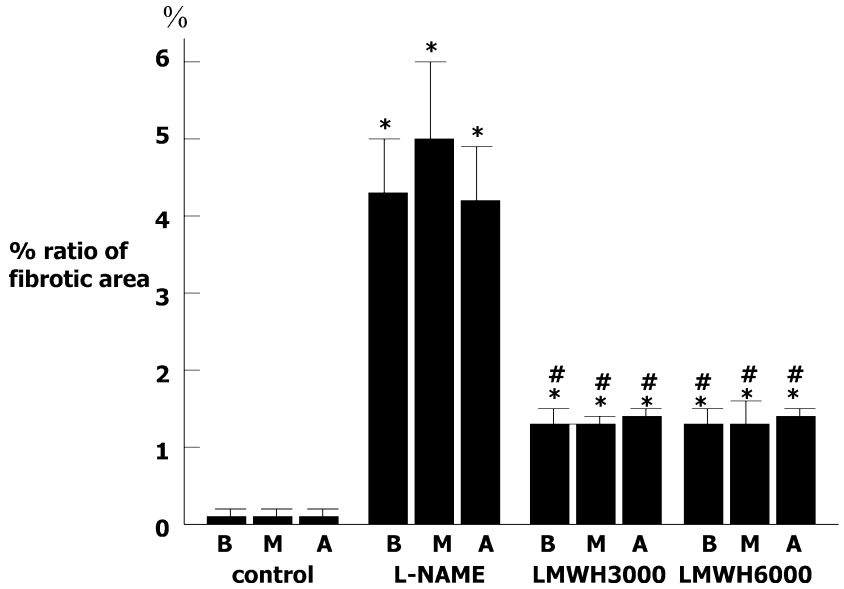

Fig. 6 Percent ratio of fibrotic area (%FR) in left ventricular cross section area. See text for detail. Data are expressed as mean±SEM. B: Base, M: Middle, A: Apex. $^{*}p<0.001$ compared with control, $^{\#}p<0.005$ compared to L-NAME.

with the control group. Further, these changes were inhibited by the coadministration of either LMWH3000 or LMWH6000. Fig. 3 shows the % ratio of the medial layer of each coronary artery in the four groups. Compared with L-NAME group, %ratio of the coronary artery medial layer was significantly reduced by the coadministration of either LMWH3000 or LMWH6000. Fig. 4 shows the %ratio of coronary artery perivascular fibrosis. Compared with L-NAME group, %ratio of perivascular fibrosis of the coronary artery was also reduced significantly by the coadministration of either LMWH3000 or LMWH6000.

Micrographs of the myocardium with Elastica-Masson staining at 4 weeks are shown in Fig 5. Myocardial fibrosis was significantly increased in the L-NAME group. Compared with the L-NAME group, the cardiac fibrosis was significantly reduced by treatment with either LMWH3000 or LMWH6000 (Fig. 6).

Discussion

This is the first study to demonstrate that LMWH prevents the cardiovascular remodeling that is induced by long-term inhibition of nitric oxide synthase with L-NAME in rat hearts. Further, this effect is independent of the anticoagulation effect of LMWH and occurred without any significant change in blood pressure.

Effects of LMWH on systolic blood pressure

Long-term inhibition of NO synthesis with L-NAME resulted in a significant increase in systolic blood pressure (Table 1). The vascular action of eNOS in modulating arterial pressure is well established, and the increase in systolic blood pressure is related to inhibition of NO synthesis, increased tissue angiotensin II (AII) activity[23] and possibly via NO interaction with the baroreflex pathways[24]. In the present study, LMWH did not affect the increase in systolic blood pressure seen with L-NAME administration. Thus, the beneficial

effects of LMWH on cardiovascular remodeling occurred independent of any change in blood pressure.

Effects of LMWH on activated coagulation time (ACT)

LMWH is characterized by a specific anti-Factor Xa effect, inducing only a small prolongation of the general clotting test, such as activated partial thromboplastin time, prothrombin time and antithrombin activity when used at high doses. LMWH fractions are approximately one-third the size of standard heparin and may be capable of greater inhibitory activity than unfractionated heparin for an equivalent anticoagulant effect. However, past studies indicate that the antiproliferative effects of LMWH appear to be independent of its molecular size and anticoagulant effect[25-27]. The present study utilized 4 mg/kg/day of LMWH (M.W.3000) and 4 mg/kg/day of LMWH (M.W.6000), which had no effect on the ACT.

Cardiovascular remodeling induced by long-term inhibition of NO synthesis

Long-term inhibition of NO synthesis with L-NAME resulted in myocardial fibrosis and marked microvascular remodeling characterized by medial thickening and perivascular fibrosis (Figs. 2-6). Use of eNOS knock-out mice has demonstrated that the effects of L-NAME are mediated via inhibition of endothelial NO synthesis as well as by activation of the tissue renin-angiotensin system[28-30], including increases in tissue angiotensin converting enzyme (ACE) activity[30] and upregulation of AII type-1 (AT1) receptors[31]. The synergistic effect of NO synthesis inhibition and increased tissue AII activity act to promote vascular inflammation via endothelial generation of superoxide anion[30,32] and secondary activation of NFκB and its downstream inflammatory pathways, including those involving monocyte chemoattractant protein-1 (MCP-1)[33]. Further, AII induces activation of the small GTPase protein, Rho[34,35], which promotes proliferation of vascular smooth muscle cells[36] and mediates activation of the proinflammatory transcription factors, NFκB and AP1[37,38]. AII can also induce the expression of transforming growth factor-β (TGFβ)[39], and MCP-1 and TGFβ may mediate microvascular remodeling induced by long-term inhibition of NO synthesis[40].

Effects of LMWH on cardiovascular remodeling

Various heparin fractions are effective in preventing smooth muscle cell hyperplasia after experimental arterial injury[10-12]. Cell culture studies have demonstrated that the antiproliferative properties are comparable or even greater for the LMWH than for unfractionated heparin, possibly because of the longer half-life and higher bioavailability of LMWH[13]. LMWH may exerts its cardioprotective effects via various mechanisms, including inhibition of nuclear transcription factors, modulation of growth factor activity[41], regulation of extracellular matrix production, direct inhibition of smooth muscle cell proliferation and migration[42], and by acting as an anti-inflammatory agent. The effects of LMWH on the local angiotensin-renin system may also underlie a portion of its beneficial effects. In fact, several studies indicate that angiotensin-converting enzyme inhibitors and angiotensin receptor blockers prevent the cardiovascular remodeling in this model[31,32].

AII-induced activation of the proinflammatory transcription factor, NFκB, contributes to pathological cardiovascular remodeling. Recent reports demonstrated that heparin inhibited NFκB activation, attenuated myocardial reperfusion injury[43] and inhibited NFκB activation in AII-stimulated mesangial cells[44]. Heparin-binding growth factor also disrupted the NFκB signaling pathway and resulted in decreased levels of inflammatory cytokines[44]. These data, in combination with the present observations, suggest that NFκB is a potential candidate to mediate the effects of LMWH on cardiovascular remodeling. Further studies to investigate this hypothesis would be of benefit.

In conclusion, the present study demonstrated

that LMWH prevents cardiovascular remodeling that was induced by long-term inhibition of nitric oxide synthase with L-NAME in rat hearts. Further, this effect is independent of the anticoagulant effects of LMWH and occurred without any significant changes in blood pressure. Therefore, LMWH, which can be administered in one or twice daily dosing, may be a viable strategy for the outpatient treatment of those at risk for pathogenic cardiovascular remodeling.

References

1) Radomski, M., Palmer, R. and Moncada, S. (1987) Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet*, **2**, 1057-1058.
2) Radomski, M., Palmer, R. and Moncada, S. (1987) Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. *Br. J. Pharmacol*., **92**, 181-187.
3) Clozel, M., Kuhn, H., Hefti, F. and Baumgartner, H. (1991) Endothelial dysfunction and subendothelial monocyte macrophages in hypertension: effect of angiotensin converting enzyme inhibition. *Hypertension*, **18**, 132-141.
4) Dubey, R. and Luscher, T. (1995) Nitric oxide inhibits angiotensin II induced migration of rat aortic smooth muscle cells. *J. Clin. Invest*, **96**, 141-149.
5) Dzau, V. and Gibbons, G. (1993) Vascular remodeling: mechanisms and implication. *J. Cardiovasc. Pharmacol*., **21** (suppl 1), S1-S5.
6) Garg, U.C. and Hassid, A. (1989) Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth mucle cells. *J. Clin. Invest*., **83**, 1774-1777.
7) Assender, J.W., Southgate, K.M. and Newby, A.C. (1991) Dose nitric oxide inhibit smooth muscle proliferation? *J. Cardiovas. Pharmacol*., **17**(3), 104-107.
8) Hasegawa, H. (1998) The effect of long-term nitric oxide synthase inhibition on coronary vasculature and myocardium in rat heart. —Angiotensin converting enzyme inhibitor prevents microvessel disease induced by nitric oxide synthase inhibitor— *Akita, J. Med*., **25**, 91-107.
9) Numaguchi, K., Egashira, K., Takemoto, M., Kadokami, T., Shimokawa, H., Sueishi, K. and Takeshita, A. (1995) Chronic inhibition of nitric oxide synthesis causes coronary microvascular remodeling in rats. *Hypertension*, **26**, 957-962.
10) Clows, A.W. and Karnovsky, M.J. (1977) Suppression by heparin of smooth muscle cell proliferation in injured arteries. *Nature*, **265**, 625-626.
11) Reilly, C.F., Fritze, L.M.S. and Rosenberg, R.D. (1986) Heparin inhibition of smooth muscle cell proliferation: a cellular site of action. *J. Cell. Physiol*., **129**, 11-19.
12) Clowes, A.W. and Clowes, M.M. (1986) Kinetics of cellar proliferation after arterial injury. IV. Heparin inhibits rat smooth muscle mitogenesis and migration. *Circ. Res*., **58**, 839-845.
13) Hoover, R., Rosenberg, R., Haering, W. and Karnovsky, M. (1980) Inhibition of rat arterial smooth muscle cell proliferation by heparin, II: in vitro studies. *Circ. Res*., **47**, 578-583.
14) Guyton, J., Rosenberg, R., Clows, A. and Karnovsky, M. (1980) Inhibition of rat arterial smooth muscle cell proliferation by heparin: in vivo studies with anticoagulant and nonanticoagulant heparin. *Circ. Res.* **46**, 625-634.
15) Castellot, J., Cochran, D. and Karnovsky, M. (1985) Effect of heparin on vascular smooth muscle cell, I: cell metabolism. *J. Cell. Physiol*., **124**, 21-28.
16) Majesky, M.W., Schwartz, S.M., Clowes, M.M. and Clowes, A.W. (1987) Heparin regulates smooth muscle S phase entry in the injured rat carotid artery. *Circ. Res*., **61**, 296-300.
17) Edelman, E.R., Adams, D.H. and Karnovsky, M.J. (1990) Effect of controlled adventitial heparin delivery on smooth muscle cell prolifer-

ation following endothelial injury. *Proc. Natl. Acad. Sci. USA*., **87**, 3773-3777.

18) Gimple, L.W., Gertz, S.D., Haber, H.L., Ragosta, M., Powers, G.R., Roberts, W.C. and Sarembock, I.J. (1992) Effect of chronic subcutaneous or intramural administration of heparin on femoral artery restenosis after balloon angioplasty in hypercholesterolemic rabbits. *Circulation*, **86**, 1536-1546.

19) Buchwald, A.B., Unterberg, C., Nebendahl, K., Grone, H.J. and Wiegand, V. (1992) Low-molecular-weight heparin reduces neointimal proliferation after coronary stent implantation in hypercholesterolemic minipigs. *Circulation*, **86**, 531-537.

20) Guyton, J., Rosenberg, R., Clowes, A. and Karnovsky, M. (1980) Inhibition of rat arterial smooth muscle cell proliferation by heparin : In vivo studies with anticoagulant and nonanticoagulant heparin. *Circ. Res*., **46**, 625-634.

21) Hammerle, H., Betz, E. and Herr. D. (1991) Human endothelial cells are stimulated and vascular smooth muscle cells are inhibited in their proliferation and migration by heparins., *V.A.S.A*., **20**, 207-215.

22) Hirsh, J. and Levine, M.N. (1992) Low molecular weight heparin. *J. Am. Soc. Hematol*., **79**, 1-17.

23) Oliveira, M., Antunes, E., Nucci, G., Lovisolo, S.M. and Zatz, R. (1992) Chronic inhibition of nitric oxide synthesis : a new model of arterial hypertension. *Hypertension*, **20**, 298-303.

24) Ramchandra, R., Barrett, C.J. and Malpas, S.C. (2004) Chronic Blockade of Nitric Oxide dose not produce hypertension in baroreceptor denervated rabbits. *Hypertension*, **42**(**5**), 974-977.

25) Frydman, A.M., Bara, P.L., Le Roux, Y., Noler, M., Chauliac, F. and Samama, M.M. (1988) The antithrombotic activity and pharmacokinetics of Enoxaparin, a low molecular weight heparin, in humans given single subcutaneous dose of 20 to 80 mg. *J. Clin. Pharmacol*., **28**, 609-618.

26) Planes, A., Vochelle, N., Mazas, C., Zucman, J., Landais, A., Pascariello, J., Weill, D. and Butel, J. (1988) Prevention of postoperative venous thrombosis : a landomized trial comparing unfractionated heparin inpatients undergoing total hip replacement. *Thromb. Haemost*., **60**, 407-410.

27) Turpie, A.G., Levine, M.N., Hirsh, J., Carter, C.J., Jay, R.M., Powers, P.J., Andrew, M., Hull, R.D. and Gent. M. (1986) A landomized controlled trial of a low molecular weight heparin to prevent deep-vein thrombosis in patients undergoing elective hip surgery. *N. Engl. J. Med*., **315**, 925-929.

28) Saito, T., Fujiwara, Y., Fujiwara, R., Hasegawa, H., Kibira, S., Miura, H. and Miura, M. (2002) Role of augmented expression of intermediate conductance Ca^{2+}-activated K^+ channels in postischaemic heart. *Clin. Exp. Pharmacol. Physiol*., **29**, 324-329.

29) Numaguchi, K., Egashira, K., Takemoto, M., Kadokami, T., Shimokawa, H., Sueishi, K. and Takeshita, A. (1995) Chronic inhibition of nitric oxide synthesis causes coronary microvascular remodeling in rats. *Hypertension*, **26**, 957-962.

30) Takemoto, M., Egashira, K., Usui, M., Numaguchi, K., Tomita, H., Tsutsui, H., Shimokawa, H., Sueishi, K. and Takeshita, A. (1997) Important role of tissue angiotensin-converting enzyme activity in the pathogenesis of coronary vascular and myocardial structural changes induced by long-term blockade of nitric oxide synthesis in rats. *J. Clin. Invest*., **99**, 278-287.

31) Moncada, S., Palmer, R.M.J. and Higgs, E.A. (1991) Nitric Oxide : Physiology, pathology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, **43**, 109-142.

32) Kurose, I., Wolf, R., Grisham, M.B., Specian, R.D. and Granger, D.N. (1995) Microvascular responses to inhibition of nitric oxide production : role of active oxidants. *Circ. Res*., **76**, 30-39.

33) Collins, T., Read, M.A., Neish, A.S., Whitley, M.Z., Thanos, D. and Maniatis, T. (1995) Transcriptional regulation of endothelial cell

adhesion molecules: NF-kappa B and cytokine-inducible enhancers. *F.A.S.E.B. J.*, **9**, 899-909.

34) Aoki, H., Izumo, S. and Sadoshima, J. (1998) Angiotensin II activates RhoA in cardiac myocytes: a critical role of RhoA in angiotensin II-induced premyofibril formation. *Circ. Res.*, **87**, 666-676.

35) Yamakawa, T., Tanaka, S., Numaguchi, K., Yamakawa, Y., Motley, E.D., Ichihara, S. and Inagami T. (2000) Involvement of Rho-kinase in angiotensin II-induced hypertrophy of rat vascular smooth muscle cells. *Hypertension*, **35**, 313-318.

36) Wagner, A.H., Kohler, T., Ruckschloss, U., Just, I. and Hecker, M. (2000) Improvement of nitric oxide-dependent vasodilatation by HMG-CoA reductase inhibitors through attenuation of endothelial superoxide anion formation. *Arterioscler. Thromb. Vasc. Biol.*, **20**, 61-69.

37) Pruefer, D., Scalia, R. and Lefer, A.N. (1999) Simvastatin inhibits leukocyte-endothelial cell interactions and protects against inflammatory processes in normocholesterolemic rats. *Arterioscler. Thromb. Vasc. Res.*, **19**, 2894-2900.

38) Naftilan, A.J., Gilliand, G.K., Eldridge, C.S. and Kraft, A.S. (1990) Induction of the proto-oncogene c-jun by angiotensin II. *Mol. Cell. Biol.* **10**(**10**), 5536-5540.

39) Sadoshima, J. and Izumo, S. (1993) Molecular characterization of angiotensin II-induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. *Circ. Res.*, **73**, 413-423.

40) Koyanagi, M., Egashira, K., Kubo-Inoue, M., Usui, M., Kitamoto, S., Tomita, H., Shimokawa, H. and Takeshita, A. (2000) Role of transforming growth factor-β1 in cardiovascular inflammatory changes induced by chronic inhibition of nitric oxide synthesis. *Hypertension*, **35**, 86-90.

41) Majesky, M.W., Schwartz, S.M., Clowes, M.M. and Clowes, A.W. (1987) Heparin regulates smooth muscle S phase entry in the injured rat carotid artery. *Circ. Res.*, **61**, 246-300.

42) Castellot, J.J., Cochran, D.L. and Karnovsky, M.J. (1985) Effect of heparin on vascular smooth muscle cells, I: cell metabolism. *J. Cell. Physiol.*, **124**, 21-28.

43) Vinod, H.T., Sukhdev, S.B., Thomas, P.K., Lisa, R.T., John, A.W., Russell, S.R., Zhi-Qing, Z., Anne, L.S., John, R.H. and Jakob, V.J. (2000) Nonanticoagulant heparin inhibits NFκB activation and attenuates myocardial reperfusion injury. *Am. J. Physiol. Heart. Circ. Physiol.*, **278**, H2084-2093.

44) Koppel, H., Yard, B.A., Christ, M., Wehling, M., van der Woude, F.J. (2003) Modulation of angiotensin II-mediated signaling by heparin sulphate glycosaminoglycans. *Nephrol. Dial. Transplant.*, **18**(**11**), 2240-2247.