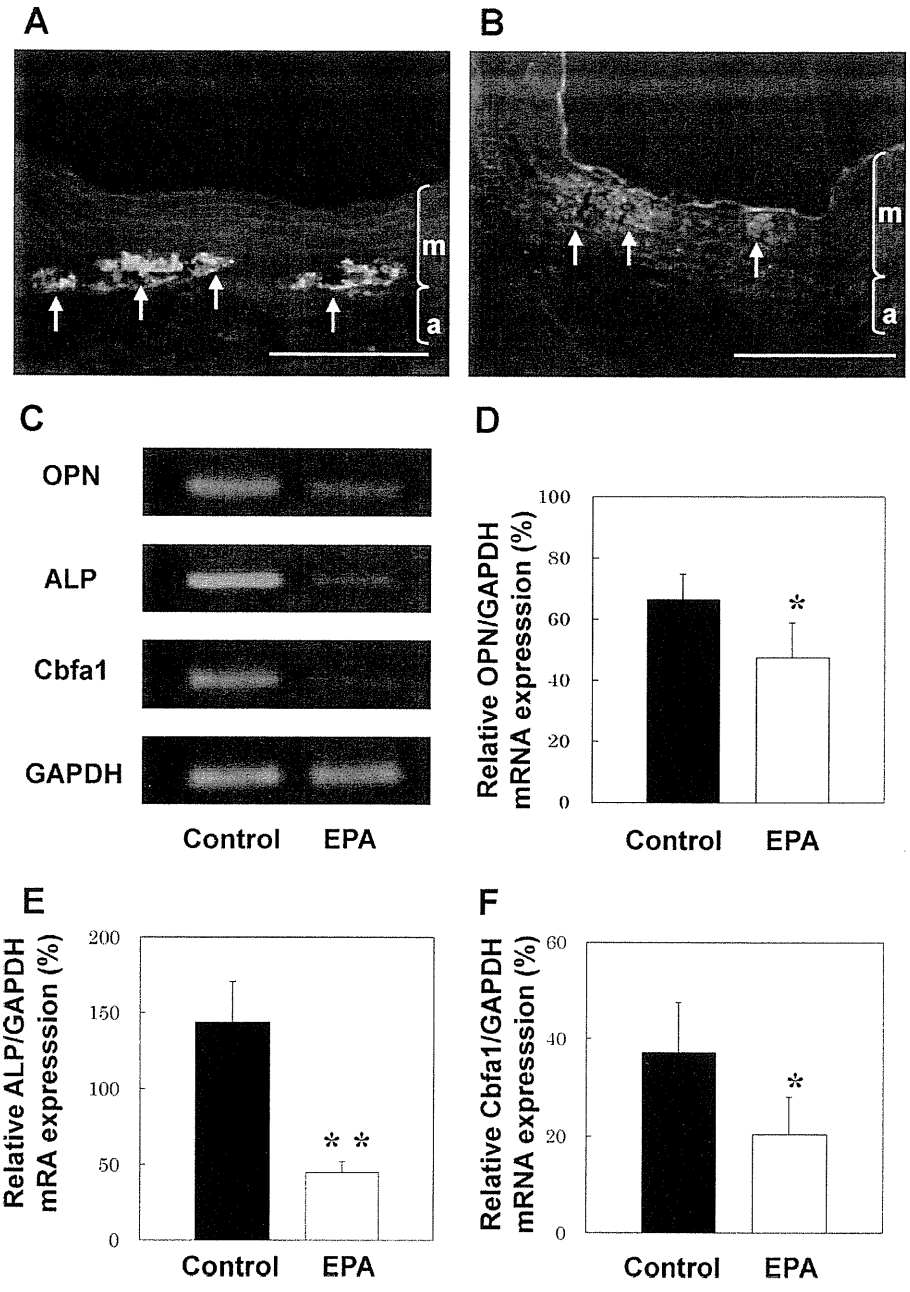

Fig. 2. EPA attenuates osteogenetic signals in the calcified aorta. (A and B) Immunohistochemical detection of osteopontin (OPN) (A) and alkaline phosphatase (ALP) (B) colocalizing with the calcification in the common iliac artery of the control group. Arrows denote areas of positive staining. m, media; a, adventitia. Scale bar, 100 μm. (C) Representative mRNA expressions assessed by RT-PCR. OPN (D), ALP (E), and Cbfa1 (F) mRNA expressions are normalized to GAPDH and evaluated densitometrically ($n=6$ per group). $^{*}p<0.05$, $^{**}p<0.01$.

3. Discussion

We set out to determine whether EPA significantly inhibits AMC and to determine whether EPA decreases osteogenesis-related gene expression and adventitial macrophage infiltration with MMP-9 in the calcified aorta.

The major finding of this present study is that EPA reduces AMC in vivo. We used a warfarin-induced AMC model established by Price in 1998 [11] The mechanism of this model is inhibiting γ-carboxylation of MGP, a calcium-binding and vitamin K-dependent protein that inhibits vascular calcification by antagonizing bone morphogenetic protein and binding elastin. A typical form of morphology of calcifications is linear deposit along the elastic lamina in the abdominal aorta to the iliac arteries a common site of AMC in humans, and those lesions progress to massive AMC similar to Mönckeberg's sclerosis without atherosclerosis. Although MGP-deficient mice show similar AMC, they have osteogenic disorders and calcification progresses faster than warfarin-treated rats and result in death from aortic rupture within 6 weeks [19]. Therefore, it is difficult to use MGP-deficient mice for suppression experiments of AMC. There have been other murine models to study inhibition of vascular calcification such as treating low-density lipoprotein receptor (LDL)-deficient mice or apolipoprotein E-deficient mice with high-fat or high-phosphate diet combined nephrectomy [5,17,20]. However, mainly intimal calcification occurs in these models and its pathogenesis is complicated. Although periadventitial application of $CaCl_2$, which causes medial calcification, has also been used in several studies [15], it requires surgery. In this

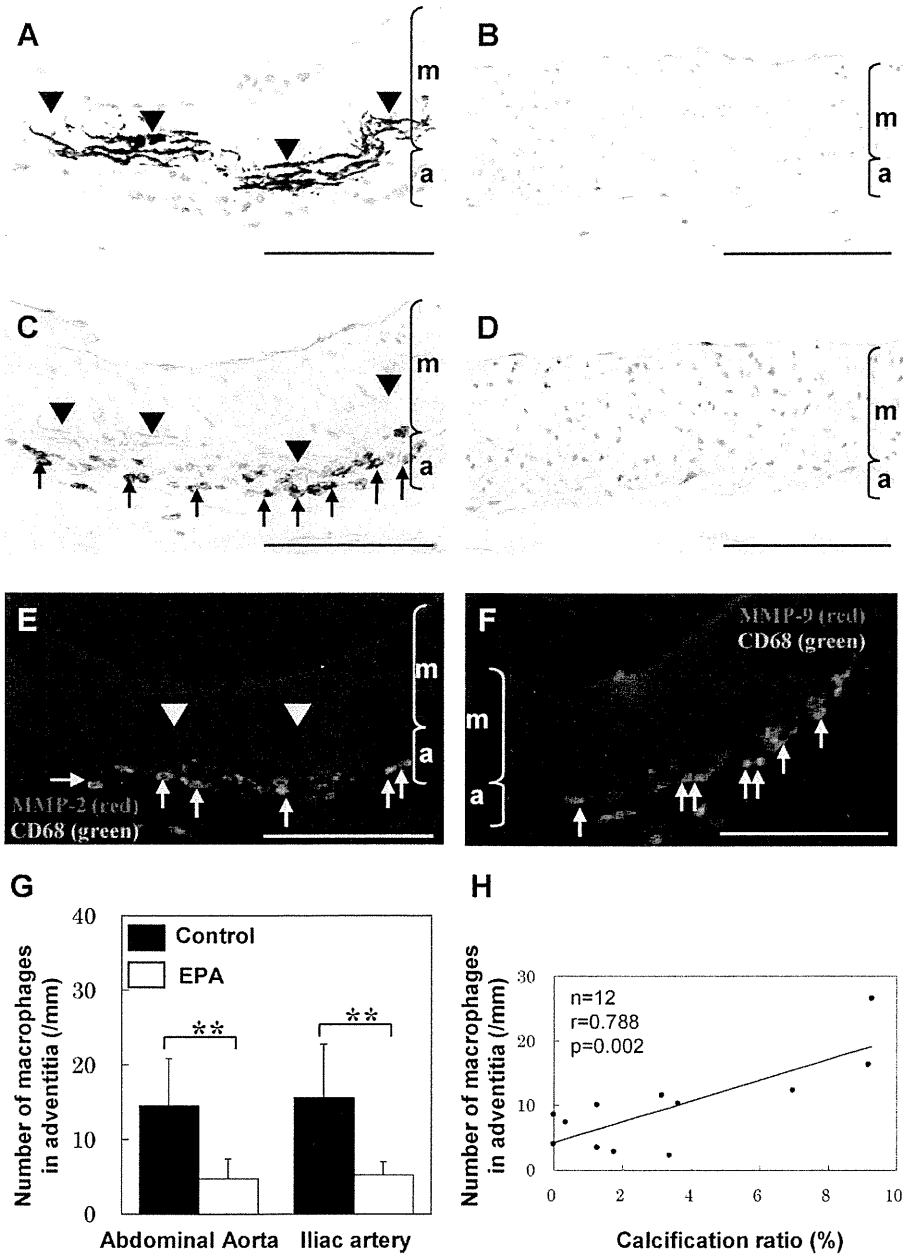

Fig. 3. EPA suppresses adventitial macrophage infiltration in rats treated with warfarin. (A–D) von Kossa stained sections (A and B) and immunostaining for CD68 (C and D) of the calcified common iliac artery (A and C) and non calcified common iliac artery (B and D) of rats in the control (A and C) or EPA (B and D) group. Numerous macrophages showing positive staining for CD68 (arrows) were found in adventitia correlated with medial calcified area (arrow heads, A and C), but not around non-calcified lesions (D). (E and F) Co localization of MMP-2 (red) and CD68 (green) (E) and MMP-9 (red) and CD68 (green) (F) in adventitia along medial calcification of common iliac artery of rat in the control group. Arrows indicate macrophages double positive for MMP-2 and CD68 (E) and MMP-9 and CD68 (F). Arrow heads indicate VSMC positive for MMP-2 (E). (G) Quantitative evaluations of macrophages positive for CD68 in adventitia ($n=5$ in the iliac artery of the control group. $n=6$ in others). (H) Correlation between number of macrophages and calcification ratio of abdominal aorta in both control and EPA groups ($n=12$). A, C, E and F, B and D are serial sections, respectively. m, media; a, adventitia. Scale bar, 100 μm. $^{**}p<0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

study, warfarin treatment requires only 2 weeks to induce calcification, and provides less-invasive and highly reproducible model of AMC. For this reason, it has been used in several preventive AMC experiments [21,22].

ω-3 PUFA has pleiotropic effects and has been shown to decrease the risk of major cardiovascular events, such as myocardial infarction [23], sudden cardiac death [24], arrhythmias [25], and death in patients with heart failure [26]. Recent large randomized trials have documented that EPA reduced the incidence of major coronary events in patients with hyperlipidemia without affecting serum LDL cholesterol [27]. Although detailed action mechanisms of EPA have not been clarified, 2 basic mechanisms, the effects on atherothrombosis and ion channels, are thought to be important. However, there have been few reports on the effects of EPA in vascular calcification, much less AMC.

AMC has demonstrated similar processes to intramembranous bone formation, unlike intimal calcification, which forms via a process similar to endochondral ossification [3]. Our finding that EPA decreased the expressions of osteogenetic markers in the aorta indicates that suppression of AMC by EPA might occur via inhibiting transition of VSMC into osteoblast-like cells. However, EPA was reported to have opposite effects on osteoblast, increasing osteo-

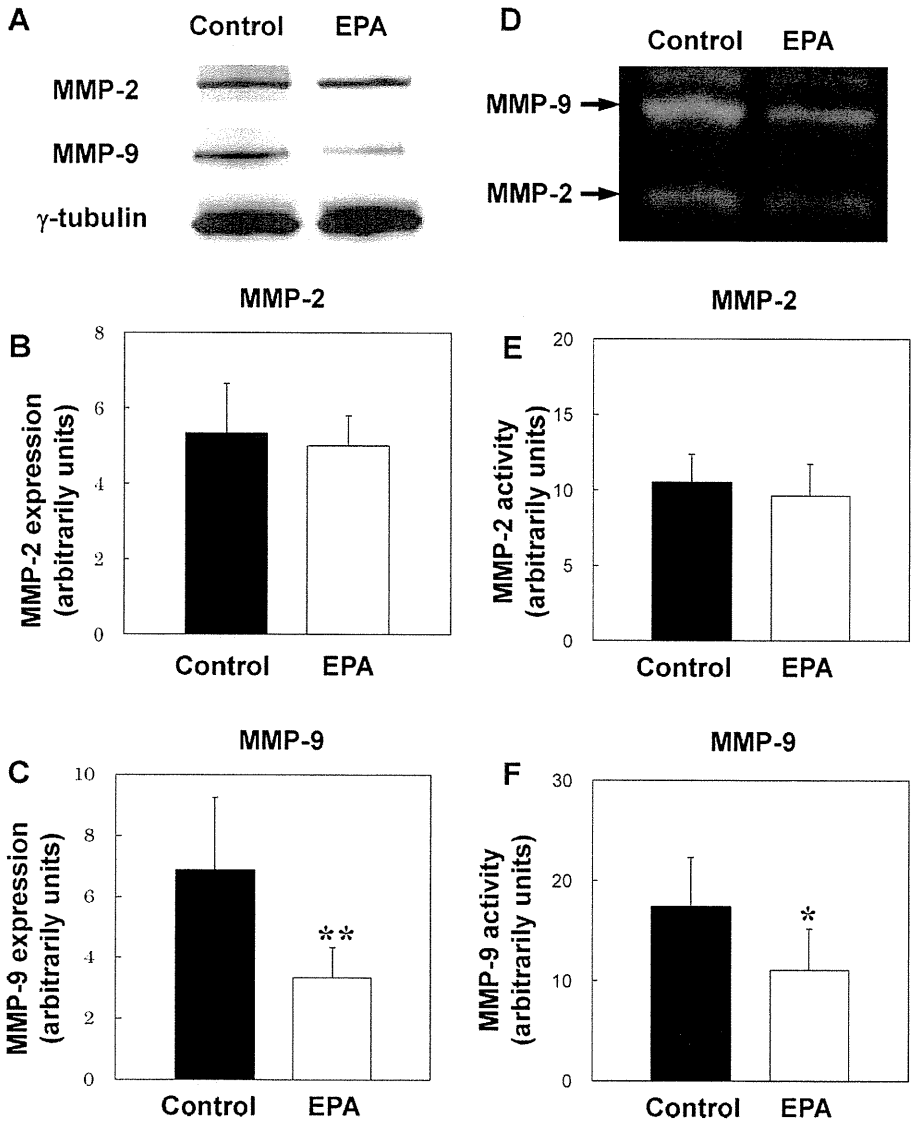

Fig. 4. Inhibitory effects of EPA on MMP expressions in the aorta. (A and D) Representative MMP-2 and MMP-9 levels in the aorta assessed by Western blotting (A, $n = 5$ per group) and gelatin zymography (D, $n = 6$ per group). Protein expressions of MMP-2 (B) and MMP-9 (C), and enzyme activity of MMP-2 (E) and MMP-9 (F) were evaluated by densitometry and expressed as arbitrarily units. $^{*}p < 0.05$, $^{**}p < 0.01$.

genetic activity and prevention of loss of bone mineral density [28]. There seems to be a difference in the effect of EPA on osteoblast-like VSMC and osteoblast in the bone. Our findings are supported by in vitro observations that EPA inhibited osteoblastic differentiation and mineralization of vascular cells by managing the p38-MAPK and PPAR-γ pathways [29]. Furthermore, Schlemmer reported that EPA reduced calcium glubionate-induced ectopic calcification of rat aortas [30].

This osteoblast-like phenotypical change of VSMC is speculated to follow after preceding elastin degradation and activation of MMP-2 and transforming growth factor (TGF)-β [31]. Our results that calcium deposition was localized in elastic fibers with elastin degeneration and MMP-9 elevation agree with a previous report that elastase activity and extracellular matrix degradation are essential to the early process of AMC, accompanied by the change of MMP-9 and TGF-β in warfarin-treated rat models [32]. Although the type of elastase which contributes to the pathogenesis of AMC may differ depending on the experimental methodology, inhibiting MMP activity may have important implications for the treatment of AMC [15,16]. We speculate that EPA plays an inhibitory role mainly in the early process of AMC through suppressing MMP activity. In addition, EPA may also have some benefits in secondary prevention of AMC as shown by the results of late EPA group.

Inflammation may be an important contributor to vascular calcification [5], especially, as macrophages contribute to elastin degeneration and vascular calcification via expressing elastase such as MMPs and cathepsin S [17], and TNF-α, a pleiotropic cytokine that is reported to promote osteoblastic differentiation of VSMC [18]. One striking result of our study was the presence of numerous macrophages in adventitia around both tiny calcification in early stage and progressive calcification. Moreover, some of these macrophages expressed MMP-2 and MMP-9. These observations indicate that adventitial macrophage may play an important role in the process of AMC. Furthermore, EPA also inhibited MCP-1, a chemokine inducing recruitment of monocytes, which was detected in VSMC and adventitial macrophages. Taken together, suppression of macrophage infiltration into adventitia via inhibition of MCP-1 might be in part responsible for the effect of EPA on AMC. Further studies are needed to clarify the role of macrophages in the pathogenesis of AMC.

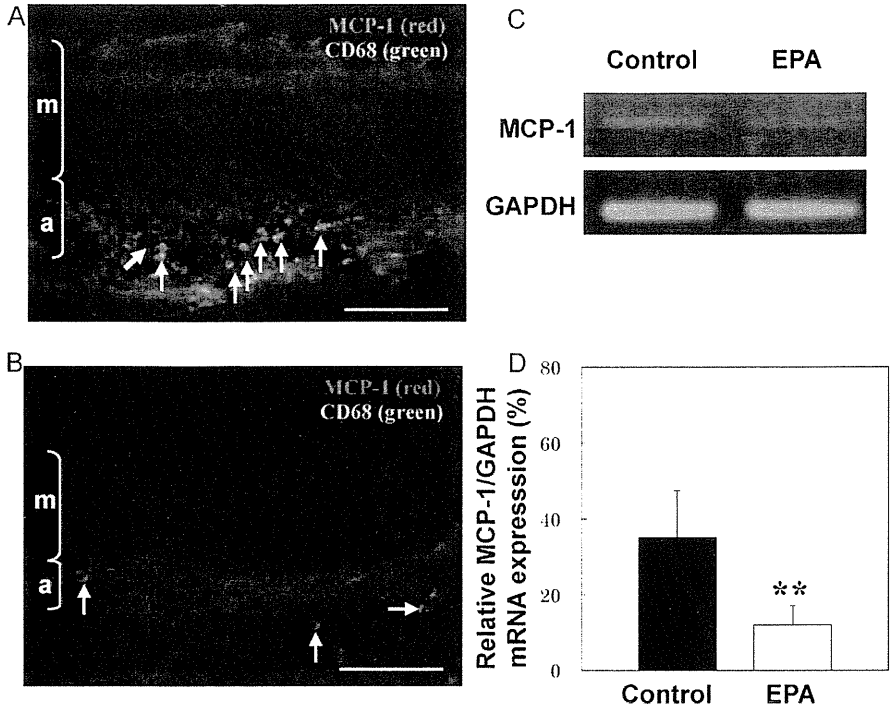

Fig. 5. MCP-1 expression in the calcified aorta. (A and B) Representative immunostaining for MCP-1 (red) and CD68 (green) of common iliac artery of rats in the control group (A) and EPA group (B). Macrophage is shown by an arrow (↑). Scale bar, 100 μm. (C) Representative MCP-1 mRNA expression assessed by RT-PCR. (D) MCP-1 mRNA expressions evaluated densitometrically and normalized to GAPDH ($n=6$ per group). m, media; a, adventitia. $^{**}p<0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

In conclusion, we showed that EPA reduces AMC in warfarin-treated rats. Multiple effects of EPA may be beneficial for AMC caused by various mechanisms.

Conflict of interest

The authors report no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2010.12.001.

References

[1] Everhart JE, Pettitt DJ, Knowler WC, Rose FA, Bennett PH. Medial arterial calcification and its association with mortality and complications of diabetes. Diabetologia 1988;31:16–23.
[2] London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. Nephrol Dial Transplant 2003;18:1731–40.
[3] Hruska KA, Mathew S, Saab G. Bone morphogenetic proteins in vascular calcification. Circ Res 2005;97:105–14.
[4] Demer LL, Tintut Y. Vascular calcification: pathobiology of a multifaceted disease. Circulation 2008;117:2938–48.
[5] Aikawa E, Nahrendorf M, Figueiredo JL, et al. Osteogenesis associates with inflammation in early-stage atherosclerosis evaluated by molecular imaging in vivo. Circulation 2007;116:2841–50.
[6] Clarke MC, Littlewood TD, Figg N, et al. Chronic apoptosis of vascular smooth muscle cells accelerates atherosclerosis and promotes calcification and medial degeneration. Circ Res 2008;102:1529–38.
[7] Mizobuchi M, Towler D, Slatopolsky E. Vascular calcification: the killer of patients with chronic kidney disease. J Am Soc Nephrol 2009;20:1453–64.
[8] Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. JAMA 2006;296:1885–99.
[9] von Schacky C. n-3 PUFA in CVD: influence of cytokine polymorphism. Proc Nutr Soc 2007;66:166–70.
[10] Harris WS, Miller M, Tighe AP, Davidson MH, Schaefer EJ. Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives. Atherosclerosis 2008;197:12–24.
[11] Price P, Faus S, Williamson M. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. Arterioscler Thromb Vasc Biol 1998;18:1400–7.
[12] Price PA, Kaneda Y. Vitamin K counteracts the effect of warfarin in liver but not in bone. Thromb Res 1987;46:121–31.
[13] Giachelli. Molecular and cellular biology of osteopontin:: Potential role in cardiovascular disease. Trends Cardiovasc Med 1995;5:88.
[14] Aubin JE, Liu F, Malaval L, Gupta AK. Osteoblast and chondroblast differentiation. Bone 1995;17:77S–83S.
[15] Qin X, Corriere MA, Matrisian LM, Guzman RJ. Matrix metalloproteinase inhibition attenuates aortic calcification. Arterioscler Thromb Vasc Biol 2006;26:1510–6.
[16] Vyavahare N, Jones PL, Tallapragada S, Levy RJ. Inhibition of matrix metalloproteinase activity attenuates tenascin-C production and calcification of implanted purified elastin in rats. Am J Pathol 2000;157:885–93.
[17] Aikawa E, Aikawa M, Libby P, et al. Arterial and aortic valve calcification abolished by elastolytic cathepsin S deficiency in chronic renal disease. Circulation 2009;119:1785–94.
[18] Tintut Y, Patel J, Territo M, Saini T, Parhami F, Demer LL. Monocyte/macrophage regulation of vascular calcification in vitro. Circulation 2002;105:650–5.
[19] Luo G, Ducy P, McKee MD, et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature 1997;386:78–81.
[20] Towler DA, Bidder M, Latifi T, Coleman T, Semenkovich CF. Diet-induced diabetes activates an osteogenic gene regulatory program in the aortas of low density lipoprotein receptor-deficient mice. J Biol Chem 1998;273:30427–34.
[21] Price PA, Faus SA, Williamson MK. Bisphosphonates alendronate and ibandronate inhibit artery calcification at doses comparable to those that inhibit bone resorption. Arterioscler Thromb Vasc Biol 2001;21:817–24.
[22] Essalihi R, Zandvliet ML, Moreau S, et al. Distinct effects of amlodipine treatment on vascular elastocalcinosis and stiffness in a rat model of isolated systolic hypertension. J Hypertens 2007;25:1879–86.
[23] Daviglus ML, Stamler J, Orencia AJ, et al. Fish consumption and the 30-year risk of fatal myocardial infarction. N Engl J Med 1997;336:1046–53.
[24] Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. N Engl J Med 2002;346:1113–8.
[25] London B, Albert C, Anderson ME, et al. Omega-3 fatty acids and cardiac arrhythmias: prior studies and recommendations for future research: a report from the National Heart, Lung, and Blood Institute and Office Of Dietary Supplements Omega-3 Fatty Acids and their Role in Cardiac Arrhythmogenesis Workshop. Circulation 2007;116:e320–35.
[26] Tavazzi L, Maggioni AP, Marchioli R, et al. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. Lancet 2008;372:1223–30.
[27] Yokoyama M, Origasa H, Matsuzaki M, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet 2007;369:1090–8.

[28] Salari P, Rezaie A, Larijani B, Abdollahi M. A systematic review of the impact of n-3 fatty acids in bone health and osteoporosis. Med Sci Monit 2008;14: RA37–44.
[29] Abedin M, Lim J, Tang T, Park D, Demer L, Tintut Y. N-3 fatty acids inhibit vascular calcification via the p38-mitogen-activated protein kinase and peroxisome proliferator-activated receptor-gamma pathways. Circ Res 2006;98: 727–9.
[30] Schlemmer CK, Coetzer H, Claassen N, et al. Ectopic calcification of rat aortas and kidneys is reduced with n-3 fatty acid supplementation. Prostaglandins Leukot Essent Fatty Acids 1998;59: 221–7.
[31] Simionescu A, Philips K, Vyavahare N. Elastin-derived peptides and TGF-beta1 induce osteogenic responses in smooth muscle cells. Biochem Biophys Res Commun 2005;334:524–32.
[32] Bouvet C, Moreau S, Blanchette J, de Blois D, Moreau P. Sequential activation of matrix metalloproteinase 9 and transforming growth factor beta in arterial elastocalcinosis. Arterioscler Thromb Vasc Biol 2008;28: 856–62.

The Pleiotropic Effects of ARB in Vascular Endothelial Progenitor Cells

Katsuhisa Matsuura* and Nobuhisa Hagiwara

Department of Cardiology, Tokyo Women's Medical University, Tokyo, Japan

Abstract: Angiotensin II regulates blood pressure and contributes to endothelial dysfunction and the progression of atherosclerosis. Bone marrow-derived endothelial progenitor cells (EPCs) in peripheral blood contribute to postnatal vessel repair and neovascularization. Impaired EPC function in patients with hypertension and diabetes inhibits the endogenous repair of vascular lesions and leads to the progression of atherosclerosis. The number of EPCs in peripheral blood is inversely correlated with mortality and the occurrence of cardiovascular events. Angiotensin II-mediated signaling is implicated in oxidative stress, inflammation and insulin resistance, factors that cause EPC dysfunction. Blockade of the angiotensin II type 1 receptor may therefore present a new therapeutic target for enhancing EPC function.

Keywords: EPC, angiotensin II, ARB, oxidative stress, PPARγ.

INTRODUCTION

The renin-angiotensin system (RAS) plays a major role in the physiological regulation of the cardiovascular system. Angiotensin II (AngII) is a pivotal molecule in the RAS. AngII causes vasoconstriction and increased blood pressure and is implicated in inflammation, endothelial dysfunction, atherosclerosis, hypertension, and congestive heart failure. Most of the pathophysiological actions of AngII in the cardiovascular system are mediated through the AngII type 1 (AT_1) receptor. Pharmacological inhibition of the RAS is one of the great success stories of cardiovascular medicine. Evidence accumulated over the past decade shows that RAS blockade with angiotensin converting enzyme (ACE) inhibitors and AngII type1 receptor blockers (ARBs) prevents progression of cardiac hypertrophy and atherosclerosis and reduces morbidity and mortality in patients with heart failure [1]. Although RAS blockade is thought to reduce cardiovascular events by lowering blood pressure, evidence suggests that ARBs also protect the cardiovascular system by mechanisms independent of their antihypertensive effect, including anti-atherogenic, anti-diabetic, anti-platelet aggregating, anti-arrhythmic and hypouricemic actions [2].

Repairing injured vessels and promoting neovascularization are promising strategies for the treatment of ischemic heart disease. Angiogenesis, the proliferation and migration of preexisting endothelial cells, was thought to be the major mechanism of postnatal vessel repair and neovascularization. Recent evidence shows that bone marrow-derived endothelial progenitor cells (EPCs) in peripheral blood also contribute to these processes [3]. EPCs migrate to injured areas and differentiate into mature functional endothelial cells *in situ* [4]. Cardiovascular risk factors, such as hypertension, diabetes, dyslipidemia, smoking, and aging, influence EPC number and functions, including migration and colony-forming ability [5, 6]. In diseases of the vessel wall, such as atherosclerosis, EPCs show impaired function and a reduction in number of up to 40 % [5]. Vasa *et al.* demonstrated that EPCs from patients with coronary artery disease (CAD) have an impaired migratory function that is negatively correlated with the number of vascular risk factors [5]. In patients with CAD bone marrow-derived mononuclear cells (BM-MNCs), presumed to include EPCs, have a reduced capacity for neovascularization [7]. Hill *et al.* report that EPC numbers are inversely correlated with endothelial function [6]. These findings suggest that EPC number and function are surrogate markers for endothelial function. Impaired EPC function may limit the endogenous repair of vascular lesions and cause progression of atherosclerosis. As the number and colony-forming ability of EPCs predict cardiovascular events, a strategy for improving EPC function may present a novel therapeutic target for reducing vascular risk.

In this article, we review recent experimental and clinical data that support the benefits of ARB treatment on EPC function as a therapeutic target for cardiovascular disease. We focus particularly on hypertension and diabetes.

EPC IN HYPERTENSION

Increased arterial blood pressure is associated with microvascular dysfunction, increased peripheral vascular resistance, and impaired post ischemic neovascularization in clinical studies and animal models of hypertension [8, 9]. While low levels of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) [10, 11], and defective endothelial function [12] contribute to impaired angiogenesis in hypertensive animals, EPC dysfunction may also contribute to the pathogenesis of hypertension. Vasa *et al.* report that the number and migratory capacity of EPCs are reduced in patients with hypertension [5], and Umemura *et al.* report that hypertension is an independent predictor of reduced EPC numbers [13]. Hypertension is associated with an increase in reactive oxygen species (ROS). ROS are thought to reduce nitric ox-

*Address correspondence to this author at the Department of Cardiology, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku, Tokyo, 162-8666, Japan; Tel: +81-3-3353-8111; Fax: +81-3-3356-0441;
E-mail: mkatu2002@yahoo.co.jp

1570-1611/11 $58.00+.00

ide (NO) bioavailability, which may lead to defective mobilization of EPCs from bone marrow [14]. Imanishi *et al.* report that ROS also affect the proliferation, senescence and apoptosis of EPCs [15, 16]. You *et al.* report that hypertension-induced increases in ROS inhibit the differentiation of BM-MNCs into cells with an endothelial phenotype *in vitro* [9], leading to a reduced therapeutic effect *in vivo*. These findings suggest ROS may be a major cause of impaired EPC function in hypertension. AngII increases oxidative stress, inflammation, and alters endothelial function *via* the AT_1 receptor. Kobayashi *et al.* report that an AngII infusion reduces the number and accelerates senescence of EPCs in rats [17]. AngII is also reported to accelerate EPC senescence by a gp91 phox–mediated increase in oxidative stress in humans [16]. Accordingly, ARBs decrease oxidative stress in endothelial cells [18]. It is thus possible that ARBs might improve EPC function by inhibiting AngII-mediated ROS. Valsartan, an ARB, inhibits the senescence of EPC caused by AngII-mediated oxidative stress *in vitro* [15]. ARBs such as losartan [9, 19] and candesartan [20] improve impaired EPC function in hypertensive animals by attenuating oxidative stress *via* the reduced expression of gp91-phox, p22-phox, and p47-phox. In a prospective study in normotensive and moderately hypertensive individuals, Bahlmann *et al.* found that olmesartan increases EPC numbers [21]. These findings support the important role of the RAS in the regulation of EPC bioactivity in hypertensive patients.

EPC IN DIABETES

In patients with diabetes, atherosclerosis progression is accelerated by direct endothelial damage and by the reduced availability and function of EPCs. EPC numbers are reduced in patients with type 1 and type 2 diabetes mellitus and EPCs from diabetic patients have an impaired capacity for adhesion, proliferation, and tubulization [22]. Uncontrolled plasma glucose levels, assessed by glycated hemoglobin and free plasma glucose levels, are inversely correlated with the number of EPCs. In contrast, improvement in glycemic control after treatment is associated with increased EPC numbers [23]. Chen *et al.* report that advanced glycation end products (AGE) impair the function of EPCs by affecting Akt and cyclooxygenase-2 [24]. Recent reports suggest that high glucose levels decrease the number of human EPCs *in vitro* through the reduced expression of SIRT1 [25]. SIRT1 down-regulates p53 activity and prolongs the lifespan of cells [26]. Hyperglycemia also impairs the proliferation and increases the apoptosis of EPCs through up-regulation of p16Ink-4a and p21Waf-1 [27]. Krankel *et al.* report that hyperglycemia causes reduced MMP-9 activity leading to a decreased capability of EPCs to invade a target tissue and incorporate into tubular structures [27]. Hyperglycemia also enhances protein phosphatase 2A activity in EPCs, causing a reduction in eNOS phosphorylation at Ser^{1177} and a decline in NO production [27]. In addition, hyperglycemia shifts the endothelial differentiation of EPCs to a pro-inflammatory phenotype [28]. The degree of impairment of EPC function is related to the severity of diabetic vasculopathies such as peripheral artery disease [29]. EPCs are thus thought to play an important role in the pathogenesis of diabetic vasculopathy.

AngII-mediated signaling is also important in the pathogenesis of the vascular complications of diabetes. As hyperglycemia-mediated endothelial dysfunction is largely attributed to oxidative stress *via* arachidonic acid metabolism, glucose oxidation, and AGE formation [30], blockade of RAS signaling is a promising potential therapeutic target for preventing diabetic complications. In clinical trials, inhibition of the RAS prevents the progression of diabetic nephropathy [31, 32]. Consistent with the evidence that ARB inhibition of oxidative stress improves EPC function in hypertension, olmesartan and irbesartan increase EPC numbers in diabetic patients 12 weeks after treatment [21].

Recent evidence suggests that endothelial dysfunction is already present in humans with insulin resistance and hyperinsulinemia before they become diabetic [33]. In insulin-resistant patients, the progression of atherosclerosis is associated with down-regulation of the phosphatidylinositol 3 kinase (PI3K)/Akt/eNOS pathway [34]. Inactivation of the PI3K/Akt/eNOS pathway is also reported to reduce mobilization of EPCs from bone marrow through a decrease in NO bioavailability [35]. Su *et al.* report that valsartan induces NO production in endothelial cells through Src/PI3K/Akt-dependent phosphorylation of eNOS [36]. As activation of the PI3K/Akt signal contributes to statin-induced EPC proliferation and inhibition of the senescence of EPCs [37, 38], a strategy to activate the PI3K/Akt signal by ARB treatment could present a target for preventing EPC dysfunction in patients with insulin resistance. AngII infusion decreases insulin sensitivity in diabetic and non-diabetic mice [39]. ARBs reduce insulin resistance by promoting the insulin-induced tyrosine phosphorylation of the insulin receptor substrate (IRS)-1, the association of IRS-1 with p85, and the translocation of GLUT4 [40]. Several clinical trials report that ARB treatment inhibits the new occurrence of diabetes in patients with hypertension [41, 42] and CAD [43]. Recently Lee *et al.* have reported that ARBs improve glucose tolerance in OLETF rats, an animal model of type 2 diabetes [44]. They also report that ARB treatment increases the number of small differentiated adipocytes that produce adiponectin. Adiponectin is the major adipokine that sensitizes the body to insulin [45] and it also promotes the migration of EPC through the PI3K/Cdc42/Rac1 pathway [46]. These findings suggest that ARBs may not only directly improve EPC function in diabetes by inhibiting oxidative stress, but also indirectly affect EPC function by improving insulin sensitivity and up-regulating adiponectin production.

THE PPARγ DEPENDENT EFFECTS OF ARB

Telmisartan has recently been identified as a partial agonist of peroxisome proliferator-activated receptor gamma (PPARγ) [47]. Other clinically approved ARBs have little or no effect on PPARγ activity with the exception of irbesartan and a metabolite of losartan, both of which are less potent activators of PPARγ than telmisartan [48, 49]. PPARs are transcription factors belonging to the nuclear receptor superfamily that heterodimerize with the retinoid X receptor and bind to PPAR-responsive elements in target gene promoters. The activation of PPARγ in adipose tissue promotes adipose differentiation and increases the number of small insulin-sensitive adipocytes [50]. Thiazolidinediones (TZD), full agonists of PPARγ, increase endothelium-derived NO pro-

duction [51] and reduce vascular inflammation [52], suggesting that PPARγ activation might be anti-atherosclerotic. Telmisartan is thought to functionally activate PPARγ and to induce adiponectin expression *via* PPARγ activation [53]. We recently reported that telmisartan increases the number of human peripheral blood-derived EPC *in vitro via* a PPARγ dependent pathway *in vitro* [54]. Our results are consistent with evidence that TZD increases EPC numbers [55, 56]. By contrast, valsartan treatment does not affect the EPC numbers [54], suggesting that different ARBs have differing effects on EPC proliferation. We also found that the telmisartan-mediated increase in EPCs is regulated by the PI3K/Akt pathway [54]. As down-regulation of the PI3K/Akt/eNOS pathway in patients with diabetes mellitus increases endothelial dysfunction and reduces mobilization of EPC from bone marrow, activation of the PI3K/Akt signal by telmisartan may be a novel therapeutic target for improving endothelial function. Pioglitazone, a TZD, attenuates AngII-induced cellular senescence and oxidative stress in endothelial cells *in vitro* [57]. Pioglitazone treatment also increases the number of circulating EPCs in type 2 diabetics and non-diabetic patients with CAD [58, 59]. As telmisartan causes AT_1 receptor blockade and PPARγ activation, it might be expected to improve vascular function and promote neovascularization *via* the proliferation of EPCs in ischemic tissue in the clinical setting.

CONCLUSIONS

Accumulating data suggest that oxidative stress and inflammation in patients with cardiovascular risk factors impair the proliferation, migration, and differentiation of EPCs. ARBs improve EPC function by reducing oxidative stress and inflammation, increasing insulin sensitivity and activating PPARγ. Impaired EPC bioactivity is thought to play the critical role in the progression of atherosclerosis and reduced EPC numbers and impaired EPC function are associated with increased mortality in patients with cardiovascular risk factors. However, treatments that improve EPC bioactivity have not yet been shown to prevent cardiovascular death or new myocardial infarction. Further basic and clinical research is thus required to elucidate the interaction between pharmacological interventions such as ARB treatment and the occurrence of cardiovascular events in terms of the effects on EPC function. Improving our understanding of EPC biology will help us develop new treatments for ischemic cardiovascular disease.

ACKNOWLEDGEMENTS

This study was supported in part by a Grant-in-Aid for Scientific Research, Developmental Scientific Research, and Scientific Research from the Ministry of Education, Science, Sports, and Culture, and Takeda Science Foundation (to K.M.).

NON-STANDARD ABBREVIATIONS

ACE	=	Angiotensin converting enzyme
AGE	=	Advanced glycation end products
AngII	=	Angiotensin II
ARBs	=	Angii typel receptor blockers
AT_1	=	AngII type 1
BM-MNCs	=	Bone marrow-derived mononuclear cells
CAD	=	Coronary artery disease
EPCs	=	Endothelial progenitor cells
HGF	=	Hepatocyte growth factor
IRS	=	Insulin receptor substrate
NO	=	Nitric oxide
PI3K	=	Phosphatidylinositol 3 kinase
PPARγ	=	Peroxisome proliferator-activated receptor gamma
RAS	=	Renin-angiotensin system
ROS	=	Reactive oxygen species
TZD	=	Thiazolidinediones
VEGF	=	Vascular endothelial growth factor

REFERENCES

[1] Zaman MA, Oparil S, Calhoun DA. Drugs targeting the renin-angiotensin-aldosterone system. Nat Rev Drug Discov 2002; 1: 621-6.

[2] Jankowski P, Safar ME, Benetos A. Pleiotropic effects of drugs inhibiting the renin-angiotensin-aldosterone system. Curr Pharm Des 2009; 15: 571-84.

[3] Asahara T, Murohara T, Sullivan A, *et al.* Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997; 275: 964-7.

[4] Tateishi-Yuyama E, Matsubara H, Murohara T, *et al.* Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone marrow cells: a pilot study and a randomized controlled trial. Lancet 2002; 360:427-35.

[5] Vasa M, Fichtlscherer S, Aicher A, *et al.* Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res 2001; 89: E1-7.

[6] Hill JM, Zalos G, Halcox JP, *et al.* Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 2003; 348: 593-600.

[7] Heeschen C, Lehmann R, Honold J, *et al.* Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. Circulation 2004; 109: 1615-22.

[8] Feihl F, Liaudet L, Waeber B, Levy BI. Hypertension: a disease of the microcirculation? Hypertension 2006; 48: 1012-7.

[9] You D, Cochain C, Loinard C, *et al.* Hypertension impairs postnatal vasculogenesis: role of antihypertensive agents. Hypertension 2008; 51: 1537-44.

[10] Emanueli C, Salis MB, Stacca T, *et al.* Rescue of impaired angiogenesis in spontaneously hypertensive rats by intramuscular human tissue kallikrein gene transfer. Hypertension 2001; 38: 136-41.

[11] Nakano N, Moriguchi A, Morishita R, *et al.* Role of angiotensin II in the regulation of a novel vascular modulator, hepatocyte growth factor (HGF), in experimental hypertensive rats. Hypertension 1997; 30: 1448-54.

[12] Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med 1990; 323: 22-7.

[13] Umemura T, Soga J, Hidaka T, *et al.* Aging and hypertension are independent risk factors for reduced number of circulating endothelial progenitor cells. Am J Hypertens 2008; 21: 1203-9.

[14] Aicher A, Heeschen C, Mildner-Rihm C, *et al.* Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. Nat Med 2003; 9: 1370-6.

[15] Imanishi T, Hano T, Nishio I. Angiotensin II accelerates endothelial progenitor cell senescence through induction of oxidative stress. J Hypertens 2005; 23: 97-104.

[16] Imanishi T, Moriwaki C, Hano T, Nishio I. Endothelial progenitor cell senescence is accelerated in both experimental hypertensive rats and patients with essential hypertension. J Hypertens 2005; 23: 1831-7.

[17] Kobayashi K, Imanishi T, Akasaka T. Endothelial progenitor cell differentiation and senescence in an angiotensin II-infusion rat model. Hypertens Res 2006; 29: 449-55.

[18] Rueckschloss U, Quinn MT, Hotz J, Morawietz H. Dose-dependent regulation of NAD(P)H oxidase expression by angiotensin II in human endothelial cells. Protective effect of angiotensin II type 1 receptor blockade in patients with coronary artery disease. Arterioscler Thromb Vasc Biol 2002; 22: 1845-51.

[19] Yao EH, Fukuda N, Matsumoto T, *et al.* Losartan improves the impaired function of endothelial progenitor cells in hypertension *via* an antioxidant effect. Hypertens Res 2007; 30: 1119-28.

[20] Yu Y, Fukuda N, Yao EH, *et al.* Effects of an ARB on endothelial progenitor cell function and cardiovascular oxidation in hypertension. Am J Hypertens 2008; 21: 72-7.

[21] Bahlmann FH, de Groot K, Mueller O, Hertel B, Haller H, Fliser D. Stimulation of endothelial progenitor cells: a new putative therapeutic effect of angiotensin II receptor antagonists. Hypertension 2005; 45: 526-9.

[22] Tepper OM, Galiano RD, Capla JM, *et al.* Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation 2002; 106: 2781-6.

[23] Kusuyama T, Omura T, Nishiya D, *et al.* Effects of treatment for diabetes mellitus on circulating vascular progenitor cells. J Pharmacol Sci 2006; 102: 96-102.

[24] Chen Q, Dong L, Wang L, Kang L, Xu B. Advanced glycation end products impair function of late endothelial progenitor cells through effects on protein kinase Akt and cyclooxygenase-2. Biochem Biophys Res Commun 2009; 381: 192-7.

[25] Balestrieri ML, Rienzo M, Felice F, *et al.* High glucose downregulates endothelial progenitor cell number *via* SIRT1. Biochim Biophys Acta 2008; 1784: 936-45.

[26] Orimo M, Minamino T, Miyauchi H, *et al.* Protective role of SIRT1 in diabetic vascular dysfunction. Arterioscler Thromb Vasc Biol 2009; 29: 889-94.

[27] Krankel N, Adams V, Linke A, *et al.* Hyperglycemia reduces survival and impairs function of circulating blood-derived progenitor cells. Arterioscler Thromb Vasc Biol 2005; 25: 698-703.

[28] Loomans CJ, van Haperen R, Duijs JM, *et al.* Differentiation of bone marrow-derived endothelial progenitor cells is shifted into a proinflammatory phenotype by hyperglycemia. Mol Med 2009; 15: 152-9.

[29] Fadini GP, Sartore S, Albiero M, *et al.* Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy Arterioscler Thromb Vasc Biol 2006; 26: 2140-6.

[30] Hadi HA, Suwaidi JA. Endothelial dysfunction in diabetes mellitus. Vasc Health Risk Manag 2007; 3: 853-76.

[31] Brenner BM, Cooper ME, de Zeeuw D, *et al.* Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med 2001; 345: 861-9.

[32] Lewis EJ, Hunsicker LG, Clarke WR, *et al.* Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. N Engl J Med 2001; 345: 851-60.

[33] Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. J Clin Invest 1996; 97: 2601-10.

[34] Zdychová J, Komers R. Emerging role of Akt kinase/protein kinase B signaling in pathophysiology of diabetes and its complications. Physiol Res 2005; 54: 1-16.

[35] Avogaro A, Fadini GP, Gallo A, Pagnin E, de Kreutzenberg S. Endothelial dysfunction in type 2 diabetes mellitus. Nutr Metab Cardiovasc Dis 2006; 16(Suppl. 1): S39-45.

[36] Su KH, Tsai JY, Kou YR, *et al.* Valsartan regulates the interaction of angiotensin II type 1 receptor and endothelial nitric oxide synthase *via* Src/PI3K/Akt signalling. Cardiovasc Res 2009; 82: 468-75.

[37] Dimmeler S, Aicher A, Vasa M, *et al.* HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells *via* the PI 3-kinase/Akt pathway. J Clin Invest 2001; 108: 391-7.

[38] Assmus B, Urbich C, Aicher A, *et al.* HMG-CoA reductase inhibitors reduce senescence and increase proliferation of endothelial progenitor cells *via* regulation of cell cycle regulatory genes. Circ Res 2003; 92: 1049-55.

[39] Shiuchi T, Iwai M, Li HS, *et al.* Angiotensin II type-1 receptor blocker valsartan enhances insulin sensitivity in skeletal muscles of diabetic mice. Hypertension 2004; 43: 1003-10.

[40] Horiuchi M, Mogi M, Iwai M. Signaling crosstalk angiotensin II receptor subtypes and insulin. Endocr J 2006; 53: 1-5.

[41] Dahlöf B, Devereux RB, Kjeldsen SE, *et al.* Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Lancet 2002; 359: 995-1003.

[42] Ogihara T, Nakao K, Fukui T, *et al.* Effects of candesartan compared with amlodipine in hypertensive patients with high cardiovascular risks: candesartan antihypertensive survival evaluation in Japan trial. Hypertension 2008; 51: 393-8.

[43] Kasanuki H, Hagiwara N, Hosoda S, *et al.* Angiotensin II receptor blocker-based *vs.* non-angiotensin II receptor blocker-based therapy in patients with angiographically documented coronary artery disease and hypertension: the Heart Institute of Japan Candesartan Randomized Trial for Evaluation in Coronary Artery Disease (HIJ-CREATE). Eur Heart J 2009; 30: 1203-12.

[44] Lee MH, Song HK, Ko GJ, *et al.* Angiotensin receptor blockers improve insulin resistance in type 2 diabetic rats by modulating adipose tissue. Kidney Int 2008; 74: 890-900.

[45] Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006; 116: 1784-92.

[46] Nakamura N, Naruse K, Matsuki T, *et al.* Adiponectin promotes migration activities of endothelial progenitor cells *via* Cdc42/Rac1. FEBS Lett 2009; 583: 2457-63.

[47] Benson SC, Pershadsingh HA, Ho CI, *et al.* Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPAR gamma-modulating activity. Hypertension 2004; 43: 993-1002.

[48] Benson SC, Pershadsingh HA, Ho CI, *et al.* Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPAR gamma-modulating activity. Hypertension 2004; 122: 130-9.

[49] Schupp M, Janke J, Clasen R, Unger T, Kintscher U. Angiotensin type 1 receptor blockers induce peroxisome proliferator-activated receptor-γ activity. Circulation 2004; 109: 2054-7.

[50] Yamauchi T, Kamon J, Waki H, *et al.* The mechanisms by which both heterozygous peroxisome proliferator-activated receptor gamma (PPARgamma) deficiency and PPARgamma agonist improve insulin resistance. J Biol Chem 2001; 276: 41245-54.

[51] Polikandriotis JA, Mazzella LJ, Rupnow HL, Hart CM. Peroxisome proliferator-activated receptor gamma ligands stimulate endothelial nitric oxide production through distinct peroxisome proliferator-activated receptor gamma-dependent mechanisms. Arterioscler Thromb Vasc Biol 2005; 25: 1810-6.

[52] Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 1998; 391: 82-6.

[53] Clasen R, Schupp M, Foryst-Ludwig A, *et al.* PPARgamma-activating angiotensin type-1 receptor blockers induce adiponectin. Hypertension 2005; 46: 137-43.

[54] Honda A, Matsuura K, Fukushima N, Tsurumi Y, Kasanuki H, Hagiwara N. Telmisartan induces proliferation of human endothelial progenitor cells *via* PPARgamma-dependent PI3K/Akt pathway. Atherosclerosis 2009; 205: 376-84.

[55] Gensch C, Clever YP, Werner C, Hanhoun M, Bohm M, Laufs U. The PPAR-gamma agonist pioglitazone increases neoangiogenesis and prevents apoptosis of endothelial progenitor cells. Atherosclerosis 2007; 192: 67-74.

[56] Pistrosch F, Herbrig K, Oelschlaegel U, Richter S, Passauer J, Fischer S. Gross P. PPARgamma-agonist rosiglitazone increases number and migratory activity of cultured endothelial progenitor cells. Atherosclerosis 2005; 183: 163-7.

[57] Imanishi T, Kobayashi K, Kuroi A, Ikejima H, Akasaka T. Pioglitazone inhibits angiotensin II-induced senescence of endothelial progenitor cell. Hypertens Res 2008; 31: 757-65.

[58] Werner C, Kamani CH, Gensch C, Böhm M, Laufs U. The peroxisome proliferator-activated receptor-gamma agonist pioglitazone increases number and function of endothelial progenitor cells in pa-

tients with coronary artery disease and normal glucose tolerance. Diabetes 2007; 56: 2609-15.

[59] Makino H, Okada S, Nagumo A, *et al.* Pioglitazone treatment stimulates circulating CD34-positive cells in type 2 diabetes patients. Diabetes Res Clin Pract 2008; 81: 327-30.

Received: September 4, 2009 **Revised: October 9, 2009** **Accepted: October 10, 2009**