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COMMENT

Pleiotropic effects of calcium channel blockers

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Recently identified cardiovascular-active agents, including HMG-CoA reductase inhibitors (statins) and renin-angiotensin system inhibitors such as angiotensin-converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARBs) are known to exert cardiovascular protective effects independent of their original pharmacologic actions, namely, pleiotropic effects [1]. The pleiotropic effects include improving vascular endothelial function, anti-inflammatory effects, antioxidative effects, antiplatelet actions, and plaque stabilization. Statins have been demonstrated to exhibit outstanding effects on the primary and secondary prevention of cardiovascular diseases. Although such effects of statins are believed to mainly depend on their considerable lipid-lowering effects, pleiotropic effects, especially vascular protective effects, have also been emphasized [2]. ACE inhibitors/ARBs also have pleiotropic effects that are independent of their blood pressure-lowering effects [3], but this class of agents has been focused on for their cardioprotective effects rather than their vascular protective effects. Additionally, calcium channel blockers (CCBs), especially dihydropyridines, also have pleiotropic effects. Regarding pleiotropic effects, the anti-atherosclerotic effects of CCBs have been demonstrated, although these effects have been given less attention than those of statins and ACE inhibitors/ARBs. Sueta et al. [4] described such pleiotropic effects of CCBs in their review article published in the current issue of *Hypertension Research*.

Impaired endothelial function has been proposed to be an initial event that leads to the development of atherosclerosis. Interactions of inflammation and oxidative stress are also important atherogenic processes that lead to endothelial cell damage. Endothelial cells are targets for cytokines and growth factors that are released during inflammation [1]. Recently, nifedipine, one of the widely used dihydropyridine-based CCBs for the treatment of patients with hypertension and angina pectoris, especially coronary spastic angina, has been demonstrated to inhibit vascular inflammation and subsequently improve endothelial function in many cardiovascular diseases, which thus slows the development and progression of atherosclerosis. Nifedipine prevents monocyte chemoattractant protein (MCP)-1 production from endothelial cells, which is elicited by tumor necrosis factor-alpha (TNF-alpha) through its antioxidative properties. Yamagishi et al. [5] observed in in vitro experiments using human umbilical vein endothelial cells (HUVECs) that nifedipine blocked TNF-alpha-induced inhibition of endothelial cell proliferation. Nifedipine has also been found to inhibit apoptotic cell death in TNF-alpha-exposed HUVECs. Additionally, nifedipine blocks TNF-alpha-induced intracellular reactive oxygen species (ROS) generation and TNF-alpha-induced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in HUVECs [6]. Furthermore, nifedipine has also been found to significantly inhibit the upregulation of MCP-1 messenger RNA levels in TNF-alpha-exposed HUVECs. These results suggest that nifedipine might inhibit TNF-alpha-induced MCP-1 overexpression in HUVECs by suppressing NADPH oxidase-mediated ROS generation. In contrast, nifedipine inhibits the TNF-alpha-induced upregulation of vascular cell adhesion molecule (VCAM)-1 mRNA levels in HUVECs and blocks the adhesion of the human lymphoblastic cell line MOLT-3 to TNF-alpha-exposed HUVECs [7]. These results suggest that nifedipine can inhibit TNF-alpha-induced leukocyte adhesion to endothelial cells by suppressing VCAM-1 expression. These results illustrate a novel beneficial aspect of nifedipine in atherogenesis.

Azelnidipine is another dihydropyridine-based L-type CCB that was developed in Japan and has unique anti-atherogenic properties. Azelnidipine has been demonstrated to inhibit

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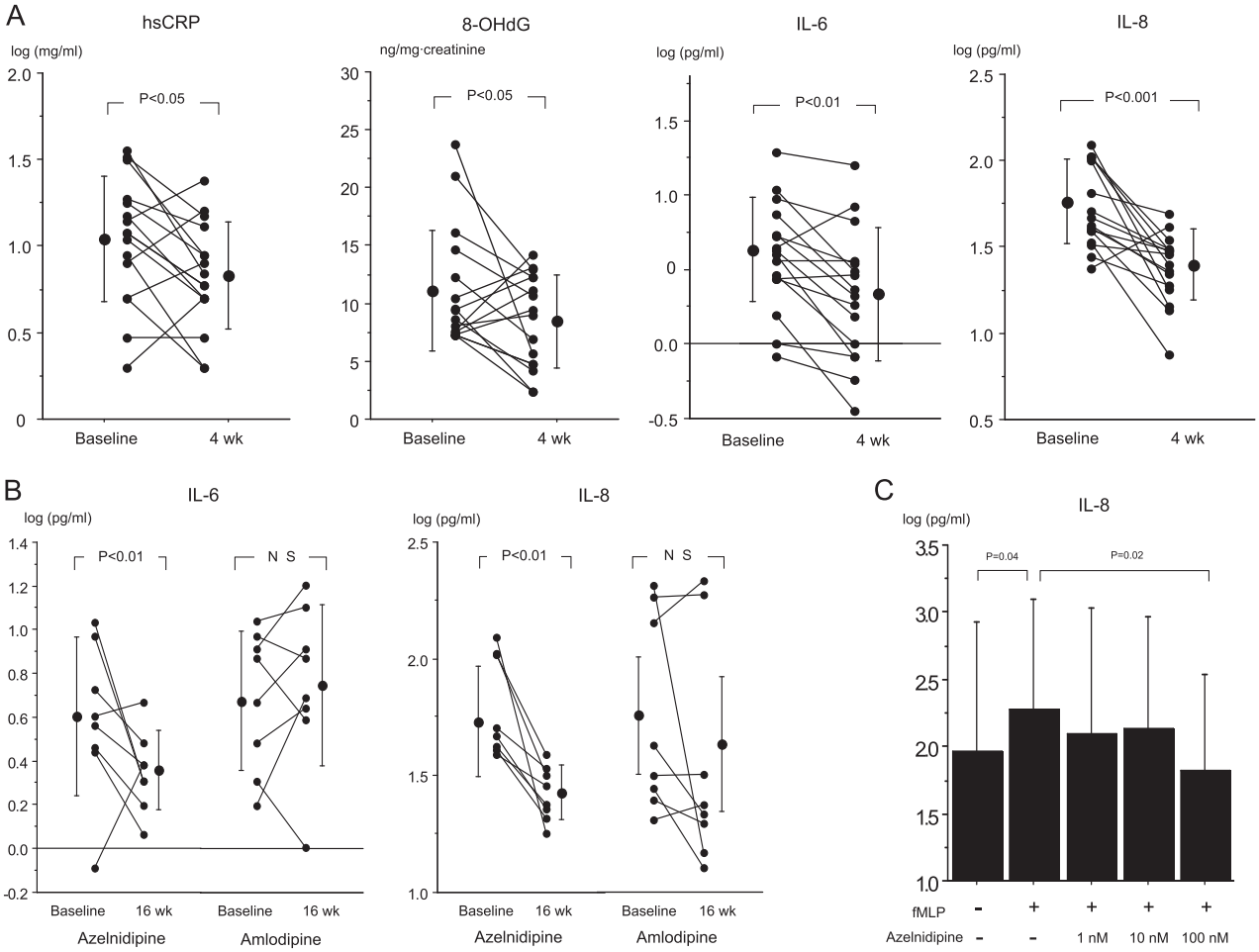

Fig. 1 **a** Changes in the serum hsCRP levels, urinary 8-OHdG levels, and serum levels of IL-6 and IL-8. The values of hsCRP, IL-6, and IL-8 were log-transformed for statistical analysis. All of the values significantly decreased after 4 weeks of treatment with 16 mg/day of azelnidipine ($n = 16$). **b** Comparison of the changes in the IL-6 and IL-8 levels between azelnidipine and amlodipine. The levels of IL-6 and IL-8 decreased after 4 weeks of treatment with azelnidipine ($n = 8$) but did not change after treatment with amlodipine ($n = 8$). **c** Effect of azelnidipine on IL-8 production in fMLP-stimulated human-isolated mononuclear cells. The fMLP stimulation increased the IL-8 production, but azelnidipine dose-dependently inhibited the fMLP-induced increase in IL-8 production. This inhibitory effect of azelnidipine was significant at a concentration of 100 nM, which is similar to the plasma levels attained when a dose of 16 mg/day is prescribed. hsCRP high-sensitivity C-reactive protein, 8-OHdG 8-hydroxy-2-deoxyguanosine, IL interleukin, fMLP formyl-methyonyl leucyl phenylalanine

endothelial inflammatory responses via an antioxidant mechanism [8, 9]. There are several reports that this agent inhibits the migration and proliferation of vascular smooth muscle cells in vitro [10] and neointimal formation after arterial injury in non-human primates [11]. Azelnidipine has been demonstrated to inhibit the TNF-alpha-induced redox-sensitive transcriptional factor activator protein-1 and the activation and subsequent overexpression of interleukin (IL)-8 in HUVECs [8]. These effects have been attributed to the drug suppressing the ROS generation that is mediated by NADPH oxidase reduction. We previously investigated the in vivo and in vitro effects of azelnidipine on inflammatory responses in the clinical setting. First, we recruited 16 high-risk patients with hypertension (systolic blood pressure ≥140 or diastolic blood pressure ≥90 mm Hg) who had atherosclerotic complications, such as coronary artery disease or cerebrovascular disease, or one or more other atherosclerotic risk factors such as diabetes or dyslipidemia. These patients were administered azelnidipine at 16 mg/day in the following two arms: first arm, antihypertensive medication as a new therapy ($n = 8$); second arm, additive therapy in combination with antihypertensive drugs other than CCBs, such as beta-blockers, ACE inhibitors, or ARBs in patients with poorly controlled blood pressure ($n = 8$). We also selected 8 patients who were receiving 5 mg/day amlodipine as a new therapy who were matched for age, sex, and risk factors for comparison with the first arm patients ($n = 8$) who were receiving azelnidipine (16 mg/day). We measured the serum levels of inflammatory markers, including high-sensitivity C-reactive protein (hsCRP) and various pro-inflammatory cytokines and the urine levels of the oxidative stress marker 8-hydroxy-2-deoxyguanosine (8-OHdG). Consequently, among the

overall 16 patients receiving azelnidioine, 4 weeks of treatment significantly reduced the levels of hsCRP (1.04 ± 0.36–0.83 ± 0.31 log (μg/ml), $P < 0.05$), 8-OHdG (11.1 ± 5.2–8.5 ± 4.0 ng/mg creatinine, $P < 0.05$), IL-6 (0.60 ± 0.36–0.33 ± 0.44 log (pg/ml), $P < 0.01$), and IL-8 (1.73 ± 0.24–1.38 ± 0.20 log (pg/ml), $P < 0.001$; Fig. 1a). Regarding the comparison with amlodipine, the levels of IL-6 (0.59 ± 0.35–0.35 ± 0.18 log (pg/ml), $P < 0.01$) and IL-8 (1.79 ± 0.21–1.42 ± 0.12 log (pg/ml), $P < 0.001$) were significantly decreased in the azelnidipine group but did not change (0.68 ± 0.32–0.75 ± 0.37 log (pg/ml) and 1.75 ± 0.25–1.73 ± 0.31 log (pg/ml), respectively, for IL-6 and IL-8) in the amlodipine group (Fig. 1b). Next, we performed an in vitro experiment using isolated human leukocytes to assess the anti-inflammatory action of azelnidipine. Venous blood was collected from 6 healthy volunteers to isolate the mononuclear cells. The isolated mononuclear cells were cultured, and azelnidipine was added at concentrations of 1, 10, and 100 nM. Then, the cells were stimulated with 10 nM formyl-methyonyl leucyl phenylalanine (fMLP). Finally, the levels of the pro-inflammatory cytokines were measured in the cultured medium. Consequently, the fMLP stimulation caused significant increases in the IL-8 levels in the incubated mononuclear cell medium (1.92 ± 0.98–2.27 ± 0.88 log (pg/ml), $P < 0.05$). Azelnidipine dose-dependently inhibited the fMLP-induced increase in the IL-8 level, and the degree of this inhibition was statistically significant at the azelnidipine concentration of 100 nM (1.84 ± 0.76 log (pg/ml), $P < 0.05$, vs. fMLP stimulation; Fig. 1c) [12].

Although our data from a very small cohort suggest that amlodipine, the most widely used dihydropyridine-based CCB, has less anti-inflammatory and antioxidant power than azelnidipine, a number of reports suggest that amlodipine has promising pleiotropic effects. Shima et al. [13] investigated differences in neutrophil activation processes between amlodipine and azelnidipine in in vitro experiments. Consequently, granulocyte-macrophage colony-stimulating factor (GM-CSF)-induced superoxide anion release from neutrophils was suppressed by amlodipine but not by azelnidipine, whereas TNF-alpha-induced superoxide anion release from neutrophils was suppressed by both amlodipine and azelnidipine. TNF-alpha-induced phosphorylation of extracellular signal-regulated kinase and Akt, but not p38 mitogen-activated protein kinase, was attenuated by azelnidipine. In contrast, GM-CSF-induced neutrophil migration was suppressed by amlodipine but not by azelnidipine. Mason et al. [14] demonstrated that amlodipine increased nitric oxide (NO) bioavailability and decreased nitroxidative stress. These authors measured NO and peroxynitrite ($ONOO^-$) from the aortic and glomerular endothelial cells ex vivo following stimulation with a calcium ionophore in spontaneously hypertensive rats that were treated with vehicle or amlodipine. Consequently, the vehicle treatment reduced the endothelial NO release and increased $ONOO^-$ compared with baseline. The NO/$ONOO^-$ ratio, which is a comprehensive index of endothelial NO synthase function, was also reduced in the vehicle group. In contrast, amlodipine treatment restored NO, decreased $ONOO^-$, and increased the NO/$ONOO^-$ ratio independently of blood pressure reduction.

Recently, Liu et al. [15] demonstrated a unique pleiotropic effect of the CCB lacidipine, which is not available in Japan but can be used in some Asian and Western countries. Endothelial progenitor cells (EPCs) play a critical role in maintaining the integrity of the vascular endothelium following arterial injury and thus act to protect arteries against atherosclerosis. These authors examined the in vivo re-endothelialization capacity of EPCs from hypertensive patients with or without in vitro lacidipine treatment in a nude mouse model of carotid artery injury and evaluated the expression of the C-X-C chemokine receptor type 4 (CXCR4) and the alteration in the migration and adhesion functions of EPCs. These authors found that lacidipine promoted CXCR4/Janus kinase (JAK)-2 signaling expression in in vitro EPCs. Transplantation of EPCs that were pretreated with lacidipine significantly accelerated in vivo re-endothelialization. The enhanced in vitro function and in vivo re-endothelialization capacity of EPCs were inhibited by short hairpin RNA-mediated knockdown of CXCR4 expression or pretreatment with the JAK-2 inhibitor AG490. In hypertensive patients, lacidipine treatment for 4 weeks also results in an upregulation of CXCR4/JAK-2 signaling of EPCs, which is associated with augmented EPC-mediated re-endothelialization and improved endothelial function.

Representative clinical evidence for the anti-atherosclerotic actions of CCBs comes from the pleiotropic effect identified in the comparison of the Amlodipine vs. Enalapril to Limit Occurrences of Thrombosis (CAMELOT) trial [16]. In the CAMELOT trial, the CCB amlodipine or the ACE inhibitor enalapril were compared with placebo in 1991 patients with angiographically documented coronary artery disease (>20% stenosis by coronary angiography) and a diastolic blood pressure <100 mm Hg. A substudy of 274 patients measured coronary artery atherosclerosis progression by intravascular ultrasound (IVUS). The results revealed that compared with baseline, the IVUS showed progression in the placebo group ($P < 0.001$), there was a trend toward progression in the enalapril group ($P = 0.08$), and there was no progression in the amlodipine group ($P = 0.31$). Regarding the amlodipine group, the correlation between blood pressure reduction and progression was $R = 0.19$, $P = 0.07$. These results suggest that amlodipine, but not enalapril, exhibited evidence of slowing atherosclerosis progression independently of blood pressure reduction.

In addition to nifedipine, azelnidipine, and amlodipine, the other CCBs, including nicardipine and benidipine, have also been demonstrated to have various pleiotropic effects. Specifically, CCBs have been demonstrated to exert more favorable vascular effects in combination with statins or ACE inhibitors/ARBs than either drug alone [17–19]. From this perspective, ARB/CCB combination pills and statin/CCB combination pills, which we used in daily clinical practice, would be rational. The pleiotropic effects of CCBs have not been fully elucidated, and it is unknown whether such effects are specific for each CCB or the class effects of CCBs. Further studies in this field could establish the position of CCBs as anti-atherosclerotic agents in addition to antihypertensive or anti-angina agents.

Compliance with ethical standards

Conflict of interest TI has received honorariums from Mochida, Bayer, and Boehringer Ingelheim. KN has received honorariums from Boehringer Ingelheim, Daiichi Sankyo, Astellas, MSD, Takeda, Mitsubishi Tanabe and Sanofi, and research grants from Sanwa Kagaku Kenkyusho, Astellas, Takeda, Boehringer Ingelheim, Teijin, and Mitsubishi Tanabe. The remaining authors declare that the have no conflict of interest.

References

1. Inoue T, Node K. Vascular failure—a new clinical entity for vascular disease. J Hypertens. 2006;24:2121–30.
2. Inoue T, Node K. Statin therapy for vascular failure. Cardiovasc Drug Ther. 2007;21:281–95.
3. Taguchi I, Inoue T, Kikuchi M, Toyoda S, Arikawa T, Abe S, Node K. Pleiotropic effects of angiotensin receptor blockers on dyslipidemia. Curr Vasc Pharmacol. 2011;9:129–35.
4. Sueta D, Tabata N, Hokimoto S. Clinical roles of calcium channel blockers in ischemic heart diseases. *Hypertens Res*. 2017;40:423–8.
5. Yamagishi S, Inagaki Y, Abe R, Kikuchi S, Sasaki N, Takeuchi M. Nifedipine inhibits apototic cell death of cultured endothelial cells induced by tumor necrosis factor-alpha. Drug Exp Clin Res. 2003;29:141–5.
6. Yamagishi S, Inagaki Y, Kikuchi S. Nifedipine inhibit tumor necrosis factor-alpha-induced monocyte chemoattractant protein-1 overexpression by blocking NADPH oxidase-mediated reactive oxygen species generation. Drug Exp Clin Res. 2003;29:147–52.
7. Yamagishi S, Takeuchi M. Nifedipine inhibit tumor necrosis factor-alpha-induced leukocyte adhesion to endothelial cells by suppressing vascular cell adhesion molecule (VCAM)-1 expression. Drug Exp Clin Res. 2004;30:163–8.
8. Yamagishi S, Inagaki Y, Nakamura K, Imaizumi T. Azelnidipine, a newly developed long-acting calcium antagonist, inhibits tumor necrosis factor-alpha-induced interleukin-8 expression in endothelial cells through its anti-oxidative properties. J Cardiovasc Pharmacol. 2004;43:724–30.
9. Matsui T, Yamagishi S, Nakamura K, Kikuchi S, Inoue H. Azelnidipine, a dihydropyridine-based calcium antagonist, inhibits angiotensin II-induced oxidative stress generation and downregulation of pigment epithelium-derived factor mRNA levels in microvascular endothelial cells. Drugs Exp Clin Res. 2005;31:215–9.
10. Nakano K, Egashira K, Ohtani K, Gang Z, Iwata E, Miyagawa M, Sunagawa K. Azelnidipine has anti-atherosclerotic effects independent of its blood pressure-lowering actions in monkeys and mice. Atherosclerosis. 2008;196:172–9.
11. Nakano K, Egashira K, Tada H, Kohjimoto Y, Hirouchi Y, Kitajima S, Endo Y, Li XH, Sunagawa K. A third-generation, long-acting, dihydropyridine calcium antagonist, azelnidipine, attenuates stent-associated neointimal formation in non-human primates. J Hypertens. 2006;24:1881–9.
12. Komoda H, Inoue T, Node K. Anti-inflammatory properties of azelnidipine, a dihydropyridine-based calcium channel blocker. Clin Exp Hypertens. 2010;32:121–8.
13. Shima E, Katsube M, Kato T, Kitagawa M, Hato F, Hino M, Takahashi T, Fujita H, Kitagawa S. Calcium channel blockers suppress cytokine-induced activation of human neutrophils. Am J Hypertens. 2008;21:78–84.
14. Mason RP, Jacob RF, Corbalan JJ, Kaliszan R, Malinski T. Amlodipine increased endothelial nitric oxide and decreased nitroxidative stress disproportionately to blood pressure changes. Am J Hypertens. 2014;27:482–8.
15. Liu X, Zhang GX, Zhang XY, Xia WH, Yang Z, Su C, Qiu YX, Xu SY, Zhan H, Tao J. Lacidipine improves endothelial repair capacity of endothelial progenitor cells from patients with essential hypertension. Int J Cardiol. 2013;168:3317–26.
16. Nissen SE, Tuzcu EM, Libby P, Thompson PD, Ghali M, Garza D, Berman L, Shi H, Buebendorf E, Topol EJ, CAMELOT Investigators. Effect of antihypertensive agents on cardiovascular events in patients with coronary disease and normal blood pressure: the CAMELOT study: a randomized controlled trial. JAMA. 2004;292:2217–25.
17. Kubota N, Miyauchi K, Kasai T, Iesaki T, Kajimoto K, Sumiyoshi K, Dohi T, Daida H. Synergistic effects of calcium-channel and angiotensin-receptor blockers on endothelial function and inflammatory responses in a porcine drug-eluting stent mode. Circ J. 2010;74:1704–10.
18. Nagasawa K, Takahashi K, Matsuura N, Takatsu M, Hattori T, Watanabe S, Harada E, Niinuma K, Murohara T, Nagata K. Comparative effects of valsartan in combination with cilnidipine or amlodipine on cardiac remodeling and diastolic dysfunction in Dahl salt-sensitive rats. Hypertens Res. 2015;38:39–47.
19. Okamura T, Tawa M, Geddawy A, Shimosato T, Iwasaki H, Shintaku H, Yoshida Y, Masada M, Shinozaki K, Imamura T. Effects of atorvastatin, amlodipine, and their combination on vascular dysfunction in insulin-resistant rats. J Pharmacol Sci. 2014;124:76–85.