Cilostazol Inhibits Vascular Smooth Muscle Cell Growth by Downregulation of the Transcription Factor E2F

Mi-Jung Kim, Keun-Gyu Park, Kyeong-Min Lee, Hye-Soon Kim, So-Yeon Kim, Chun-Soo Kim, Sang-Lak Lee, Young-Chae Chang, Joong-Yeol Park, Ki-Up Lee, In-Kyu Lee

Abstract—Neointimal formation, the leading cause of restenosis, is caused by proliferation of vascular smooth muscle cells (VSMCs). Patients with diabetes mellitus have higher restenosis rates after coronary angioplasty than nondiabetic patients. Cilostazol, a selective type 3 phosphodiesterase inhibitor, is currently used to treat patients with diabetic vascular complications. Cilostazol is a potent antiplatelet agent that inhibits VSMC proliferation. In the present study, we examine whether the antiproliferative effect of cilostazol on VSMCs is mediated by inhibition of an important cell cycle transcription factor, E2F. Cilostazol inhibited the proliferation of human VSMCs in response to high glucose in vitro and virtually abolished neointimal formation in rats subjected to carotid artery injury in vivo. Moreover, the compound suppressed high-glucose–induced E2F–DNA binding activity, and the expression of E2F1, E2F2, cyclin A, and PCNA proteins. These data suggest that the beneficial effects of cilostazol on high-glucose–stimulated proliferation of VSMCs are mediated by the downregulation of E2F activity and expression of its downstream target genes, including E2F1, E2F2, cyclin A, and PCNA. **(*Hypertension*. 2005;45:552-556.)**

Key Words: diabetes mellitus ■ hyperplasia ■ vascular smooth muscle cells

Neointimal hyperplasia and restenosis are major problems limiting the long-term efficacy of percutaneous transluminal coronary angioplasty.[1,2] Common events in vascular response to arterial injury include proliferation and migration of vascular smooth muscle cells (VSMCs) within arterial intimal and neointimal formation in injured vessels.[2,3] Patients with diabetes mellitus have higher restenosis rates after coronary angioplasty than nondiabetic patients.[2,4]

The transcription factor, E2F, has been implicated in the periodic regulation of cellular genes required for transition through G1 and entry into the S phase, including dihydrofolate reductase, c-myc, DNA polymerase, cdc2, and proliferating cell nuclear antigen (PCNA).[5–7] E2F activity is regulated by interactions with RB family members. As cells progress toward S phase, RB family proteins are phosphorylated by G1 cyclin-complexes, resulting in the release of transcriptionally active E2F, which then leads to the activation of genes required for cell cycle progression.[8–10] We recently showed that high glucose activates the DNA-binding activity of E2F, and decoy oligodeoxynucleotides against E2F inhibit the proliferation of VSMCs.[11] These data suggest that downregulation of E2F could constitute a therapeutic target to prevent restenosis after angioplasty in patients with diabetes.

Cilostazol increases intracellular cAMP concentrations by selectively blocking phosphodiesterase type III. The clinical implications and pharmacokinetics with respect to the effects and safety of this drug have been well-established, especially in peripheral vascular disease.[12] Cilostazol is a potent antiplatelet agent currently used in clinical practice to treat patients with diabetic vascular complications.[13–15] Several lines of evidence indicate that cilostazol additionally inhibits the proliferation of VSMCs, reduces neointimal formation in balloon-injured rat carotid arteries,[16–18] and inhibits restenosis after percutaneous transluminal coronary angioplasty.[19,20] One mechanism by which cilostazol may inhibit VSMC proliferation is via an increase in intracellular cAMP, because cAMP inhibits the proliferation of VSMCs by induction of p53-mediated and p21-mediated apoptosis.[21] However, Nadri et al demonstrated that increased cAMP leads to inhibition of phosphorylation of pRB, which regulates the activity of the E2F family, and consequently leads to arrest of cells at G1 in human lymphocytes.[22] Data from this investigation suggest another mechanism by which cilostazol inhibits VSMC proliferation, specifically through the suppression of E2F-mediated transcription. In the present study, we examine whether the antiproliferative effect of cilostazol on VSMCs is mediated by inhibition of E2F, which is regulated by pRB phosphorylation.

Received October 7, 2004; first decision November 4, 2004; revision accepted January 24, 2005.

From the Departments of Internal Medicine (M.-J.K., K.-G.P., K.-M.L., H.-S.K., I.-K.L.) and Institute for Medical Science, Physiology (S.-Y.K.), and Pediatrics (C.-S.K., S.-L.L.), Keimyung University School of Medicine, Daegu; Department of Pathology (Y.-C.C.), Catholic University of Daegu, School of Medicine, Daegu; and the Department of Internal Medicine (J.-Y.P., K.-I.U.), University of Ulsan College of Medicine, Seoul, Korea.

M.-J.K. and K.-G.P. contributed equally to this work.

Correspondence to In-Kyu Lee, MD, PhD, Department of Internal Medicine, Keimyung University School of Medicine, 194 Dongsan-dong, Jung-gu, Daegu, 700-712, South Korea. E-mail inkyulee@dsmc.or.kr

Hypertension **is available at http://www.hypertensionaha.org** **DOI: 10.1161/01.HYP.0000158263.64320.eb**

Materials and Methods

Animals

Nine- to 10-week-old male Sprague-Dawley rats (Hyochang, Daegu, Korea) weighing 280 to 320 grams were used in the experiments. All procedures were used in accordance with the institutional guidelines for animal research.

Rat Carotid Artery Balloon Injury

We examined the possible beneficial effects of cilostazol on neointimal formation by using a rat carotid artery balloon-injury model.[11] Cilostazol (100, 30, and 10 mg/d per kg) was administered daily by gavage, and balloon injury was performed on day 4. Rats were anesthetized with 50 mg/kg of sodium pentobarbital (Entobar; Hanlim Pharmaceutical, Yong-In, Korea). Cilostazol treatment was continued until rats were euthanized at 2 weeks after balloon injury.

Cell Culture

Human VSMCs (HVSMCs) were isolated from the thoracic aorta of kidney transplantation donors by the explant method as described previously.[11] Tissue collection was approved by the local Ethics Committee. HVSMCs were cultured in DMEM (Gibco BRL, Grand Island, NY) containing 20% fetal bovine serum (Gibco BRL). In each preparation, HVSMC purity was determined by staining with smooth muscle-specific α-actin monoclonal antibodies (Sigma, St Louis, Mo). Cells from the third and fifth passages were used in all experiments.

Growth Assay

HVSMCs were seeded into 96-well tissue culture plates. At 30% confluence, cells were rendered quiescent by incubation for 24 hours in defined serum-free medium. Cilostazol was donated by Otsuka Pharmaceutical Co (Osaka, Japan). The indicated dose of cilostazol was added to the wells, and cells were incubated at 37°C for a further 5 hours. After 48 hours, indices of cell proliferation were determined with a WST cell counting kit (Wako, Osaka, Japan).

Electrophoretic Mobility Shift Assay

Nuclear extracts were prepared from HVSMCs, as described previously.[11] Briefly, the DNA probe for E2F was labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. After end-labeling, the probe was purified with a NAP-5 column. Protein–DNA binding reactions were performed at room temperature for 20 minutes in a total volume of 20 μL. After incubation, samples were loaded onto a 4% native polyacrylamide gel in 0.5 × Tris-borate-EDTA buffer, and performed at 150 V for 2 hours. The gels were dried and visualized by autoradiography.

Luciferase Assay

The [E2F]×4 luciferase reporter construct, which contains 4 E2F sites with the TTTCGCGC sequence, was used in transient transfection assays, as described previously.[23]

Histological Analysis

Immunohistochemistry and morphological analysis of neointima were performed as described previously.[11]

Western Blot Analysis

Total protein extraction, Western blotting, and densitometric measurement of bands were performed as described previously.[24]

Statistical Analysis

All results are expressed as means±SEM. Analysis of variance was performed with Duncan test and used to determine significant differences in multiple comparisons. Values of $P<0.05$ were taken as statistically significant. All experiments were performed at least 3 times.

Figure 1. Effect of cilostazol on neointimal formation after balloon injury to the rat carotid artery. A representative cross-section of the left common carotid artery of a control rat 14 days after balloon injury, and 14 days after balloon injury with 100, 30, and 10 mg·d^{-1}·kg^{-1} cilostazol. The bars represent the intimal area of common carotid arteries after balloon injury in each group of animals (n=5). Statistical significance was determined as *$P<0.01$, compared with control, #$P<0.01$, and **$P<0.05$, compared with balloon-injured rats.

Results

Effect of Cilostazol on Neointimal Formation in Balloon-Injured Rat Carotid Arteries

As shown in Figure 1, neointimal formation was detected in the vessels 2 weeks after injury. Treatment with cilostazol reduced neointimal formation in a dose-dependent manner.

Effects of Cilostazol and cAMP Stimulants on Inhibition of HVSMC Growth In Vitro

As expected, high glucose (22 mmol/L D-glucose) significantly increased the proliferation of cultured HVSMCs, compared with normal glucose ($P<0.05$). Cilostazol (100 μmol/L, 10 μmol/L, and 1 μmol/L) inhibited this stimulation in a *dose*-dependent manner ($P<0.01$, $P<0.05$, and $P<0.05$, respectively). Similarly, cAMP stimulants, such as forskolin (100 μmol/L) and 8-bromo-cAMP (3 mmol/L), also suppressed HVSMC growth stimulated by high glucose ($P<0.01$) (Figure 2).

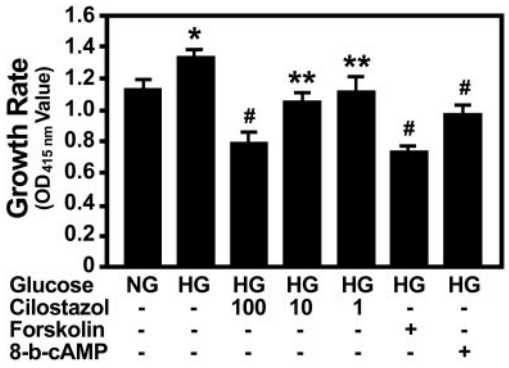

Figure 2. The inhibitory effect of cilostazol on HVSMC proliferation. HVSMCs were treated with cilostazol (100, 10, and 1 μmol/L), forskolin (100 μmol/L), and 8-bromo-cAMP (3 mmol/L). Proliferation is presented as means±SEM of 3 separate measurements. NG indicates HVSMCs cultured with normal glucose (5.5 mmol/L D-glucose); HG, HVSMCs cultured with high glucose (22 mmol/L D-glucose). *$P<0.05$ compared with NG; #$P<0.01$ compared with HG; **$P<0.05$ compared with HG.

Effect of Cilostazol on the DNA-Binding Activity of E2F

In view of the finding that cilostazol inhibits the proliferation of HVSMCs and neointimal formation, we examined its effect on the cell cycle regulatory transcription factor, E2F. The increase in E2F–DNA binding activity under high glucose conditions was attenuated by cilostazol at a dose of 10 μmol/L ($P<0.05$). Cilostazol (100 μmol) completely attenuated E2F–DNA binding activity (Figure 3A).

Effects of Cilostazol and cAMP Stimulants on Promoter Activity in HVSMCs

To further confirm the inhibition of E2F–DNA binding activity by cilostazol, we used E2F-responsive promoter constructs containing 4 E2F binding sites in the promoter. High glucose markedly stimulated E2F responsive promoter activity, compared with normal glucose ($P<0.05$). This increased luciferase activity was attenuated by cilostazol in a dose-dependent manner (100 μmol/L, 10 μmol/L, and 1 μmol/L) ($P<0.01$, $P<0.05$, and $P<0.05$, respectively). Similarly, forskolin (100 μmol/L) and 8-bromo-cAMP (3 mmol/L) inhibited luciferase gene expression ($P<0.01$) (Figure 3B).

Effects of Cilostazol and cAMP Stimulants on Expression of E2F and PCNA Proteins

We next examined the expression patterns of E2F downstream genes, such as cyclin A and PCNA. Treatment with high glucose increased the expression of E2F1, E2F2, cyclin A, and PCNA proteins. This glucose-induced stimulation of protein expression was inhibited by cilostazol and cAMP stimulants, such as forskolin and 8-bromo-cAMP (Figure 4).

To demonstrate the inhibitory effect of cilostazol on E2F downstream gene expression in vivo, we stained the neointimal region with PCNA. A marked increase in PCNA-positive cells in the neointimal region and dividing endothelial cells were noted 2 weeks after injury. The number of PCNA-positive cells in vessels treated with cilostazol was significantly lower than that in untreated vessels (Figure 5).

Discussion

Patients with diabetes mellitus have higher restenosis rates after coronary angioplasty than nondiabetic patients.[2,4] Hyperglycemia is believed to play a pivotal role in this vascular response, the major pathogenesis being the proliferation of VSMCs.[2,4,25] Multiple mechanisms involving protein kinase C and NF-κB have been implicated in the genesis of high-glucose–induced VSMC proliferation.[26,27] Over the past decade, the transcription factor E2F has emerged as a key component of cellular proliferation, during which it controls the expression of genes required for cell cycle progression,[28,29] especially in high-glucose–stimulated VSMCs.[11,30] However, only a few investigators have examined the effects of pharmacological agents on E2F activity and VSMC proliferation.[31,32] Here, we show that cilostazol effectively reduces high-glucose–stimulated E2F activity, as well as proliferation of HVSMCs in vitro and in vivo. In agreement with

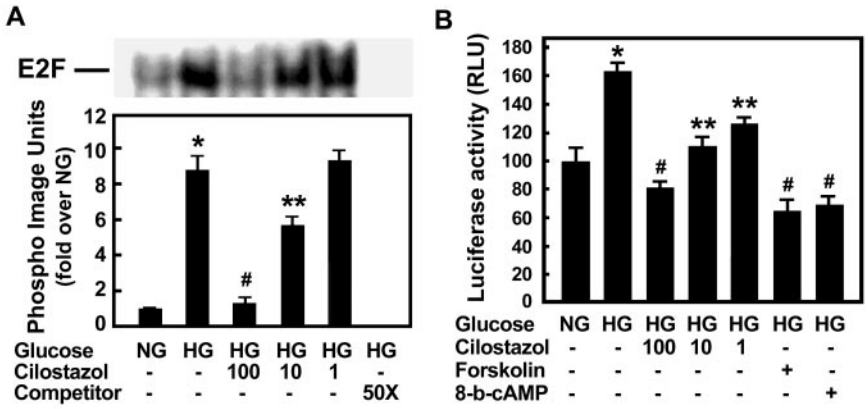

Figure 3. Effect of cilostazol on E2F–DNA binding activity and promoter activity. A, Effect of cilostazol on E2F–DNA binding activity. Typical gel shift assay of HVSMC lysates treated with cilostazol (upper). Electrophoretic mobility shift assay results are expressed as means±SEM of 3 separate measurements (lower). B, Effect of cilostazol on promoter activity. HVSMCs were treated with cilostazol (100, 10, and 1 μmol/L), forskolin (100 μmol/L), and 8-bromo-cAMP (3 mmol/L), and each lysate was assayed for β-galatosidase and luciferase. NG indicates HVSMCs cultured with normal glucose (5.5 mmol/L D-glucose); HG, HVSMCs cultured with high glucose (22 mmol/L D-glucose); 8-b-cAMP, 8-bromo-cAMP. Data are presented as means±SEM of 3 separate measurements. Statistical significance was determined as *$P<0.05$ compared with NG; #$P<0.01$ compared with HG; and **$P<0.05$ compared with HG.

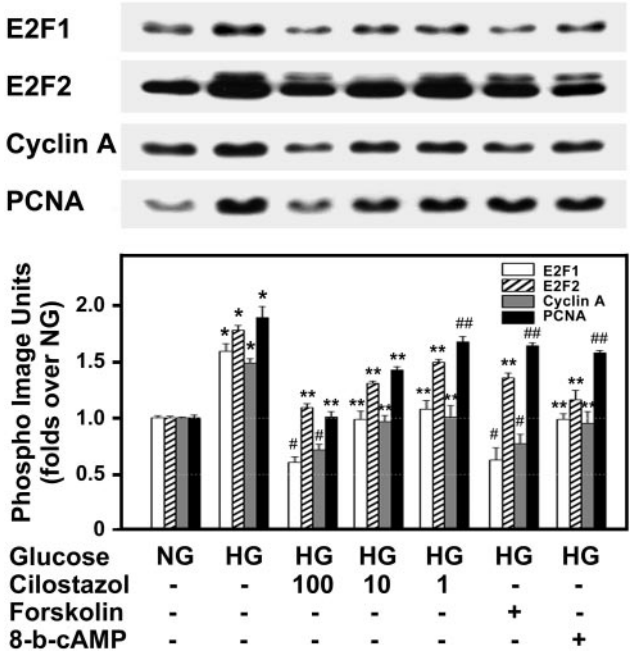

Figure 4. Effect of cilostazol on E2F1, E2F2, cyclin A, and PCNA protein expression. Typical Western blot of E2F1, E2F2, cyclin A, and PCNA in HVSMCs treated with cilostazol (100, 10, and 1 μmol/L), forskolin (100 μmol/L), and 8-bromo-cAMP (3 mmol/L). NG indicates HVSMCs cultured with normal glucose (5.5 mmol/L D-glucose); HG, HVSMCs cultured with high glucose (22 mmol/L D-glucose). Data are presented as means±SEM of 3 separate measurements. Statistical significance was determined as $^{*}P<0.01$ compared with NG; $^{\#}P<0.001$ compared with HG; $^{**}P<0.01$ compared with HG, and $^{\#\#}P<0.05$ compared with HG.

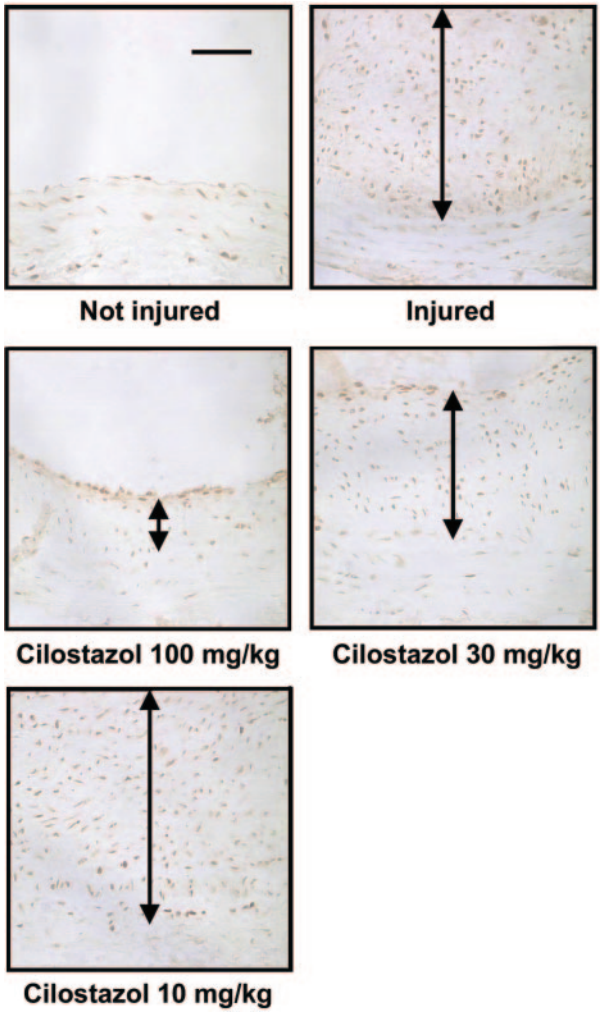

Figure 5. Effect of cilostazol on PCNA expression of the neointimal region after balloon injury. PCNA staining of control vessel, balloon-injured vessel without cilostazol, and balloon-injured vessels with 100, 30 and 10 mg·d^{-1}·kg^{-1} cilostazol are shown. PCNA-positive cells appear brown–black, and the number of PCNA-positive cells in the neointimal region in vessels treated with cilostazol was lower than that in untreated vessels. All figures are depicted at 200× magnification. The arrow represents the neointimal region. The scale bar represents 50 μm.

our previous data,[11] high glucose increases E2F–DNA binding and luciferase activity of the [E2F]×4-luciferase construct, consequently stimulating VSMC proliferation. These results provide a theoretical background for the use of cilostazol in the treatment of vascular disease in diabetic patients.

In mammals, 6 members of the E2F family have been identified (E2F1 to E2F6), of which transcriptionally active E2F proteins are structurally and functionally divided into 2 groups. Expression of E2F1 to E2F3 is low in quiescent cells, and increases during growth stimulation, whereas E2F4 and E2F5 accumulate in quiescent cells or during differentiation.[28,33,34] Several studies show that E2F proteins stimulate their own activity directly through the presence of binding sites in the promoters (E2F1 and E2F2),[35] and E2F downstream transcription is mediated by the synthesis of new E2F1 and E2F2.[36–38] Moreover, a previous study by our group demonstrated that high glucose increased the expression of endogenous targets of E2F, cyclin A, and PCNA. This expression was successfully inhibited by E2F decoy oligodeoxynucleotides. Accordingly, we investigated whether cilostazol could attenuate the increased expression of E2F1, E2F2, cyclin A, and PCNA mediated by high-glucose–stimulated E2F–DNA binding activity. Our data establish that cilostazol inhibits the high-glucose–stimulated increase in E2F1 and E2F2 proteins and their endogenous downstream targets, cyclin A, and PCNA. This study additionally shows that cilostazol attenuates the increase in PCNA-positive cells in the neointimal region and completely abolishes neointimal formation induced by balloon injury.

Recently, the mechanisms by which cAMP suppresses the proliferation and migration of VSMCs and prevents postangioplasty neointimal formation have been investigated.[21,39,40] Newman et al reported that cAMP inhibits the production of IL-6 and migration of HVSMCs.[39] Andolfi et al showed that the activation of cAMP-dependent protein kinase inhibits neointimal formation after vascular injury in a model of restenosis after angioplasty.[40] Hayashi et al reported that cAMP directly inhibits abnormal VSMC growth and induces the expression of the anti-oncogenes, p53 and p21, and apoptosis.[21] In addition, results from the present study strongly suggest that the inhibitory effect of cAMP on VSMC proliferation is mediated by downregulation of E2F activity.

Perspectives

The present study has clearly demonstrated that phosphodiesterase type 3 inhibitor, cilostazol, effectively reduces high-glucose–stimulated E2F activity and proliferation of HVSMCs in vitro and in vivo. The data presented herein are consistent with other reports showing a role for cAMP in VSMCs proliferation and neointimal formation. Taken together, these studies suggest that these agents increase cAMP in the vasculature, which thereby helps to prevent the development of restenosis after percutaneous transluminal coronary angioplasty, especially in patients with diabetes.

Acknowledgments

This work has been supported by The Advanced Medical Technology Cluster for Diagnosis and Prediction at KNU, which carries out one of the R&D Projects sponsored by the Korea Ministry of Commerce, Industry and Energy and was supported by a grant from Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (00-PJ3-PG6-GN07-001).

References

1. Currier JW, Faxon DP. Restenosis after percutaneous transluminal coronary angioplasty: have we been aiming at the wrong target? *J Am Coll Cardiol*. 1995;25:516–520.
2. Liu MW, Roubin GS, King SB III. Restenosis after coronary angioplasty. Potential biologic determinants and role of intimal hyperplasia. *Circulation*. 1989;79:1374–1387.
3. Pauletto P, Sartore S, Pessina AC. Smooth-muscle-cell proliferation and differentiation in neointima formation and vascular restenosis. *Clin Sci*. 1994; 87:467–479.
4. Massi-Benedetti M, Federici MO. Cardiovascular risk factors in type 2 diabetes: the role of hyperglycaemia. *Exp Clin Endocrinol Diabetes.* 1999; S107:S120–S123.
5. Dalton S. Cell cycle regulation of the human cdc2 gene. *EMBO J*. 1992;11: 1797–1804.
6. Thalmeier K, Synovzik H, Mertz R, Winnacker EL, Lipp M. Nuclear factor E2F mediates basic transcription and trans-activation by E1a of the human MYC promoter. *Genes Dev*. 1989;3:527–536.
7. Watson RJ, Dyson PJ, McMahon J. Multiple c-myb transcript cap sites are variously utilized in cells of mouse haemopoietic origin. *EMBO J*. 1987;6: 1643–1651.
8. Pagano M, Pepperkok R, Verde F, Ansorge W, Draetta G. Cyclin A is required at two points in the human cell cycle. *EMBO J*. 1992;11:961–971.
9. Weintraub SJ, Prater CA, Dean DC. Retinoblastoma protein switches the E2F site from positive to negative element. *Nature*. 1992;358:259–261.
10. Pagano M, Draetta G, Jansen-Durr P. Association of cdk2 kinase with the transcription factor E2F during S phase. *Science*. 1992;255:1144–1147.
11. Ahn JD, Morishita R, Kaneda Y, Kim HS, Chang YC, Lee KU, Park JY, Lee HW, Kim YH, Lee IK. Novel E2F decoy oligodeoxynucleotides inhibit in vitro vascular smooth muscle cell proliferation and in vivo neointimal hyperplasia. *Gene Ther*. 2002;9:1682–1692.
12. Tjon JA, Riemann LE. Treatment of intermittent claudication with pentoxyifylline and cilostazol. *Am J Health Syst Pharm*. 2001;58:485–496.
13. Dawson DL, Cutler BS, Meissner MH, Strandness DE Jr. Cilostazol has beneficial effects in treatment of intermittent claudication: results from a multicenter, randomized, prospective, double-blind trial. *Circulation*. 1998; 98:678–686.
14. Tanaka K, Gotoh F, Fukuuchi Y, Amano T, Uematsu D, Kawamura J, Yamawaki T, Itoh N, Obara K, Muramatsu K. Effects of a selective inhibitor of cyclic AMP phosphodiesterase on the pial microcirculation in feline cerebral ischemia. *Stroke*. 1989;20:668–673.
15. Fujitani K, Kambayashi J, Murata K, Yano Y, Shinozaki K, Yukawa M, Sakon M, Murata T, Kawasaki T, Shiba E, Mori T. Clinical evaluation on combined administration of oral prostacyclin analogue beraprost and phosphodiesterase inhibitor cilostazol. *Pharmacol Res*. 1995;31:121–125.
16. Souness JE, Hassall GA, Parrott DP. Inhibition of pig aortic smooth muscle cell DNA synthesis by selective type III and type IV cyclic AMP phosphodiesterase inhibitors. *Biochem Pharmacol*. 1992;44:857–866.
17. Meinkoth JL, Montminy MR, Fink JS, Feramisco JR. Induction of a cyclic AMP-responsive gene in living cells requires the nuclear factor CREB. *Mol Cell Biol*. 1991;11:1759–1764.
18. Ishizaka N, Taguchi J, Kimura Y, Ikari Y, Aizawa T, Togo M, Miki K, Kurokawa K, Ohno M. Effects of a single local administration of cilostazol on neointimal formation in balloon-injured rat carotid artery. *Atherosclerosis*. 1999;142:41–46.
19. Tsuchikane E, Fukuhara A, Kobayashi T, Kirino M, Yamasaki K, Kobayashi T, Izumi M, Otsuji S, Tateyama H, Sakurai M, Awata N. Impact of cilostazol on restenosis after percutaneous coronary balloon angioplasty. *Circulation*. 1999;100:21–26.
20. Take S, Matsutani M, Ueda H, Hamaguchi H, Konishi H, Baba Y, Kawaratani H, Sugiura T, Iwasaka T, Inada M. Effect of cilostazol in preventing restenosis after percutaneous transluminal coronary angioplasty. *Am J Cardiol*. 1997;79:1097–1099.
21. Hayashi S, Morishita R, Matsushita H, Nakagami H, Taniyama Y, Nakamura T, Aoki M, Yamamoto K, Higaki J, Ogihara T. Cyclic AMP inhibited proliferation of human aortic vascular smooth muscle cells, accompanied by induction of p53 and p21. *Hypertension*. 2000;35:237–243.
22. Naderi S, Gutzkow KB, Christoffersen J, Smeland EB, Blomhoff HK. cAMP-mediated growth inhibition of lymphoid cells in G1: rapid down-regulation of cyclin D3 at the level of translation. *Eur J Immunol*. 2000;30: 1757–1768.
23. Chang YC, Illenye S, Heintz NH. Cooperation of E2F-p130 and Sp1-pRb complexes in repression of the Chinese hamster dhfr gene. *Mol Cell Biol*. 2001;21:1121–1131.
24. Ahn JD, Kim CH, Magae J, Kim YH, Kim HJ, Park KK, Hong S, Park KG, Lee IK, Chang YC. E2F decoy oligodeoxynucleotides effectively inhibit growth of human tumor cells. *Biochem Biophys Res Commun*. 2003;310: 1048–1053.
25. Feener EP, King GL. Vascular dysfunction in diabetes mellitus. *Lancet.* 1997;S350:S9–S13.
26. Ishii H, Jirousek MR, Koya D, Takagi C, Xia P, Clermont A, Bursell SE, Kern TS, Ballas LM, Heath WF, Stramm LE, Feener EP, King GL. Amelioration of vascular dysfunctions in diabetic rats by an oral PKC beta inhibitor. *Science*. 1996;272:728–731.
27. Yerneni KK, Bai W, Khan BV, Medford RM, Natarajan R. Hyperglycemia-induced activation of nuclear transcription factor kappaB in vascular smooth muscle cells. *Diabetes*. 1999;48:855–864.
28. Black AR, Azizkhan-Clifford J. Regulation of E2F: a family of transcription factors involved in proliferation control. *Gene*. 1999;237:281–302.
29. Bernards R. E2F: a nodal point in cell cycle regulation. *Biochim Biophys Acta*. 1997;1333:M33–M40.
30. Fujita N, Furukawa Y, Du J, Itabashi N, Fujisawa G, Okada K, Saito T, Ishibashi S. Hyperglycemia enhances VSMC proliferation with NF-kappaB activation by angiotensin II and E2F-1 augmentation by growth factors. *Mol Cell Endocrinol*. 2002;192:75–84.
31. Bruemmer D, Yin F, Liu J, Kiyono T, Fleck E, Van Herle AJ, Law RE. Rapamycin inhibits E2F-dependent expression of minichromosome maintenance proteins in vascular smooth muscle cells. *Biochem Biophys Res Commun*. 2003;303:251–258.
32. Goukassian D, Sanz-Gonzalez SM, Perez-Roger I, Font de Mora J, Urena J, Andres V. Inhibition of the cyclin D1/E2F pathway by PCA-4230, a potent repressor of cellular proliferation. *Br J Pharmacol*. 2001;132:1597–1605.
33. Ferreira R, Magnaghi-Jaulin L, Robin P, Harel-Bellan A, Trouche D. The three members of the pocket proteins family share the ability to repress E2F activity through recruitment of a histone deacetylase. *Proc Natl Acad Sci U S A*. 1998;95:10493–10498.
34. Dyson N. The regulation of E2F by pRB-family proteins. *Genes Dev*. 1998; 12:2245–2262.
35. Yamasaki L. Growth regulation by the E2F and DP transcription factor families. *Results Probl Cell Differ*. 1998;22:199–227.
36. Hsiao KM, McMahon SL, Farnham PJ. Multiple DNA elements are required for the growth regulation of the mouse E2F1 promoter. *Genes Dev*. 1994;8: 1526–1537.
37. Neuman E, Flemington EK, Sellers WR, Kaelin WG Jr. Transcription of the E2F-1 gene is rendered cell cycle dependent by E2F DNA-binding sites within its promoter. *Mol Cell Biol*. 1994;14:6607–6615.
38. Sears R, Ohtani K, Nevins JR. Identification of positively and negatively acting elements regulating expression of the E2F2 gene in response to cell growth signals. *Mol Cell Biol*. 1997;17:5227–5235.
39. Newman WH, Castresana MR, Webb JG, Wang Z. Cyclic AMP inhibits production of interleukin-6 and migration in human vascular smooth muscle cells. *J Surg Res*. 2003;109:57–61.
40. Indolfi C, Di Lorenzo E, Rapacciuolo A, Stingone AM, Stabile E, Leccia A, Torella D, Caputo R, Ciardiello F, Tortora G, Chiariello M. 8-chloro-cAMP inhibits smooth muscle cell proliferation in vitro and neointima formation induced by balloon injury in vivo. *J Am Coll Cardiol*. 2000;36:288–293.

Cilostazol Inhibits Vascular Smooth Muscle Cell Growth by Downregulation of the Transcription Factor E2F

Mi-Jung Kim, Keun-Gyu Park, Kyeong-Min Lee, Hye-Soon Kim, So-Yeon Kim, Chun-Soo Kim, Sang-Lak Lee, Young-Chae Chang, Joong-Yeol Park, Ki-Up Lee and In-Kyu Lee

Hypertension. 2005;45:552-556; originally published online February 21, 2005;
doi: 10.1161/01.HYP.0000158263.64320.eb
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0194-911X. Online ISSN: 1524-4563