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Cilostazol, Not Aspirin, Prevents Stenosis of Bioresorbable Vascular Grafts in a Venous Model

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Objective—Despite successful translation of bioresorbable vascular grafts for the repair of congenital heart disease, stenosis remains the primary cause of graft failure. In this study, we investigated the efficacy of long-term treatment with the antiplatelet drugs, aspirin and cilostazol, in preventing stenosis and evaluated the effect of these drugs on the acute phase of inflammation and tissue remodeling.

Approach and Results—C57BL/6 mice were fed a drug-mixed diet of aspirin, cilostazol, or normal chow during the course of follow-up. Bioresorbable vascular grafts, composed of poly(glycolic acid) mesh sealed with poly(l-lactide-co-ε-caprolactone), were implanted as inferior vena cava interposition conduits and followed up for 2 weeks (n=10 per group) or 24 weeks (n=15 per group). Both aspirin and cilostazol suppressed platelet activation and attachment onto the grafts. On explant at 24 weeks, well-organized neotissue had developed, and cilostazol treatment resulted in 100% graft patency followed by the aspirin (67%) and no-treatment (60%) groups ($P<0.05$). Wall thickness and smooth muscle cell proliferation in the neotissue of the cilostazol group were decreased when compared with that of the no-treatment group at 24 weeks. In addition, cilostazol was shown to have an anti-inflammatory effect on neotissue at 2 weeks by regulating the recruitment and activation of monocytes.

Conclusions—Cilostazol prevents stenosis of bioresorbable vascular graft in a mouse inferior vena cava implantation model up to 24 weeks and is accompanied by reduction of smooth muscle cell proliferation and acute inflammation. **(*Arterioscler Thromb Vasc Biol*. 2015;35:2003-2010. DOI: 10.1161/ATVBAHA.115.306027.)**

Key Words: antiplatelet drugs ■ constriction, pathologic ■ inflammation ■ mice ■ monocytes

Approximately 0.6% of live births are affected by moderate to severe forms of congenital heart disease,[1] many of which require surgical intervention with various prosthetics to restore normal cardiac function. However, synthetic materials, such as polytetrafluoroethylene and polyethylene terephthalate, lack growth potential, and their use requires reoperation to up-size the conduit as the child grows. To address this challenge, novel tissue engineering techniques allow the implantation of bioresorbable vascular grafts that restore function and transform into biologically active blood vessels.[2] A bioresorbable vascular graft is entirely reconstituted by host-derived cells over the course of its degradation via an inflammation-mediated process.[3] This technique has been successfully applied in the clinical arena, and evidence has shown that this therapy is safe and effective in pediatric patients.[4,5] The application of bioresorbable vascular grafts has several advantages, such as growth potential, favorable biocompatibility, and low risk of infection or rejection; however, the incidence of stenosis because of neotissue hyperplasia, which is thought to be related to excessive inflammation, platelet activation, and smooth muscle cell (SMC) proliferation, is nearly equivalent to that of polytetrafluoroethylene grafts currently used in the Fontan surgery.[6] Therefore, the top priority in the development of second-generation bioresorbable vascular grafts is to safely reduce the incidence of stenosis.

Aspirin, a widely used antiplatelet drug, is routinely used as a therapeutic in our clinical trial to prevent platelet aggregation on the graft directly after implantation. Aside from its antiplatelet effects, aspirin has been shown to inhibit SMC migration and proliferation in blood vessels,[7] to protect endothelial cells (ECs),[8] and to suppress vascular inflammation.[9] The phosphodiesterase 3 inhibitor cilostazol is another antiplatelet drug, which can reduce platelet aggregation and can improve peripheral vasodilation by increasing intracellular cAMP content.[10]

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Nonstandard Abbreviations and Acronyms	
α-SMA	α-smooth muscle actin
BM-MNC	bone marrow–derived mononuclear cell
EC	endothelial cell
ECM	extracellular matrix
IFN-γ	interferon-gamma
iNOS	inducible nitric oxide synthase
IVC	inferior vena cava
Ly6C	lymphocyte antigen 6C
SMC	smooth muscle cell

Similar to aspirin, cilostazol has been reported to exert pleiotropic effects on SMCs, ECs, and vascular inflammation.[11–14]

Although previous findings support the potential of the antiplatelet drugs, aspirin and cilostazol, to suppress excessive neotissue formation during the process of vascular remodeling, the effect of these drugs on preventing the development of stenosis in bioresorbable vascular grafts is currently unknown. The purpose of this study was to clarify the impacts of long-term (24 weeks) administration of aspirin and cilostazol on neotissue hyperplasia–causing stenosis after the implantation of bioresorbable vascular grafts as inferior vena cava (IVC) interposition conduits in a mouse model. Furthermore, our previous findings also suggest that the natural history of graft stenosis in the murine model begins within 2 weeks after implantation, and that this time point is a critical window to assess vascular inflammation and neotissue formation in implanted bioresorbable grafts.[15] Thus, we also investigated the acute phase (2 weeks) effect of antiplatelet treatment with aspirin and cilostazol on the inflammation of and tissue remodeling processes in the bioresorbable vascular grafts.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Aspirin and Cilostazol Reduce Platelet Activation and Attachment Onto Bioresorbable Grafts In Vitro

To confirm the antiplatelet effects of aspirin and cilostazol administration in our mouse model, we examined the activation potential of platelets isolated from mice of each experimental group (aspirin, cilostazol, or no treatment) by evaluation of P-selectin and glycoprotein IIb expression on platelets with flow cytometry under both resting and thrombin-activated conditions. Platelet activation was suppressed in both aspirin and cilostazol groups (no treatment, 44.6%; aspirin, 11.3%; and cilostazol, 7.58%; Figure 1A). Furthermore, platelet attachment onto bioresorbable grafts after thrombin activation was reduced by both aspirin ($P<0.05$) and cilostazol ($P<0.01$; Figure 1B). These effects were not observed in resting, nonactivated conditions (Figure 1A and 1B). These results indicate that both aspirin and cilostazol reduced platelet function in our mouse model to the extent that they inhibited platelet attachment onto the bioresorbable graft.

Cilostazol Prevents Stenosis of Bioresorbable Vascular Grafts

A total of 75 mice received bioresorbable vascular grafts as IVC interposition conduits and were followed up for 2 weeks (n=10 per group) to evaluate the effects of aspirin and cilostazol on tissue remodeling and inflammation during the acute phase, or for 24 weeks (n=15 per group) to clarify the impact of long-term administration of these drugs on graft stenosis and neotissue formation. All mice survived during the course of observation.

Macroscopically, implanted grafts were still distinguishable from native IVC at the 2-week time point but were fully integrated with native IVC by 24 weeks (Figure 2A). Serial ultrasonographic imaging was performed on all mice that received grafts for 24 weeks, and graft patency was determined with color Doppler and pulse Doppler in the graft lumen. Graft patency of the cilostazol group was sustained from 2 to 24 weeks after implantation, whereas that of the aspirin group gradually decreased. The no-treatment group experienced the lowest patency rate at each time point (Figure 2B). There was a statistically significant difference in patency between the no-treatment and the cilostazol groups at 8 weeks (no treatment, 46.7% versus cilostazol, 93.3%; $P=0.014$; Figure 2B).

On explant, sufficient cell infiltration and cell growth were observed in all groups at the 24-week time point (Figure 3A). Cilostazol treatment resulted in 100% graft patency followed by the aspirin (67%) and the no-treatment groups (60%) at 24 weeks (Figure 3B). In addition, wall thickness was

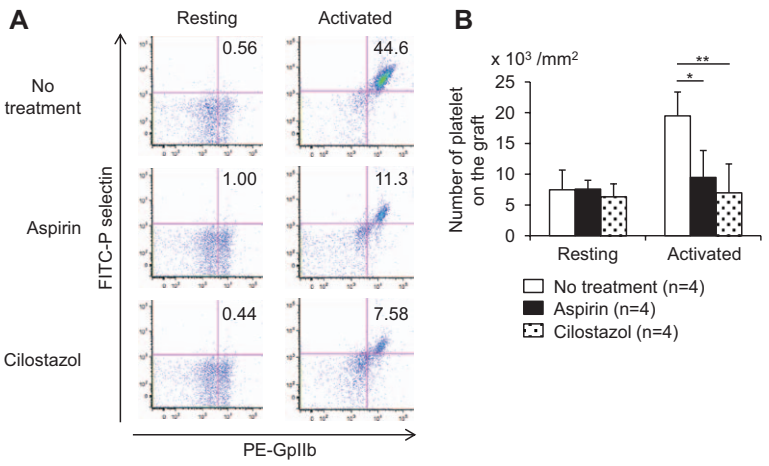

Figure 1. Platelet function evaluated by activation potential and graft attachment. **A**, Activated platelets were defined by expression both of P-selectin and glycoprotein IIb (GpIIb) with flow cytometry. Both aspirin and cilostazol treatments suppressed platelet activation. **B**, Aspirin and cilostazol treatments reduced thrombin-activated platelet attachment to grafts. Data are shown as mean±SD and evaluated by 1-way ANOVA followed by Tukey HSD. *$P<0.05$, **$P<0.01$. FITC indicates fluorescein isothiocyanate.

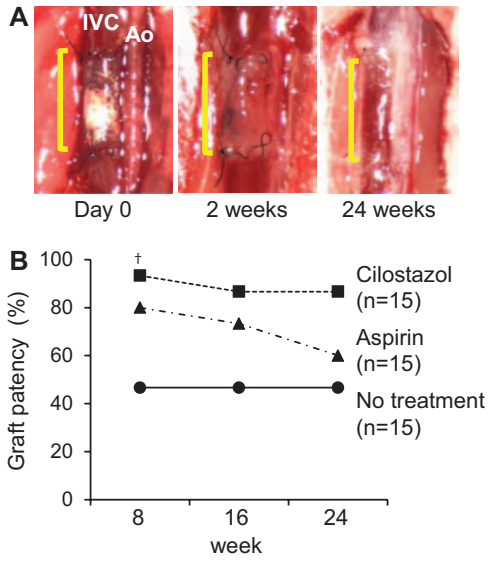

Figure 2. Serial monitoring of implanted grafts. **A**, Macroscopic assessment showed an integration of the implanted graft with native vein. Yellow bars indicate implanted grafts. **B**, Ultrasound evaluation demonstrated statistically significant differences in graft patency between no-treatment and cilostazol groups at 8 weeks, when evaluated by Fisher exact probability test with Bonferroni–Holm correction (P<0.016 was considered statistically significant). †P=0.014.

significantly less in the cilostazol group at the 24-week time point (no treatment, 633.2±250.3 μm; aspirin, 454.4±330.2 μm; cilostazol, 202.5±50.9 μm; P<0.001; Figure 3C).

Cilostazol Suppresses SMC Proliferation

Endothelialization on the luminal surface is thought to be a crucial step in the development of well-organized neotissue of a bioresorbable vascular graft.[16] To evaluate endothelialization of implanted bioresorbable grafts, immunostaining for the EC marker CD31 was used. EC coverage on the graft progressed before the 2-week time point, and favorable endothelialization was achieved in all groups by the 24-week time point (Figure 4A). For the quantitative comparison of endothelialization between groups at the 24-week time point, gene expression of platelet endothelial cell adhesion molecule-1 and endothelial nitric oxide synthase in explanted grafts was measured. No statistically significant differences between groups were observed (Figure 4B).

Abundant SMCs, which were defined by α-smooth muscle actin (α-SMA) immunolabeling, surrounded the endothelium at the 2-week time point, and the total number of SMCs decreased during the course of tissue remodeling in the aspirin and the control groups (Figure 4A). A significant decrease in the number of SMCs was observed in the neotissue of the cilostazol group when compared with that of the no-treatment group at the 24-week time point (no treatment, 481.5±127.3; cilostazol, 313.3±78.6 per mm^2; P<0.05; Figure 4C). Interestingly, some α-SMA–positive cells were coincident with CD31 at the 2-week time point, and these double-positive cells decreased at the 24-week time point (Figure 4A).

Aspirin and Cilostazol Do Not Affect Extracellular Matrix Deposition in Neotissue

Extracellular matrix (ECM) is the primary determinant of the biomechanical properties of a neovessel. Consequently, we evaluated ECM components including collagen and elastin by histology. Masson's trichrome and Alcian Blue staining showed a gradual increase in collagen deposition. Hart's staining showed no elastin deposition during neotissue formation (Figure I in the online-only Data Supplement). However, no differences were identified among groups (Figure II in the online-only Data Supplement), suggesting that both aspirin and cilostazol do not affect the development of a robust ECM in the neotissue of bioresorbable vascular grafts.

To quantify collagen deposition in the grafts and to confirm graft polymer degradation at the 2- and the 24-week time points, the proportion of thin and thick collagen fibers was measured using Picrosirius red staining visualized with polarized light microscopy. On the basis of previous reports,

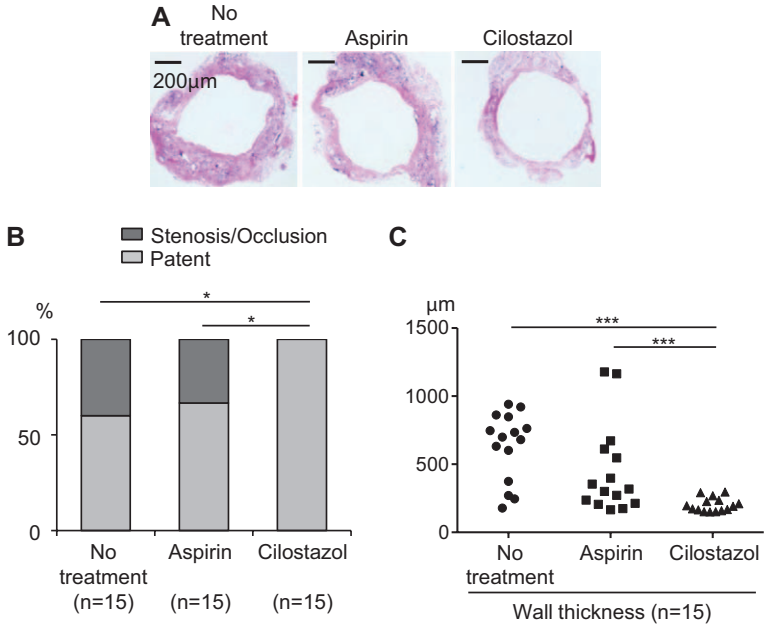

Figure 3. Comparison of morphometric analysis of explanted grafts at 24 weeks after implantation. **A**, Representative hematoxylin-eosin staining images from each group. **B**, Cilostazol treatment resulted in 100% graft patency followed by aspirin (67%) and no-treatment groups (60%). Data were evaluated by Fisher exact probability test. **C**, Wall thickness was significantly lower in the cilostazol group than in the no-treatment and aspirin groups. For comparisons among multiple groups, data were evaluated by nonparametric Kruskal–Wallis test. A post hoc Mann–Whitney test was performed to detect significant difference between groups with Bonferroni–Holm correction (P<0.016 was considered statistically significant). *P<0.05, ***P<0.001.

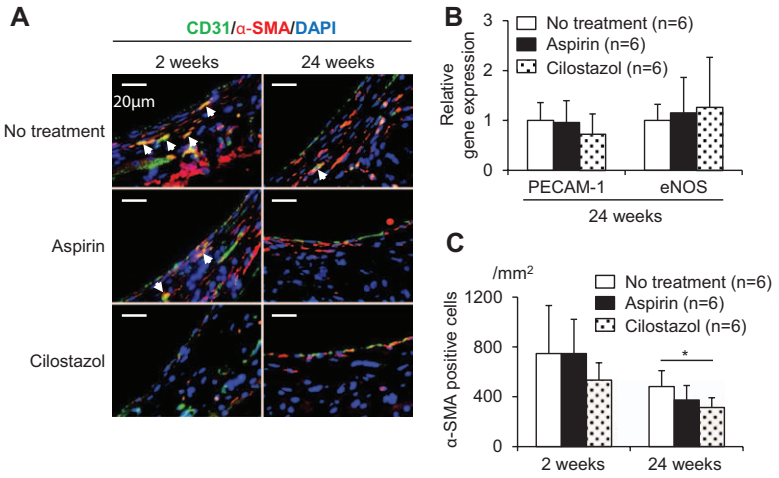

Figure 4. Endothelialization and smooth muscle cell proliferation at 2- and 24-week time points. **A**, CD31-positive endothelial cells covered the luminal surface in each group at the 2-week time point. CD31-positive cells were also identified in the neotissue, and these cells were coincident with α-smooth muscle actin (SMA). At the 24-week time point, favorable endothelialization was achieved in all groups, and double-positive cells for CD31 and α-SMA had decreased. Arrows indicate double-positive CD31 and α-SMA cells. **B**, There was no statistically significant difference in gene expression of platelet endothelial cell adhesion molecule (PECAM)-1 and endothelial nitric oxide synthase (eNOS) between groups. **C**, α-SMA–positive cells in the cilostazol group decreased significantly in comparison with the no-treatment group at the 24-week time point. Data are shown as mean±SD and evaluated by 1-way ANOVA followed by Tukey HSD. *P<0.05.

we correlated orange and yellow (thick fibers) with collagen type I, green (thin fibers) with collagen type III, and attributed white regions to remaining scaffold fibers or suture material.[17] Although scaffold fibers were present at 2 weeks, all scaffold material had been completely resorbed at 24 weeks. Collagen type I deposition increased over the time course of neotissue formation (Figure IIIA in the online-only Data Supplement). In addition, no differences in the distribution of collagen type I or type III at any time point were observed among experimental groups (Figure IIIB in the online-only Data Supplement).

Vascular basement membranes are a specialized form of ECM and are important structural and functional components of a blood vessel.[18] A main component of vascular basement membrane is collagen type IV, and collagen IV deposition has been demonstrated in similar mouse models.[19] To this end, we performed immunofluorescent staining for collagen type IV at the 24-week time point and demonstrated deposition of luminal collagen type IV in all experimental groups (Figure IV in the online-only Data Supplement).

Cilostazol Regulates Monocyte Recruitment and Activation in the Acute Phase of Tissue Remodeling

Monocyte- and macrophage-mediated inflammation is understood to play a crucial role in the formation of both vascular neotissue and the development of stenosis in a bioresorbable vascular graft.[3,15]

Immunohistochemical F4/80 staining showed a decrease in infiltration of monocytes/macrophages in the grafts from the cilostazol group when compared with that from the no-treatment groups at the 2-week time point (P<0.05; Figure 5A). Flow cytometric analysis revealed that recruited monocytes ($CD115^{+}F4/80^{+low}$) are present in the graft explants 2 weeks after implantation. Fewer absolute numbers of inflammatory lymphocyte antigen 6C positive high ($Ly6C^{+high}$) monocytes were observed in the aspirin and cilostazol groups than in the no-treatment group, but the difference between groups was only significant at P=0.19 (Figure 5B). On the contrary, no discernible macrophage populations ($CD115^{+}$ $F4/80^{+high}$ $Ly6C^{+low}$) were identified in any 2-week graft explants with our staining methods and gating strategies for flow cytometric analysis (Figure VA in the online-only Data Supplement).

Because inducible nitric oxide synthase (iNOS) is a marker for classical activation of inflammatory cells,[20] we determined iNOS expression in neotissue of the grafts by immunohistochemistry. Fewer activated inflammatory cells in the neotissue of the cilostazol group were found when compared with that of the either aspirin or no-treatment groups, and statistical significance in the number of iNOS-positive cells was detected between the cilostazol and the no-treatment groups at the 2-week time point (P<0.01; Figure 5C). Negative and positive controls for iNOS staining are shown in Figure VI in the online-only Data Supplement. Because our gating strategy for flow cytometric analysis indicated that monocytes accounted for most of the infiltrating leukocytes (CD45-positive cells) in the 2-week graft explants (Figure VA in the online-only Data Supplement), we think that the majority of iNOS-positive cells at the 2-week time point are activated monocytes.

To confirm the functional effect of aspirin and cilostazol in the activation of monocytes in vitro, we induced classical activation of bone marrow monocytes ($Ly6C^{+high}$ and $Ly6C^{+low}$) by stimulation with lipopolysaccharide and interferon-gamma (IFN-γ) after incubation with aspirin, cilostazol, or vehicle control and determined iNOS expression by flow cytometry. Lipopolysaccharide /IFN-γ stimulation increased the number of iNOS-positive cells in both $Ly6C^{+high}$ and $Ly6C^{+low}$ monocytes. Interestingly, only cilostazol prevented iNOS expression in both monocyte subsets after lipopolysaccharide /IFN-γ stimulation in a dose-dependent manner (Figure 6A–6D).

Discussion

The primary finding of our study was that cilostazol treatment, in contrast to aspirin, achieved 100% patency of bioresorbable vascular grafts up to 24 weeks with favorable neotissue formation. Bone marrow–derived mononuclear cells (BM-MNCs) have the potential to reduce the incidence of stenosis when seeded onto grafts before implantation.[15,21] However, previous clinical data demonstrate that 16% of patients who have received bioresorbable vascular grafts with BM-MNC seeding still developed stenosis requiring angioplasty,[5] even when aspirin is used postoperatively. In the present study, we implanted BM-MNC–free (unseeded) bioresorbable vascular grafts in a mouse IVC interposition graft model. We have previously demonstrated that unseeded grafts result in a higher incidence

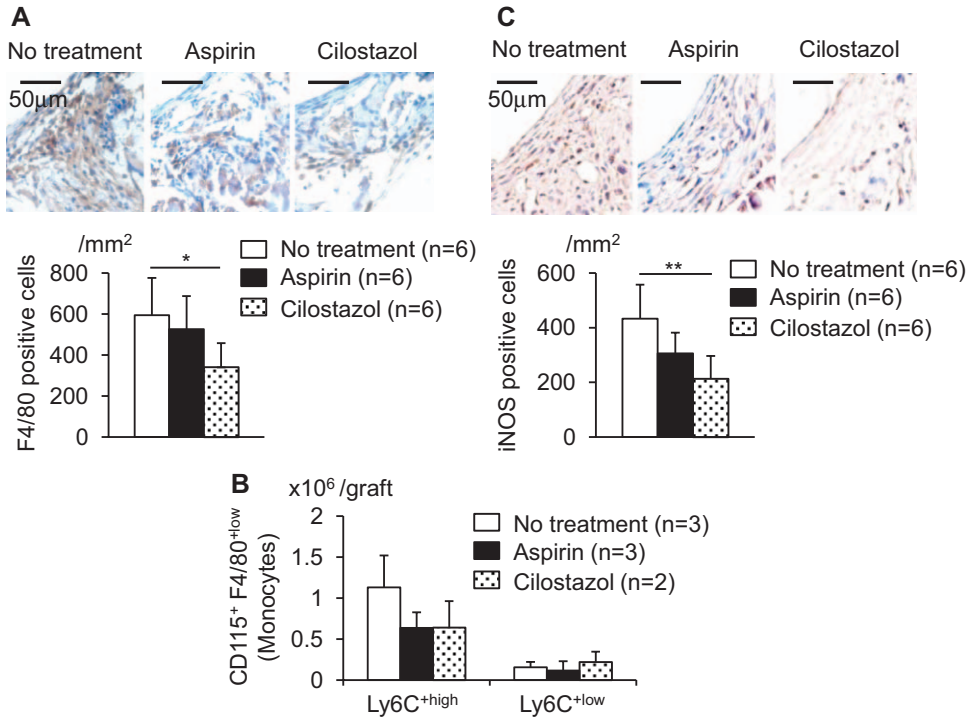

Figure 5. Inflammation in neotissue at 2 weeks. **A**, F4/80 staining showed a decrease in macrophage lineage cells in neotissue of cilostazol group, and statistical significance in the number of F4/80-positive cells between no-treatment and cilostazol groups was detected. **B**, Flow cytometric analysis revealed fewer inflammatory Ly6C^{+high} monocytes in graft explants in both aspirin and cilostazol groups when compared with the no-treatment group, but the difference between groups was only significant at P=0.19. **C**, Inducible nitric oxide synthase (iNOS) staining demonstrated migration of activated inflammatory cells into neotissue. Statistical significance in the number of iNOS-positive cells between no-treatment and cilostazol groups was detected. Data are shown as mean±SD and evaluated by 1-way ANOVA followed by Tukey HSD. *P<0.05, **P<0.01.

of stenosis than seeded grafts, and we selected the unseeded graft model for the current study to focus on the impact of antiplatelet drugs in preventing the graft stenosis and demonstrated the efficacy of cilostazol in this application. We suggest that combined therapy of BM-MNC seeding and systemic cilostazol treatment, instead of aspirin, has a potential to reduce the incidence of stenosis after the implantation of bioresorbable vascular grafts. However, seeding BM-MNCs onto the grafts may affect the response of the graft to these antiplatelet drugs. Additional studies to evaluate the combined effect of

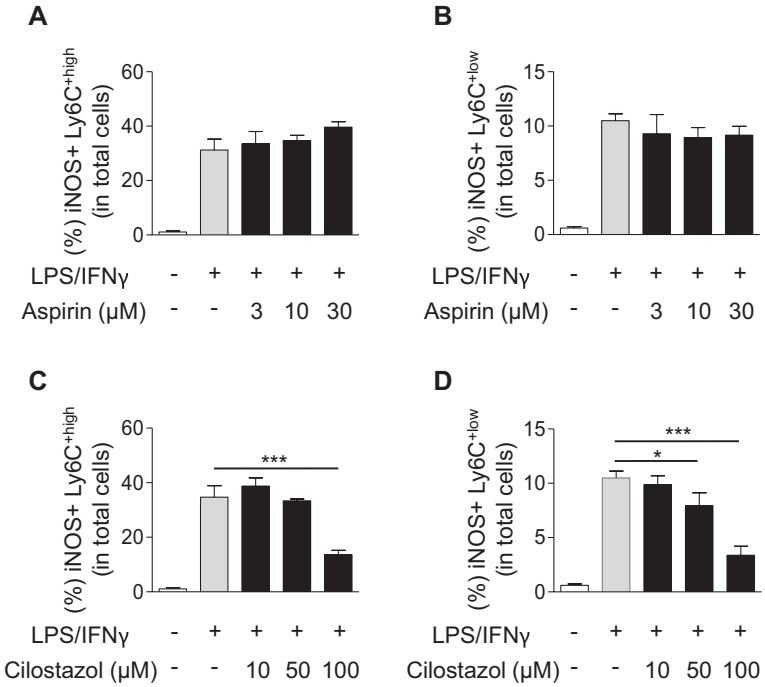

Figure 6. Flow cytometric analysis to evaluate the anti-inflammatory effect of aspirin and cilostazol on bone marrow isolated monocytes. **A** and **B**, Aspirin did not alter inflammatory inducible nitric oxide synthase (iNOS) expression of Ly6C^{+high} and Ly6C^{+low} monocytes after lipopolysaccharide (LPS)/interferon-gamma (IFN-γ) stimulation (n=3 in each group). **C** and **D**, However, high-dose cilostazol prevented iNOS expression of both monocyte subsets (n=3 in each group). Data are shown as mean±SD and evaluated by 1-way ANOVA followed by Tukey HSD. *P<0.05, ***P<0.001.

BM-MNC seeding and antiplatelet treatment are, therefore, required before clinical translation can be advocated.

In the present study, both aspirin and cilostazol treatments reduced platelet activation and attachment on the grafts in vitro. Because activated platelets release several growth factors, such as transforming growth factor-beta and platelet-derived growth factor, which promote SMC recruitment and proliferation leading to neotissue hyperplasia, prevention of platelet activation and aggregation on the graft surface was expected to attenuate stenosis. Ultrasound assessment demonstrated that both aspirin and cilostazol had a similar effect on the development of stenosis at early time points (≤8 weeks), indicating that the antiplatelet effect of these drugs may work to prevent stenosis because of thrombosis during the acute phase. Cilostazol treatment sustained this high patency rate throughout our observation period. On the contrary, the patency rate of the aspirin group decreased gradually, supporting similar results in the literature in which oral administration of aspirin has been shown ineffective in amelioration of neointimal lesions in a mouse vein graft model.[22] Interestingly, clinical findings demonstrated that cilostazol successfully prevented neointimal hyperplasia after implantation of arterial stents[23,24] even when compared with aspirin.[25] On the basis of these findings, we propose that multiple effects of cilostazol, including anti-SMC proliferation, EC protection, and anti-inflammation, in addition to its antiplatelet effect, may have worked in concert to prevent neotissue hyperplasia throughout the time course of its formation in our bioresorbable vascular graft.

Wall thickness is an important metric by which to evaluate neotissue formation and the development of stenosis in a bioresorbable vascular graft. Size mismatch is routinely used in clinical application of vascular grafts in the pediatric population to minimize reoperation to up-size the conduit because of somatic overgrowth. Over time, the scaffold materials degrade, and the graft wall is expected to remodel into a neovessel that closely resembles native IVC. We demonstrated that wall thickness 24 weeks after implantation was least in the cilostazol group, with a reduction in the number of α-SMA–positive SMCs in the developing neotissue although there was no difference in graft material absorption, endothelialization, and ECM deposition between groups at this time point. Although vascular SMCs are essential for the functional integrity of the neovessel, excessive proliferation of SMCs leads to neointimal hyperplasia followed by graft stenosis and occlusion. Cilostazol is understood to inhibit the proliferation of SMCs directly by increased intracellular cAMP.[11,26] SMCs are complex cells capable of existing in heterogeneous populations and switching phenotypes on a variety of stimuli (ie, contractile to synthetic).[27] The synthetic dedifferentiated phenotype of SMCs, which have characteristics of migration, proliferation, and ECM synthesis in the vascular wall, promotes neointimal hyperplasia and can be identified by the expression of α-SMA, which is detectable early in the developing vasculature.[28] On the contrary, differentiated SMCs have a contractile apparatus with less potential for proliferation and are distinguished by the expression of differentiated SMC markers, such as smooth muscle-myosin heavy chain.[28] In this study, much fewer smooth muscle-myosin heavy chain–positive cells were observed in the neotissue of implanted grafts (Figure VII in the online-only Data Supplement) than α-SMA–positive cells (Figure 4A), indicating that synthetic SMCs account for most of the SMCs in the neotissue of the bioresorbable grafts during the remodeling process in our model. Breakdown products from bioresorbable poly(glycolic acid), which was used in the present study, may have induced dedifferentiation of SMCs to the synthetic phenotype. Furthermore, the combination of scaffold geometry, biochemical, and mechanical stimulation are thought to affect SMC phenotypes. Cilostazol may exert its effect on these scaffold characteristics to suppress dedifferentiation or proliferation of SMCs.

During the acute phase (2 weeks after the graft implantation) of tissue remodeling, we demonstrated that cilostazol regulates Ly6C^{+high} monocyte recruitment to implanted grafts and decreased the number of iNOS-positive activated monocytes in neotissue. Ly6C^{+high} monocytes have been recognized to play a crucial role in inflammation, yet little is known about the role of Ly6C^{+low} monocytes in this process. Our data do not identify the function of Ly6C^{+low} monocytes in the inflammatory process of tissue remodeling. However, we consider Ly6C^{+low} monocytes to still have inflammatory properties because Ly6C^{+low} monocytes are thought to be derived from Ly6C^{+high} monocytes,[29] and these cells still express Ly6C on their surface. Recent findings suggest that Ly6C^{+low} monocytes initiate an early immune response and differentiate into macrophages.[30] We could not determine which monocyte subset is most crucial to the development of graft stenosis in the present study; however, we report that only cilostazol prevents activation of both Ly6C^{+high} and Ly6C^{+low} monocytes after inflammatory lipopolysaccharide /IFN-γ stimulation. This observation may highlight one mechanism by which cilostazol prevents stenosis of bioresorbable vascular grafts because inflammatory stimulation is known to switch the phenotype of contractile SMCs to synthetic SMCs.[31]

Cilostazol increases intracellular content of cAMP, which is a second messenger, used for intracellular signal transduction in many biological processes. Interestingly, in the present study, cilostazol prevented iNOS expression in bone marrow monocytes (Figure 6C and 6D), but not in bone marrow–derived macrophages (Figure VIII in the online-only Data Supplement). These results indicate that increased cAMP may affect iNOS expression only in monocytes, but not in macrophages, although precise role of cAMP in iNOS expression in these cells is still unknown. Indeed, cAMP activity was recently shown to be cell specific with regards to iNOS expression.[32–34]

We acknowledge some limitations in the present study. First, the precise mechanism of cilostazol's activity during neotissue formation remains to be fully elucidated because we did not investigate every possible effect of cilostazol on the development of graft stenosis. Second, we routinely use anticoagulation drugs in our clinical trial to prevent acute thrombosis because anticoagulation drugs, rather than antiplatelet drugs, are more effective in preventing venous thrombosis.[35] Thrombosis is an another possible mechanism of stenosis in bioresorbable vascular grafts although clinical data indicate that graft occlusion is primarily because of a hyperplastic intima.[5] To verify this assumption, additional studies using

anticoagulation drugs are required. Third, in the present study, we could not detect fully mature macrophages in the neotissue of implanted grafts at the 2-week time point by flow cytometric analysis, in contrast to our previous findings in which abundant F4/80+ macrophages were identified by immunohistochemical staining at this time point.[15] The different results for macrophage presence in the graft between the current study and other reports published by our group may be explained by differences in the sensitivity and specificity of the experimental methods used to identify macrophages because tissue infiltrating monocytes also express the F4/80 antigen (albeit at lower levels than resident macrophages).[29] The flow cytometric analysis used in this study more precisely distinguishes among these 2 cell populations. Fourth, an analysis of protein levels would be more appropriate than that of gene expression for the quantitative assessment of endothelialization. However, we could not prepare samples for a protein assay in this study because of a limited amount of tissue from each explant. Fifth, the optimal wall thickness after tissue remodeling of bioresorbable vascular grafts has not been established. Because in clinical application, the conduit is used for much larger vessels than that of the mouse abdominal IVC, the thick wall observed in the no-treatment group of the present study (≈600 μm) may be tolerated.

In conclusion, our work demonstrates that cilostazol prevents stenosis of bioresorbable vascular grafts for 24 weeks in mouse IVC implantation model when compared with aspirin and no-treatment groups. Cilostazol treatment effectively suppresses SMC proliferation and reduces acute phase (2 weeks) inflammation mediated mainly by monocyte infiltration and activation, and we suggest that these effects may consequently attenuate neotissue hyperplasia–causing stenosis.

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Disclosures

None.

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Significance

Bioresorbable vascular grafts offer the potential of a synthetic conduit that ultimately transforms into a neovessel capable of growth throughout the lifespan of the host patient. However, neotissue hyperplasia leading to stenosis is the primary cause of graft failure in a clinical trial, evaluating these grafts in the treatment of congenital heart disease. Aspirin is used in this application and is expected to prevent platelet aggregation on the graft, which could promote smooth muscle cell proliferation causing neotissue hyperplasia. However, we report here that aspirin failed to prevent the development of neotissue hyperplasia in a mouse inferior vena cava implantation model. On the contrary, cilostazol (a related antiplatelet drug) was shown to prevent graft stenosis up to 24 weeks and to reduce acute inflammation mediated by monocyte recruitment and activation. These findings provide further strategies of antiplatelet therapy after implantation of bioresorbable vascular grafts in the clinical setting.