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Imatinib Attenuates Diabetes-Associated Atherosclerosis

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Objective—Diabetes is associated with accelerated atherosclerosis, the major factor contributing to increased mortality and morbidity in the diabetic population. The molecular mechanisms by which diabetes promotes atherosclerosis are not fully understood. Platelet-derived growth factor has been shown to play a major role in the pathology of vascular diseases, but whether it plays a role in atherosclerosis associated with diabetes remains unknown. The aims of this study were to assess whether platelet-derived growth factor–dependent pathways are involved in the development of diabetes-induced atherosclerosis and to determine the effects of platelet-derived growth factor receptor antagonism on this disorder.

Methods and Results—Diabetes was induced by injection of streptozotocin in 6-week-old apolipoprotein E knockout mice. Diabetic animals received treatment with a tyrosine kinase inhibitor that inhibits platelet-derived growth factor action, imatinib (STI-571, 10 mg/kg per day), or no treatment for 20 weeks. Nondiabetic apolipoprotein E knockout mice served as controls. Induction of diabetes was associated with a 5-fold increase in plaque area in association with an increase in aortic platelet-derived growth factor-B expression and platelet-derived growth factor-β receptor phosphorylation as well as other pro sclerotic and proinflammatory cytokines. Imatinib treatment prevented the development of atherosclerotic lesions and diabetes-induced inflammatory cytokine overexpression in the aorta.

Conclusions—Tyrosine kinase inhibition with imatinib appears to be a novel therapeutic option to retard the development of atherosclerosis, specifically in the context of diabetes. (Arterioscler Thromb Vasc Biol. 2004;24:935-942.)

Key Words: atherosclerosis ■ diabetes mellitus ■ apolipoprotein E knockout mice ■ platelet-derived growth factor ■ vasculature

Patients with type 1 and type 2 diabetes are at greater risk for atherosclerosis than nondiabetic subjects. It has been estimated that 70% to 75% of adults with diabetes will die from cardiovascular diseases. After correction for the other major risk factors, dyslipidemia, hypertension, and obesity, diabetes remains an independent risk factor for atherosclerosis. The molecular mechanisms responsible for the accelerated progression of atherosclerosis as observed in diabetes remain to be fully delineated.

Platelet-derived growth factor (PDGF) is a cytokine that has a physiological role in the embryonic development of kidneys, blood vessels, lungs, and the central nervous system. At least 5 PDGF isoforms have been identified, although the predominant isoforms appear to be AA, AB, and BB. These isoforms bind to 2 structurally and functionally related receptors, PDGF-Rα and PDGF-Rβ, with different affinities. Ligand binding induces receptor dimerization and autophosphorylation, leading to activation of a cytoplasmic SH2 domain containing signal transduction molecules and initiation of different intracellular signaling pathways, including cellular tyrosine phosphorylation and c-fos mRNA induction. These, in turn, lead to cell growth, proliferation, chemotaxis, and differentiation.

Overactivation of the PDGF system is suggested to play a role in various pathological conditions, including several types of tumors, lung fibrosis, kidney fibrosis, liver cirrhosis, and vascular wall proliferative diseases, such as restenosis and allograft arteriosclerosis (chronic rejection). Recent studies suggest that an overactivated PDGF system may play a role in the development of atherosclerosis in the context of hyperlipidemia.

The role of PDGF in atherosclerosis in the diabetic context remains unknown. This area of research has been hampered by a lack of an appropriate animal model. Induction of experimental diabetes has, in general, resulted in less rather than more atherosclerosis in different animal species, including the rat and the rabbit. Several groups, including our own, have recently reported a model of diabetes-associated atherosclerosis in apolipoprotein E knockout (apoE-KO) mice. In these mice, induction of diabetes resulted in an increase in atherosclerosis not only in the arch but also along the entire aorta.
To explore the role of PDGF, antagonists for the PDGF receptor such as imatinib (STI-571) have been developed. This agent was initially developed to prevent restenosis after angioplasty by inhibition of abnormal PDGF receptor activation.\textsuperscript{20} Such agents have been previously used to explore the role of PDGF receptor antagonism in the vascular setting, albeit in the absence of diabetes.\textsuperscript{21,22} It should be noted that a number of other tyrosine kinases are also inhibited by these agents, although a role of these kinases such as C-abl and Kit in vascular injury has not been previously reported.

The aims of the study were first to study the status of PDGF and its receptors in diabetes-related atherosclerosis, and second to determine the effects of imatinib on the development of atherosclerosis and the processes associated with this disorder in diabetic apoE-KO mice.

Methods

Six-week-old homozygous apoE-KO male mice (back-crossed 20 times from the C57BL/6 strain; Animal Resource Centre, Canning Vale, Australia) were housed at the Biological Research Laboratory at the Austin and Repatriation Medical Centre and were studied according to the principles devised by the Animal Welfare Committee of the Austin and Repatriation Medical Centre. Thirty-four mice were rendered diabetic by 5 daily intraperitoneal injections of streptozotocin (Boehringer-Mannheim, Mannheim, Germany) at a dose of 55 mg/kg in citrate buffer.\textsuperscript{19} Only animals with blood glucose levels >15 mmol/L 2 days after the induction of diabetes were included in the study. Control mice (n = 20) received citrate buffer alone. The animals had unrestricted access to water and were maintained on a 12-hour light–dark cycle in a nonpathogen-free environment on standard mouse chow (Barastoc, Pakenham, Victoria, Australia). After the induction of diabetes, the animals were further randomized to be treated with imatinib (Novartis Pharmaceuticals, Basel, Switzerland) (n = 14) at a dose of 10 mg/kg body weight per day via gavage for 20 weeks or to receive no treatment (n = 20).

After pilot studies suggested an antitherogenic effect of imatinib in diabetic apoE-KO mice, a separate study was performed to determine the effect of the drug on atherosclerosis in nondiabetic mice. Specifically, the effects of imatinib on the development of atherosclerosis were studied in 14 nondiabetic apoE-KO mice, which were randomized to receive imatinib at a dose of 10 mg/kg body weight per day via gavage for 20 weeks or to receive no treatment (n = 20).

Systolic blood pressure was assessed by a noninvasive tail-cuff method. Mice were habituated to the device before measuring the blood pressure to assess the effect of the drug on blood pressure.

Immunohistochemistry

Frozen aortic sections were used to immunostain for macrophages with a rat anti-mouse CD68 antibody (Serotec; Oxford, UK; diluted 1:50).\textsuperscript{19} In brief, 10-μm frozen aortic sections were cut on a cryostat at −20°C. Frozen sections were fixed with cold ethanol and endogenous peroxidase was inactivated using 0.1% hydrogen peroxide (H₂O₂) in phosphate-buffered saline (PBS). The sections were incubated with Protein Blocking Agent (Lipshaw-Immunon, Pittsburgh, Pa) and endogenous nonspecific binding for biotin was blocked with incubation with Protein Blocking Agent (Lipshaw-Immunon, Pittsburgh, Pa).

Evaluation of Atherosclerotic Lesions

To evaluate the atherosclerotic lesions, 2 approaches were used: en face whole and histological section analysis.\textsuperscript{59} The en face approach was used to obtain information about distribution and extent of atherosclerosis in the aorta, whereas microscopic analysis was used to evaluate the lesion complexity. The entire aorta was cleaned of peripheral fat under a dissecting microscope, opened longitudinally and cut into 3 different segments: arch and descending thoracic and abdominal aorta. The aortas were stained with Sudan IV–Herxheimer solution (Sigma Chemical) and pinned out flat. Photographs of the stained aortas were digitized using a dissecting microscope (Olympus BX-50; Olympus Optical, Tokyo, Japan) equipped with a high-resolution camera (Fujix HC-2000; Fujifilm, Tokyo, Japan). Digitized images were then evaluated using an image analysis system (AIS; Imaging Research, St. Catherines, Ontario, Canada). Lesion area measurements were performed by calculating the proportion of aortic intimal surface area occupied by the red stain in each of the 3 aortic segments. The aortic segments were then embedded in paraffin. Then 4-μm-thick cross-sections were prepared and stained with hematoxylin and eosin to evaluate atherosclerotic lesion complexity.

Reverse-Transcription Polymerase Chain Reaction

Three micrograms of total RNA extracted from each aorta were used to synthesize cDNA with Superscript First Strand synthesis system for reverse-transcription polymerase chain reaction (RT-PCR) (Gibco BRL, Grand Island, NY). PDGF-B, connective tissue growth factor (CTGF), monocyte chemoattractant protein-1 (MCP-1), vascular cell adhesion molecule-1 (VCAM-1), and C-abl gene expression were analyzed by real-time quantitative RT-PCR using the TaqMan system based on real-time detection of accumulated fluorescence (ABI Prism 7700; Perkin-Elmer, Foster City, Calif.).\textsuperscript{24} Fluorescence for each cycle was quantitatively analyzed by an ABI Prism 7700 Sequence Detection System (Perkin-Elmer, PE Biosystems, Foster City, Calif.). To control for variation in the amount of DNA available for PCR in the different samples, gene expression of the target sequence was normalized in relation to the expression of an endogenous control, 18S ribosomal RNA (rRNA) (18S rRNA TaqMan Control Reagent kit; ABI Prism 7700). After pilot studies suggested an antiatherogenic effect of imatinib in diabetic apoE-KO mice, a separate study was performed to determine the effect of the drug on atherosclerosis in nondiabetic mice. Specifically, the effects of imatinib on the development of atherosclerosis were studied in 14 nondiabetic apoE-KO mice, which were randomized to receive imatinib at a dose of 10 mg/kg body weight per day via gavage for 20 weeks or to receive no treatment (n = 20).
blocked using an avidin/biotin blocking kit (Vector Laboratories, Burlingame, Calif). The aortic sections were incubated with primary antibody overnight at 4°C. Biotinylated rabbit anti-rat immunoglobulin (Vector Laboratories) diluted 1:200 in PBS was used as the secondary antibody for 60 minutes, followed by Vectastain ABC reagent (Vector Laboratories) for 30 minutes. Peroxidase activity was identified by reaction with 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical) as the chromogen. The slides were then counterstained with hematoxylin, dehydrated, and mounted.

Then 4-μm paraffin sections of aorta were used to stain for PDGF-B, phosphorylated PDGF-Rβ (p-PDGFR-β), smooth muscle cells, proliferating cell nuclear antigen (PCNA), MCP-1, and VCAM-1. The primary antibodies that were used included a polyclonal rabbit anti-human PDGF-B (Santa Cruz Biotechnology, Santa Cruz, Calif; diluted 1:50), a polyclonal rabbit anti-human p-PDGFR-β (Santa Cruz Biotechnology; diluted 1:50), a mouse anti-human smooth muscle α-actin antibody (Dako A/S, Copenhagen, Denmark; diluted 1:50), a mouse anti-rat PCNA antibody (Dako A/S; diluted 1:50), a mouse anti-rat MCP-1 (PharMingen San Diego, Calif; diluted 1:25), and anti-rat VCAM-1 (PharMingen, diluted 1:50). For sections stained for PDGF-B, p-PDGFR-β, smooth muscle α-actin, and PCNA, the endogenous peroxidase was neutralized with 3% H2O2 for 20 minutes followed by blocking with protein blocking agent for 20 minutes. The sections were incubated with primary antibodies at 4°C overnight. Biotinylated goat anti-rabbit immunoglobulin diluted 1:500 or biotinylated horse anti-mouse immunoglobulin diluted 1:250 in PBS (Dako A/S) was then applied as a secondary antibody for 30 minutes, followed by horseradish peroxidase-conjugated streptavidin (Dako A/S; diluted 1:500). The staining was visualized by reaction with DAB. The slides were then counterstained with hematoxylin, dehydrated, and mounted. The sections stained for MCP-1 were digested with 0.2% pepsin for 20 minutes at 37°C and blocked with H2O2 and 1% horse serum in 0.5% milk/tris-buffered saline for 30 minutes before adding the primary antibody. Biotinylated horse anti-mouse immunoglobulin (Vector Laboratories), diluted 1:250 for 10 minutes, was used as the secondary antibody, followed by Vector ABC reagent (Vector Laboratories) and visualizing with DAB. Immunostaining for VCAM-1 was performed using the DAKO Catalyzed Signal Amplification System (DAKO A/S).

The sections stained for PDGF-B, p-PDGFR-β, PCNA, MCP-1, and macrophages were examined by light microscopy (Olympus BX-50; Olympus Optical) and digitized using a high-resolution camera (Fujix HC-2000; Fujifilm). Assessment of p-PDGFR-β immunostaining in aortic medial layer and within the plaques and PDGF-B immunostaining in the aortic medial layer was performed using AIS software (Imaging Research, St. Catharines, Ontario, Canada) associated with a videocamera and computer. PCNA positive cells in aortic medial layer and within the plaques and CD68 positive cells within the plaques were counted manually. The PCNA positive cells and macrophages were expressed as a percentage of the total cells (positive nuclei/total nuclei × 100).

Statistical Analysis
Data were analyzed by ANOVA using Statview V (Brainpower, Calabasas, Calif). Comparisons of group means were performed by Fisher least significant difference method. Data are shown as mean±SEM, unless otherwise specified. A P<0.05 was viewed as statistically significant.

Results
Metabolic Parameters and Systolic Blood Pressure
Diabetic apoE-KO mice gained less weight and had increased HbA1c levels when compared with the nondiabetic control mice (online Table I, please see http://atvb.ahajournals.org). Induction of diabetes was associated with an increase in total cholesterol and triglyceride levels (online Table I). These metabolic parameters were not influenced by imatinib treat-

![Figure 1](http://atvb.ahajournals.org/)

**Figure 1.** Atherosclerotic plaque area in the whole aorta (A), aortic arch (B), and thoracic and abdominal (D) aortas in control (Con), diabetic (Diab), and imatinib-treated diabetic (Diab+) apoE-KO mice. Data are expressed as mean±SEM. *P<0.05 vs control group; #P<0.05 vs diabetic group. N=8 to 10.

Assessment of Aortic Atherosclerotic Lesion Area and Complexity
Induction of diabetes was associated with a 5-fold increase in plaque area in the entire aorta compared with nondiabetic animals (Figure 1). Evaluation of specific areas within the aorta indicated that this increase in atherosclerosis was observed in diabetic animals in the aortic arch and thoracic and abdominal aortic regions (Figure 1). In nondiabetic control mice, most plaques were fatty streaks and only occasionally were complex fibrous plaques seen at the aortic arch (Figure 1, available online at http://atvb.ahajournals.org; representative examples of en face dissection of aortic arch and thoracic and abdominal aorta showing atherosclerotic lesions stained as red with Sudan IV–Herxheimer solution in control [A], diabetic [C], and imatinib-treated [E] diabetic apoE-KO mice. Histologic cross-sections [HE] from the aorta of control [B], diabetic [D], and imatinib-treated [F] diabetic apoE-KO mice. Magnification×100). In diabetic mice, the individual lesions were predominantly complex fibrous plaques with an increased number of cells stained with smooth muscle cell α-actin (Figure 2). Fatty streaks in diabetic mice were also increased when compared with control mice. Immunostaining for macrophages showed that in diabetic mice, atherosclerosis was associated with increased macrophage infiltration when compared with nondiabetic mice (Figures 3 and 4).

Imatinib reduced the total lesion area in the diabetic apoE-KO mice. When the specific areas of the aorta were analyzed, imatinib had no effect on the lesion area in the arch, but in the thoracic and the abdominal region the lesion area was reduced by imatinib (Figure 1). Imatinib treatment ameliorated the complexity of the atherosclerotic lesions and
was associated with reduced infiltration of smooth muscle cells and macrophages in the plaque areas (online Figure I and Figures 2, 3, and 4).

Studies in nondiabetic mice showed that imatinib had no significant effect on the total lesion area (control apoE-KO versus imatinib-treated apoE-KO 4.2%±1.0% versus 3.3%±0.7%; P=0.46) or on any specific segment of the aorta (arch 11.9%±1.9% versus 9.9%±2.3%; P=0.52; thoracic 0.9%±0.2% versus 1.7%±0.8%; P=0.40; abdominal 3.0%±1.9% versus 1.5%±0.4%; P=0.47).

**PDGF-B Expression**

Diabetes was associated with increased PDGF-B mRNA levels in the aorta (Figure 5) when assessed by RT-PCR. Immunohistochemical analysis revealed that PDGF-B protein expression was increased in diabetic apoE-KO mice when compared with the nondiabetic animals (Figures 5 and 6). In the medial layer of the vascular wall, minimal PDGF-B expression was detected in the diabetic but not in the nondiabetic animals. The tyrosine kinase inhibitor of the PDGF receptor imatinib reduced PDGF-B gene and protein expression (Figures 5 and 6).

**PDGFR-β Expression**

Immunohistochemical analysis showed that PDGFR-β phosphorylation was increased in diabetic apoE-KO mice in the medial layer as well as in the plaque (Figure 5 and 6). The p-PDGF-β immunostaining was increased in the plaque area of the diabetic mice. Imatinib reduced p-PDGF-β immunostaining (Figures 5 and 6).

**C-abl Expression**

Because imatinib inhibits not only the PDGF receptor tyrosine kinase but also the C-abl kinase, the expression of this oncogene was studied. Gene expression of C-abl was not altered in diabetic animals (control group 1.0±0.42 versus diabetic group 0.71±0.23). Gene expression of C-abl was reduced by imatinib treatment (0.035±0.09; P<0.05 versus control and diabetic groups).

**Proliferative Cell Nuclear Antigen Expression**

Immunohistochemical staining for PCNA showed an increased number of PCNA positive cells in the adjacent media (control group 6.3±1.7 versus diabetic group 26.0±4.1, P<0.05) as well as in the plaque area (control group 23.3±6.4 versus diabetic group 46.5±2.0, P<0.05) of the aortas from the diabetic animals (Figure 2). PCNA protein expression was reduced by imatinib treatment (media 11.3±2.3; plaque 33.8±4.4, P<0.05 versus diabetic group at both sites; Figure 2).

**Expression of Other Cytokines**

Diabetic mice had an increase in CTGF gene expression as assessed by RT-PCR in aortic tissue when compared

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**Figure 2.** Immunohistochemical staining for proliferating cell nuclear antigen (PCNA) in aortic sections from control (A), diabetic (C), and imatinib-treated (E) diabetic apoE-KO mice. Representative photomicrographs show α-smooth muscle actin (α-SMA) immunostaining in sections of aorta from control (B), diabetic (D), and imatinib-treated (F) diabetic apoE-KO mice. Positive cells are stained as brown in the aortic medial layer and in the plaque. Sections are counterstained with hematoxylin. Magnification ×200.

**Figure 3.** Monocyte chemoattractant protein-1 (MCP-1) mRNA expression (arbitrary units, A) and MCP-1 positive area after immunohistochemical staining (B) in the aorta. Macrophage infiltration in the aortic plaques (C) is expressed as a percentage of CD68 positive cells/total cells ratio. Vascular cell adhesion molecule-1 (VCAM-1) (arbitrary units, D) and connective tissue growth factor (CTGF) (arbitrary units, E) mRNA expression in the aortic tissue. Con, control; Diab, diabetic; Diab-I, diabetic treated with imatinib apoE-KO mice. Data are expressed as mean±SEM. *P<0.05 vs control group; #P<0.05 vs diabetic group. N=4 to 10.
nondiabetic control animals (Figure 3). Imatinib treatment was associated with a reduction in the CTGF gene expression (Figure 3).

Diabetic mice displayed a marked increase in gene expression for VCAM-1 in aortic tissue when compared with nondiabetic control mice (Figure 3). Immunohistochemistry showed that expression was predominantly in the plaque area of the diabetic mice (Figure 4). This increase was significantly attenuated by imatinib (Figures 3 and 4).

Diabetes was associated with an almost 100-fold increase in MCP-1 gene expression in the aorta (Figure 3). Immunohistochemistry revealed that expression was predominantly in the plaque area and increased in the diabetic aorta (Figures 3 and 4). Imatinib prevented the diabetes-associated increase in aortic MCP-1 expression (Figures 3 and 4).

Discussion

The present study has assessed whether PDGF-dependent pathways play a role in the development of diabetes-associated atherosclerosis in apoE-KO mice. Diabetes-induced atherosclerosis was associated with increased expression of PDGF-B and PDGFR-β phosphorylation in the aorta. Furthermore, the tyrosine kinase inhibitor of the PDGF receptor, imatinib, was able to attenuate diabetes-induced atherosclerosis in association with a reduction in putative mediators of this injury including cytokines, chemokines, and adhesion molecules. These findings suggest that blockade of certain cytokine and in particular PDGF-dependent pathways is a potential therapeutic option for the treatment and/or prevention of atherosclerosis in diabetes.

The molecular mechanisms responsible for the increase in atherosclerosis in the diabetic population with its associated cardiovascular morbidity and mortality remain poorly under-
stood. In the present study it was demonstrated that induction of diabetes led to activation of the PDGF pathway as assessed by measurement of both the ligand and the receptor. It is postulated that this pathway may be an important mediator of atherosclerosis, specifically in the context of diabetes. This was further supported by the finding that PDGF inhibition effectively reduced atherosclerotic plaque area as well as the complexity of these plaques in the diabetic animals without significantly affecting blood pressure, plasma lipids, or glycemic control.

Previous studies have suggested, albeit indirectly, that PDGF may be relevant to diabetes-associated macrovascular disease. In vitro studies have demonstrated that high glucose concentrations increase PDGF-β receptor expression in cultured human endothelial cells, in human monocyte-derived macrophages, and in rabbit aortic medial smooth muscle cells. In vivo, PDGF-β receptor expression is reported to be increased in medial smooth muscle cells in an animal model of noninsulin-dependent diabetes mellitus. Furthermore, PDGF levels are increased in response to a variety of factors that have been implicated in diabetic cardiovascular disease, including angiotensin II, endothelin, inflammatory cytokines, and advanced glycation end products. Previous studies have suggested, albeit indirectly, that PDGF may be relevant to diabetes-associated macrovascular disease. In vitro studies have demonstrated that high glucose concentrations increase PDGF-β receptor expression in cultured human endothelial cells, in human monocyte-derived macrophages, and in rabbit aortic medial smooth muscle cells. In vivo, PDGF-β receptor expression is reported to be increased in medial smooth muscle cells in an animal model of noninsulin-dependent diabetes mellitus. Furthermore, PDGF levels are increased in response to a variety of factors that have been implicated in diabetic cardiovascular disease, including angiotensin II, endothelin, inflammatory cytokines, and advanced glycation end products. Previous studies have suggested, albeit indirectly, that PDGF may be relevant to diabetes-associated macrovascular disease. In vitro studies have demonstrated that high glucose concentrations increase PDGF-β receptor expression in cultured human endothelial cells, in human monocyte-derived macrophages, and in rabbit aortic medial smooth muscle cells. In vivo, PDGF-β receptor expression is reported to be increased in medial smooth muscle cells in an animal model of noninsulin-dependent diabetes mellitus. Furthermore, PDGF levels are increased in response to a variety of factors that have been implicated in diabetic cardiovascular disease, including angiotensin II, endothelin, inflammatory cytokines, and advanced glycation end products.

There is increasing evidence that suggests that an upregulated PDGF system plays a role in development of atherosclerosis in the nondiabetic context. Specifically, clinical and experimental studies have shown that PDGF has a role in the development of atherosclerosis in the context of hyperlipidemia. PDGF-A and PDGF-B mRNA levels are increased in circulating mononuclear cells of hypercholesterolemic and hyperlipidemic patients. In rabbits fed with a high-cholesterol diet, immunization against PDGF-AA or against PDGF-BB reduces the size of aortic atherosclerotic lesions. In apoE-KO mice, the effects of antibodies against PDGFR-β and PDGFR-α have been explored. Interestingly, when the investigators studied the development of early fatty streak lesions in apoE-KO mice fed a normal rodent diet, an antibody for either receptor subtype administered for 12 weeks had no significant effect. This is in agreement with our findings of no significant effect of imatinib on atherosclerotic area in nondiabetic apoE-KO mice. However, in apoE-KO mice fed with a high-fat and high-cholesterol diet from age 6 weeks, the antibody for PDGFR-β administered for 12 weeks reduced the aortic atherosclerotic lesion size and smooth muscle infiltration into the intima whereas injection of the antibody for PDGFR-α had only minimal effects. These results are in agreement with our results that PDGF and regulation of its signal transduction via PDGF receptors play a major role in the development of complex atherosclerotic lesions, particularly in the context of exacerbating factors such as hyperlipidemia and diabetes. A recent study has explored the effect of imatinib in LDL-receptor knock-out/smooth muscle-specific LDL-receptor-related protein (LRP1)-gene inactivated mice. These mice had accelerated atherosclerosis, which was attenuated by imatinib. Interestingly, no such effect was demonstrated in mice with only gene inactivation of the LDL receptor, consistent with the present study in which the effects of imatinib appear to be particularly evident in models of atherosclerosis in which there is increased PDGF and/or PDGF receptor expression/activation.

Diabetes-related atherosclerosis was associated with increased expression of proteins involved in inflammation such as the chemoattractant, MCP-1, and the adhesion molecule, VCAM-1. Both of these molecules have been shown to be important in the development of atherosclerosis. PDGF antagonism reduced atherosclerosis and also was associated with reduced inflammation. Protection from atherosclerosis by PDGF antagonism may at least be partly caused by a specific antiinflammatory effect involving MCP-1. It has been previously shown in rat aorta that PDGF, but not other growth promoters including angiotensin II, stimulated monocyte chemotaxis. This pathway was reported to be independent of Ca²⁺ mobilization, Na⁺-H⁺ exchange, protein kinase C activation, or elevation in cAMP levels, effects that are observed with PDGF and angiotensin II.

It remains to be determined if the reduction in macrophage accumulation in the plaques is purely a manifestation of less atherosclerosis after imatinib treatment or represents an important mechanism whereby less macrophage infiltration results in less accumulation of a range of cellular and molecular factors implicated in the progression of atherosclerosis. Diabetes was also associated with increased cell proliferation within the plaques. Imatinib treatment reduced the number of PCNA positive cells, suggesting that cell proliferation via PDGF-dependent pathways may play an important role in the pathogenesis of diabetes-associated atherosclerosis, particularly in this experimental model.

To further explore potential molecular effects of inhibiting PDGF-dependent pathways in diabetes-associated large vessel disease, the expression of a prosclerotic cytokine, CTGF, which has been shown to be linked to the development of atherosclerosis, was evaluated. Imatinib treatment was associated with decreased expression of the prosclerotic cytokine CTGF. This suggests that the protective effects of inhibition of PDGF signal transduction may be partly caused by reduction in CTGF. There is increasing evidence to suggest that CTGF plays a role in the development of atherosclerosis. It has been shown that this growth factor promotes adhesion, migration, and proliferation of fibroblasts and endothelial cells and induces neovascularization in vivo.

Expression of CTGF has been reported to be increased in human atherosclerotic plaques. The present findings of increased CTGF expression in the aorta of diabetic apoE-KO mice confirm our previous finding in this model. Whether PDGF regulates CTGF expression directly or indirectly remains unknown. In a cell line of retinal vascular endothelial cells, CTGF gene expression was not altered by PDGF. By contrast, in hepatic stellate cells, PDGF induced CTGF expression. This stimulation was abrogated by a neutralizing antibody for transforming growth factor-β (TGF-β), suggesting that there is an interplay among all these growth factors.

Previous studies by our group have suggested that the protective effect of various treatments such as ACE inhibitors and inhibitors of advanced glycation end-product formation on diabetic vascular complications is associated with reduced expression of cytokines such as PDGF and CTGF.
cause these cytokines are upregulated in diabetic vessels and seem to play a pivotal role in the development of these complications, a more specific approach of directly inhibiting these cytokine dependent pathways, as reported in the present study, appears to be an interesting therapeutic option. This is of particular interest because imatinib has been shown to be well tolerated with minimal side effects when used as a targeted cancer therapy in humans.\textsuperscript{12,13} In the present study, imatinib effectively reduced atherosclerosis in the lower parts of the aorta, as did ACE inhibition in our previous study.\textsuperscript{19} However, this tyrosine kinase inhibitor, unlike ACE inhibition, had no effect on atherosclerotic plaque area in the aortic arch. Indeed, a superior anti-atherosclerotic action of imatinib in the abdominal region versus aortic arch was also reported in LDL/smooth muscle LRP1 knockout mice.\textsuperscript{22} Because the ACE inhibitor\textsuperscript{19} but not imatinib lowered blood pressure, it seems likely that hemodynamic effects may play an important role in the development of atherosclerosis in the aortic arch whereas diabetes-induced cytokine pathways are important in other aortic regions where there may be a reduced role for blood flow and shear stress. It would now be of interest to determine the effects of specific cytokine inhibitors in the prevention of diabetes associated atherosclerosis when administered in combination with antihypertensive medications. Because imatinib can block other kinases, such as C-abl, one cannot exclude that the anti-atherosclerotic effects of imatinib may partly relate to its effects on these kinases. Although these other kinases, and in particular C-abl, the target for treatment of chronic myeloid leukemia, have not been extensively studied in the vasculature, one cannot exclude that C-abl may have biological effects relevant to the progression of diabetes-associated atherosclerosis. However, in the present study, there was minimal expression of this protein in the vasculature with no evidence of increased expression in diabetes. Recent studies that have explored in more detail PDGF receptor signaling in vascular integrity and atherosclerosis have provided additional evidence to suggest that the effects of imatinib in this context are primarily via its ability to control PDGF activation.\textsuperscript{23} This data on C-abl expression contrast markedly with the prominent effects of diabetes on PDGF and PDGF-receptor expression.

The vasoprotective effect observed with PDGF receptor antagonism was associated with decreased expression of PDGF-B as well as p-PDGFR-β, both of which were highly upregulated in the aorta from diabetic animals. Tyrosine kinase inhibition of the receptors would not be expected to directly reduce the ligand for these receptors, PDGF. This suggests that the reduction of PDGF-B in response to imatinib is caused by the reduction of atherosclerosis in the treated animals or that there is an autocrine loop linking PDGF signal transduction to PDGF expression. It is likely that there is complex interplay among the different cytokines and growth factors that leads to a vicious circle in which PDGF plays a central role. Blockade of this system is able to downregulate several inflammatory and proliferative pathways, including PDGF itself, which are induced by diabetes, and this ultimately translates to reduced atherosclerosis.

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