Intervention in Diabetic Vascular Disease by Modulation of Growth Factors

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Several growth factors have been implicated in the derangements of cellular metabolism and proliferation that occur in diabetes, eg, kidney mesangial expansion, retinal neovascular formation, and acceleration of atherosclerosis in large vessels. These phenomena contribute to the development and progression of diabetic microvascular and macrovascular disease. Pharmacological interventions aimed at reducing growth factor alterations, among other actions in diabetic vasculopathy, include a multitude of classes of drugs, such as angiotensin-converting enzyme (ACE) inhibitors, calcium antagonists, lipid-lowering drugs, and somatostatin analogs. New potential interventions, ie, antisense oligonucleotide local delivery, are being applied in growth factor research and may prove beneficial in diabetic macrovascular disease.

A MULTITUDE of complex components of diabetes mellitus (types I and II) are responsible for initiation and progression of microvascular and macrovascular lesions. The development of diabetic vasculopathy requires the simultaneous presence of a number of molecular perturbations. It is becoming increasingly clear that enhanced autocrine and paracrine growth factor production or action is one such perturbation.[1-3] Growth factors that are generated in response to exogenous stimuli are also elicited in response to the in vivo nonenzymatic modification of tissue components by chronically high glucose levels.[4,5] Moreover, oxidative stress induced by hyperglycemia accelerates the formation of advanced-glycosylation end products and glycoxidation products and stimulates growth factor production.[6] Recent findings have supported a putative role for localized imbalances of growth factor expression in the development of diabetic vasculopathy in the kidney, the retina, and large vessels. The purpose of this brief review is to consider the potential of current and future therapeutic interventions in the modulation of growth factors in diabetic vascular disease.

DIABETIC NEPHROPATHY

The early diabetic renal anomalies are mainly enlargement of the kidneys and increased glomerular filtration rate. Advanced diabetic nephropathy is characterized by thickening of the glomerular basement membrane, mesangial cell proliferation, expansion of mesangial matrix, and finally obstruction of the capillary lumen and loss of glomerular function. Among other candidates, growth factors have been suggested to play a central role in the pathogenesis of this pattern of injury.

The insulin-like growth factor (IGF) system has been the most widely studied in early diabetic disease. Infusion of IGF-I to normal human subjects results in increased circulating IGF-I and elevation of glomerular filtration rate.[7] IGF-I administration induces kidney growth in growth hormone (GH) and IGF-I–deficient mice and rats.[8] IGF-I is mitogenic in vitro, stimulating DNA synthesis in primary cultures of mesangial cells.[9] IGF-I also stimulates collagen and proteoglycan synthesis.[10] IGF-I accumulates in the kidney and reaches its maximum level 24 to 48 hours after induction of experimental diabetes in the rat, and then returns to normal within 4 days.[11] Both renal hypertrophy and IGF-I accumulation are prevented by strict glycemic control with insulin.[11] Accumulation of IGF-I in the kidney is not due to increased local production, but is probably the result of increased uptake of circulating IGF-I by renal IGF-binding proteins (IGF-binding protein-1).[12] However, if IGF-I is relevant to the initiation of early diabetic nephropathy, it cannot account for the progression toward advanced nephropathy. Indeed, transgenic mice overexpressing IGF-I develop glomerular hypertrophy but not glomerulosclerosis,[13] suggesting that other factors may be involved in the progression toward glomerulosclerosis.

The dominant histological feature of diabetic nephropathy is expansion of the extracellular matrix in the mesangium of the glomeruli, with resulting glomerulosclerosis and obliteration of the capillary surface area for filtration.[14] Glomerulosclerosis can be induced by in vivo transfection of transforming growth factor-β (TGF-β) into the rat kidney.[15] In vitro, TGF-β is expressed by glomerular endothelial, epithelial, and mesangial cells.[16] Exposure to high glucose concentrations doubles TGF-β mRNA in cultured mesangial cells.[17] Nakamura et al[18] and Yamamoto et al[19] have recently reported that in glomeruli of streptozotocin-diabetic rats, there is a progressive increase in the expression of TGF-β mRNA and TGF-β protein. Matrix proteins induced by TGF-β such as fibronectin, tenascin, and the proteoglycan, biglycan, were also increased.[19] In glomeruli from humans with advanced diabetic nephropathy, TGF-β and fibronectin were also found to be increased, suggesting that increased TGF-β expression in both experimental and human diabetes contributes to matrix accumulation.[19]

Recently, numerous studies have reported the growth-modulating properties of angiotensin II (Ang II). It is now recognized that the systemic vasculature contains and can synthesize all components of the renin-angiotensin system.[20,21] Ang II is mitogenic for a variety of cell types, including fibroblasts, vascular smooth muscle cells (SMC), and renal tubular epithelium.[22,23] In vascular SMC, Ang II increases c-*myc* and c-*fos* transcripts, as well as mRNAs for platelet-derived growth factor (PDGF) and TGF-β.[24,25] Infusion of Ang II into the renal artery of rats increases

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proto-oncogene levels in whole renal cortex.[26] Ang II also promotes extracellular matrix accumulation and mesangial cell hypertrophy or hyperplasia.[27]

Angiotensin-Converting Enzyme Inhibitors

Angiotensin-converting enzyme (ACE) inhibitors inhibit glomerular growth in the maturing rat kidney, an action that is independent of systemic hemodynamic changes.[28] In animals with experimental diabetes induced by streptozotocin, ACE inhibitors reverse the glomerular capillary hypertension and minimize the mild sclerosis that characterize this model.[29] Long-term ACE-inhibition therapy is efficient in postponing the development of diabetic nephropathy in normotensive type I diabetic patients with persistent microalbuminuria.[30] The effect of ACE inhibition in the diabetic kidney on growth factor expression is not well known. In the remnant-kidney model, Ang II receptor antagonist prevented the development of early glomerulosclerosis and decreased PDGF expression.[31]

Calcium Antagonists

Calcium channel blockers enhance glomerular filtration rate by antagonizing the effect of vasoactive substances at the level of the afferent renal arteriole.[32,33] Additional renal effects include attenuation of the mitogenic action of PDGF and of renal hypertrophy and reduction of mesangial entrapment of macromolecules induced by Ang II.[34,35] However, the potential effect of calcium antagonists on growth factors in the diabetic kidney and in the long-term treatment of diabetic patients with incipient nephropathy awaits further studies.

Somatostatin Analogs

Somatostatin and its analogs have a well-known antiproliferative effect that may be due to antagonism of the actions of growth factors. The effect is mediated through a tyrosine phosphatase receptor, which in turn acts by dephosphorylating the second-messenger products of the tyrosine kinase family of some growth factor receptors.[36] Furthermore, somatostatin analogs can inhibit synthesis of some growth factors. Flyvbjerg et al[37] have shown that in diabetic rats, octreotide, a somatostatin analog, reduces serum and kidney IGF-I levels and prevents kidney hypertrophy without altering glycemic control. Serri et al[38] have shown that in type I diabetic patients, long-term octreotide treatment reduces kidney hyperfiltration and hypertrophy. The effect was associated with a pronounced reduction in circulating IGF-I levels, independently of GH suppression. The suggestion that octreotide may directly inhibit IGF-I synthesis at the hepatic level has later been confirmed.[39]

Antagonists of TGF-β

Although there has been no study on the modulation of TGF-β expression in experimental or human diabetic nephropathy, there is indirect evidence that targeting TGF-β expression or action reduces matrix accumulation, thus preventing glomerulosclerosis. In a rat model of experimental glomerulonephritis, administration of anti–TGF-β reduces matrix accumulation in glomeruli.[40] Recently, Border et al[41] treated similar nephritic rats with repeated injections of decorin, a normal component of extracellular matrix. Decorin treatment inhibited TGF-β_1, TGF-β_2, and TGF-β_3 and resulted in prevention of accumulation of extracellular matrix in the glomeruli.[41] The finding that dietary protein restriction in a rat model of nondiabetic glomerulosclerosis reduces both extracellular matrix expansion and glomerular expression of TGF-β[42] may also have important therapeutic implications in this respect. However, there may be limitations in the use of pharmacological antagonists of TGF-β because of the pleiotropic actions of this growth factor. To be therapeutically useful, the antagonist should recognize mesangium with a selectivity sufficiently greater than that with which it recognizes normal sources of TGF-β.

DIABETIC RETINOPATHY

The principal hypothesis concerning the molecular basis of proliferative diabetic retinopathy has focused on the presence of diffusible angiogenic growth factors that are released from the retina under hyperglycemic conditions. The formation of new blood vessels involves at least (1) degradation of the extracellular matrix surrounding normal capillaries, (2) endothelial migration and proliferation, (3) endothelial tube formation, and (4) anastomosis of nascent tubes. However, the nature of growth-promoting mediators implicated in these phenomena is not well known. Numerous factors have been identified that can elicit new blood vessel formation in angiogenesis assays. The only data available from human studies have been limited to measurements of growth factors in intraocular fluid samples from patients with diabetic proliferative retinopathy. Attempts to correlate circulating IGF-I levels and proliferative diabetic retinopathy have yielded conflicting results.[43-47] Vitreous concentrations of IGF-I may be of more importance than systemic levels. Grant et al[48] found markedly elevated IGF-I levels in the vitreous from patients with proliferative diabetic retinopathy undergoing vitrectomy as compared with nondiabetic patients.

Basic fibroblast growth factor (bFGF) is one potentially important growth factor that can initiate mitogenesis in endothelial cells. It is stored in high concentrations within the extracellular matrix as an inactive complex, and is released when the matrix is dissolved by activated endothelial cells. Sivalingam et al[49] reported elevated levels of bFGF in vitrectomy samples from patients with active proliferative diabetic retinopathy.

Microvascular endothelial cells also synthesize and respond to PDGF. PDGF, which stimulates endothelial migration but not proliferation, is able to elicit a full angiogenic response in the rabbit corneal assay.[50]

In vitro, IGF-I and bFGF synergistically stimulate growth of human retinal endothelial cells.[51]

Somatostatin Analogs

Octreotide inhibits the in vitro growth of human retinal endothelial cells induced by IGF-I and bFGF.[51] Furthermore, somatostatin analogs have been shown to inhibit angiogenesis in the chick allantoic membrane.[52]

In a controlled trial, a 1-year continuous subcutaneous infusion of octreotide had no significant effect on early retinopathy in type I diabetic patients.[53] In four patients with type I diabetes and severe proliferative retinopathy, long-term treatment (6 to 20 months) with octreotide led to stabilization of proliferative retinopathy.[54] In one controlled study,[55] two of eight patients demonstrated improvement of proliferative retinopathy with BIM23014, a somatostatin analog, whereas control patients showed worsening of fluorescein leakage. The other six patients receiving somatostatin analog infusion showed stabilization of lesions.[55] In these studies, circulating IGF-I levels were inhibited by somatostatin analog treatment. Randomized trials are still lacking to conclude on the efficacy of these analogs. However, it appears that they cannot replace photocoagulation therapy, but rather constitute in selected cases an adjunct therapy to photocoagulation, at least in advanced proliferative lesions. The apparent limited efficacy of these drugs may be due to their use in advanced stages of retinopathy, in which damage may be irreversible. In considering future trials, timing and duration of treatment should aim to prevent or at least slow angiogenesis at preproliferative stages of retinopathy.

ACE Inhibitors

Treatment with ACE inhibitors has been shown to reduce fluorescein leakage in diabetic subjects with background retinopathy.[56] In a small series of patients, a 2-year double-blinded trial also showed decreased retinal deterioration and some improvement in normotensive diabetic subjects treated with ACE inhibitors.[57]

Miscellaneous

Thalidomide is a well-known potent teratogen. It was postulated that its teratogenicity may be related to an inhibition of blood vessel growth in the developing fetal limb bud.[58] The effect of thalidomide was examined on growing vasculature in the chicken chorioallantoic membrane and in the rabbit cornea.[58] The study showed that thalidomide is an inhibitor of angiogenesis induced by bFGF.

A novel endogenous angiogenesis inhibitor has been recently reported.[59] Angiostatin, which is a 38-kd fragment of plasminogen, potently inhibits in vitro endothelial proliferation and blocks bFGF-induced neovascularization in the chick chorioallantoic membrane. Future studies should determine whether thalidomide and angiostatin can be useful in the treatment of patients with proliferative diabetic retinopathy.

ATHEROSCLEROSIS

Atherosclerosis is a response of the artery wall to a variety of initiating agents with multiple pathogenic mechanisms contributing to the formation of the atherosclerotic plaque. One major transitional event in lesion progression is the migration to and proliferation within the intima of medial SMC, leading to the synthesis of plaque collagens, elastin, and proteoglycans that contribute further to lesion progression. Molecules controlling smooth muscle growth are derived from platelets, monocyte/macrophages, T-lymphocytes, endothelial cells, and SMC themselves. They include PDGF, TGF-β, bFGF, heparin-binding epidermal growth factor (EGF), IGF-I, interleukin-1 (IL-1), and tumor necrosis factor alpha (TNFα).

Examination of human atherosclerotic specimens has revealed mRNAs transcribed from genes for a variety of growth-promoting substances not normally expressed in arterial wall. A marked increase of PDGF has been demonstrated in SMC and adjacent macrophages in developing atherosclerotic plaques. SMC are the predominant source of PDGF-A chain,[60] whereas macrophages express mRNA for PDGF-B chain.[61] Even though PDGF is neither a necessary nor sufficient contributor to human atherogenesis, it is the prototypic SMC mitogen. PDGF by itself is a powerful chemoattractant, even in submitogenic concentrations, and is able to induce SMC migration more rapidly than proliferation.[62] It induces extensive SMC replication in conjunction with other serum factors.

IGF-I mRNA levels are also markedly increased in plaques, and in situ hybridization studies have shown that IGF-I mRNA is expressed by SMC themselves. An increase in vascular load is associated with an increase in IGF-I production by endothelial cells and SMC.[63] Moreover, enhanced IGF-I expression has also been observed during vessel restenosis and angiogenesis.[64] Because PDGF and IGF-I may function together to result in enhanced rates of SMC proliferation in vitro, localization of their respective transcripts in vessel-wall cell types suggests that they also have the potential to stimulate lesion development.

Recent studies have supported the involvement of FGFs in vascular pathobiology. These growth factors can foster re-endothelialization,[65] mediate SMC replication in balloon-injured arteries,[66] and possess chemotactic activity.[67] Increased levels of acidic FGF mRNA have been demonstrated in atherosclerotic plaques, with most of the hybridization signal for this growth factor being localized in macrophages.[68]

Although detected in minor amounts in normal arterial wall, TGF-β mRNA expression is markedly increased during atherosclerosis.[69] TGF-β exerts a powerful control over SMC proliferation.[70,71] During the early stages of SMC proliferation, TGF-β appears to be antiproliferative,[70] whereas later in the development of the fibrous plaque, TGF-β is growth-stimulatory to SMC.[72] It also strongly stimulates SMC to produce the components of the extracellular matrix.[73] By promoting angiogenesis, it may also account for the plaque disruption of late atherosclerosis.[74]

Activated monocytes release large amounts of several cytokines with growth-promoting properties in the arterial wall, including IL-1 and TNFα. Although IL-1 per se is not mitogenic for SMC, it induces autocrine expression and release of PDGF-AA from SMC.[72] IL-1 may also facilitate SMC migration[72] and induce SMC to synthesize some components of the extracellular matrix.[75] TNFα is one of the earliest-acting atherosclerotic cytokines. It stimulates SMC to release IL-1[76] and facilitates recruitment of monocytes in the arterial wall. It is also responsible for the later development of plaque neovascularization.[77]

Several key components of atherosclerotic plaque initiation are likely to be enhanced by diabetes, including intimal lipoprotein influx and accumulation, monocyte-macrophage recruitment, generation of free radicals, and lipoprotein oxidation.[78] Diabetes can also probably contribute to plaque progression by augmented SMC proliferation or connective tissue synthesis. In this regard, advanced-glycosylation end products may play a central role by enhancing PDGF and IGF-I secretion in the vascular wall.[4]

Pharmacological Modulation of Growth Factors in Atherosclerosis

Decreasing elevated total and low-density lipoprotein (LDL) cholesterol levels is a well-established therapeutic method of manipulating plasma lipids to reduce the progression of atherosclerosis. Lipid-lowering drugs are numerous and include nicotinic acid, bile acid sequestrants, fibric acid derivatives, hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase inhibitors, and probucol. In addition to their effects on lipid metabolism, some of these drugs also possess antiproliferative properties.

Fibric Acid Derivatives

A role of fenofibric acid as a PDGF antagonist was proposed in 1983 by Pascal et al,[79] who reported an inhibitory effect of this drug on DNA synthesis induced by a PDGF-rich platelet extract in cultured rat vascular SMC. Similar inhibitory effects of fenofibrate but not of fenofibric acid on human vascular SMC growth have been recently documented by Munro et al.[80] The antiproliferative effect of fibric acid derivatives could account for the regression of coronary atherosclerotic plaques observed following fenofibrate treatment.[81]

HMG CoA Reductase Inhibitors

Inhibition of HMG CoA reductase, the enzyme responsible for biosynthesis of mevalonate, can result in cell cycle arrest.[82] It has been reported that lovastatin, one inhibitor of HMG CoA reductase, may increase the frequency of regression and reduce the progression of coronary atherosclerosis by combination therapy with colestipol in clinical studies.[83] This drug also has been shown to reduce intimal hyperplasia after balloon angioplasty in the hypercholesterolemic atherosclerotic rabbit.[84] It has been proposed that lovastatin exerts these effects by inhibiting SMC proliferation. Lovastatin also attenuates induction of c-*fos* mRNA by EGF, insulin, and IGF-I, but not by bFGF or PDGF.[85] In a similar way, mevinolin also suppresses c-*fos* and c-*myc* mRNA accumulation in response to serum stimulation,[86] demonstrating that a component of the early response to growth factors is sensitive to mevalonate deprivation. Finally, inhibition of cultured vascular SMC migration by simvastatin, another HMG CoA inhibitor, has also been reported.[87] Although the mechanisms by which these drugs exert their effects are unclear, it appears that HMG CoA reductase inhibitors can attenuate activation of the cells within the vessel wall. Further studies are required to elucidate the importance of this property in the prevention of atherosclerotic lesions.

Calcium Antagonists

Experimental and clinical evidence suggests that calcium antagonists may be able to prevent or retard the progression of atherosclerosis by mechanisms independent of and in addition to blood pressure reduction.[88] A wide range of explorations for the apparent antiatherosclerotic effects of calcium antagonists have been offered. Some of these drugs may suppress vascular SMC migration[89] and proliferation.[90] They have been reported to reduce PDGF-induced growth of SMC and to inhibit Ang II– and PDGF-BB–induced DNA synthesis in the cells.[91-93] A recent study provides evidence that the antiproliferative effect of isradipine on PDGF- and Ang II–induced vascular SMC growth may be due to inhibition of expression of the transcriptional factor, c-*fos*.[93] Some calcium channel blockers such as manidipine may also enhance transcription of many genes induced by PDGF-BB such as LDL receptor, HMG CoA reductase, c-*fos*, c-*jun*, and cytokines.[94,95]

ACE Inhibitors

Ang II may play an important role in modulation of mechanisms underlying SMC proliferation by inducing the genes encoding growth factors and stimulating the expression of factors affecting activities of these cells.[96,97] A role of the local renin-angiotensin system in the myointimal proliferative processes that develop in the vascular wall is supported by several observations. It has been shown that Ang II is a potent growth factor for vascular SMC in vitro,[98] and that ACE inhibitors prevent or attenuate development of myointimal hyperplasia after endothelial denudation and vascular injury.[96,99] Some ACE inhibitors blunt PDGF-BB–stimulated de novo DNA synthesis and cell proliferation in vascular SMC,[100] and decrease de novo synthesis of mRNA of the transcription factors c-*fos* and c-*jun* achieved by PDGF-BB.[100] Finally, they intensify the transcription of LDL receptor mRNA induced by PDGF-BB.[100] Despite these experimental evidences, the clinical importance of such findings is still controversial and needs to be assessed in future investigations.

Miscellaneous

Although antioxidants, including probucol, exert their antiatherosclerotic effect by preventing oxidation of LDL, their ability to directly inhibit growth factor biosynthesis and SMC proliferation is an additional property that may contribute to their antiatherosclerotic effects. Alpha-tocopherol has been demonstrated to inhibit growth factor–induced SMC proliferation.[101] In addition, alpha-tocopherol and probucol depress IL-1β expression.[102]

Octreotide decreases chemotaxis of monocytes induced by GH in vitro[103] and inhibits release of superoxide anion from stimulated monocytes.[104] Octreotide has also recently been reported to reduce IGF-I– and bFGF-induced human coronary artery SMC proliferation.[105] Its potential clinical usefulness in reducing the incidence of restenosis remains to be evaluated.

Table 1 summarizes the principal data for growth factor modulation by different pharmacological interventions in diabetic vascular disease.

Table 1. Principal Data of Growth Factor Modulation by Pharmacological Interventions in Diabetic Vascular Disease

Agents	Nephropathy	Retinopathy	Atherosclerosis
ACE inhibitors	• Inhibit glomerular growth in maturing rat kidney[28] • Reverse glomerular capillary hypertension in experimental diabetes, and minimize glomerulosclerosis[29]	• Reduce fluorescein leakage in background retinopathy[56] • Decrease retinal deterioration in normotensive diabetic subjects[57]	• Prevent or attenuate myointimal hyperplasia[96,99] • Can blunt PDGF-stimulated SMC proliferation[100]
Calcium antagonists	• Antagonize the effect of vasoactive substances at afferent renal arteriole level[32,33] • Attenuate the mitogenic action of PDGF and renal hypertrophy, and attenuate mesangial entrapment of macromolecules induced by Ang II[34,35]		• Can suppress SMC migration and proliferation[89,90] • Reduce stimulated SMC proliferation by PDGF and inhibit stimulated DNA synthesis by Ang II in SMC[91-93]
Somatostatin analogus	• Reduce kidney hyperfiltration[38] • Reduce kidney hypertrophy[37,38] • Inhibit circulating IGF-I levels[37,38] • Reduce kidney IGF-I accumulation[37]	• Inhibit growth of human retinal endothelial cells induced by IGF-I and bFGF[51] • Inhibit angiogenesis in chick allantoic membrane[52] • Improve or stabilize proliferative retinopathy in small series of patients[54,55] • Inhibit circulating IGF-I levels[54,55]	• Decrease chemotaxis of monocytes induced by GH in vitro[103] • Inhibit release of superoxide anion from stimulated monocytes[104] • Inhibit human coronary artery SMC proliferation induced by IGF-I and bFGF[105]
HMG CoA reductase inhibitors			• Have the potential to inhibit SMC proliferation[84,87] • Lovastatin and simvastatin Arrest cells in the GI phase[82] Attenuate induction of c-*fos* by EGF, insulin, and IGF-I[85]
Potential future interventions	• Antagonists of growth factors Anti–TGF-β[40] Decorin[41] • Antagonists of Ang II receptors[31]	• Inhibitors of angiogenesis Thalidomide[58] Angiostatin[59]	• Antagonists of growth factors • Antisense oligonucleotides to proto-oncogenes [106]

FUTURE PROSPECTS FOR GROWTH FACTOR–MODULATING THERAPEUTIC STRATEGIES

It is still unclear which growth factor may be involved as a primary initiator of each specific diabetic vasculopathy and which one is a secondary but still important mediator of injury. It seems unlikely that blocking one mediator alone will reverse growth perturbations when other pathways remain active. Nevertheless, the possibility of treatment using anti–growth factor antibodies or growth factor receptor blockers should be considered. The former approach is unlikely to succeed because the dilution of the antibody will not be able to significantly reduce local production of a growth factor. Receptor blockade is more specific and more promising. Among other therapeutic interventions, a classic pharmacological approach can evaluate combinations of different classes of drugs such as ACE inhibitors or calcium antagonists with somatostatin analogs (when oral preparations are available) to confer additional benefits in the management of progressive diabetic nephropathy. Inhibition of angiogenesis in diabetic proliferative retinopathy can also be evaluated with new drugs such as thalidomide or angiostatin. A future therapeutic strategy targeting the vasculature could be local delivery of antisense oligonucleotides to inhibit gene products implicated in SMC growth.[106] This may prove beneficial in the treatment of acute atherosclerotic processes in the diabetic patient, such as restenosis following coronary artery angioplasty or bypass grafts.

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