STATE-OF-THE-ART PAPERS

The Pathogenesis of Myocardial Fibrosis in the Setting of Diabetic Cardiomyopathy

Juan Asbun, MD, PHD,* Francisco J. Villarreal, MD, PHD†

Mexico City, Mexico; and San Diego, California

Diabetes has emerged as a major threat to worldwide health. The increasing incidence of diabetes in young individuals is particularly worrisome given that the disease is likely to evolve over a period of years. In 1972, the existence of a diabetic cardiomyopathy was proposed based on the experience with four adult diabetic patients who suffered from congestive heart failure in the absence of discernible coronary artery disease, valvular or congenital heart disease, hypertension, or alcoholism. The exact mechanisms underlying the disease are unknown; however, an important component of the pathological alterations observed in these hearts includes the accumulation of extracellular matrix (ECM) proteins, in particular collagens. The excess deposition of ECM in the heart mirrors what occurs in other organs such as the kidney and peritoneum of diabetics. Mechanisms responsible for these alterations may include the excess production, reduced degradation, and/or chemical modification of ECM proteins. These effects may be the result of direct or indirect actions of high glucose concentrations. This article reviews our state of knowledge on the effects that diabetes-like conditions exert on the cells responsible for ECM production as well as relevant experimental and clinical data. (J Am Coll Cardiol 2006;47:693–700) © 2006 by the American College of Cardiology Foundation

During the past three decades epidemiological, clinical, and laboratory studies have confirmed the existence of a cardiomyopathy associated with diabetes mellitus (DM). The diagnosis of a diabetic cardiomyopathy is made in patients in whom no other known etiological factors, such as coronary artery disease, alcoholism, or hypertension, are present (1,2). In these patients, a higher incidence of congestive heart failure is observed (3). Pathological alterations include those relevant to muscle tissue and cells, such as the presence of myocardial hypertrophy and impaired contraction, those associated with extracellular matrix (ECM) proteins, such as excess deposition of collagens, abnormal glycosylation/crosslinking, and alterations in diastolic compliance (4). Other abnormalities include those associated with the vascular compartment, such as the thickening of the endothelium basal lamina (5). This review focuses on the current knowledge of pathogenesis of myocardial fibrosis in diabetics. It also includes observations relevant to the topic of tissue fibrosis in diabetics as derived from various cells and organs.

ORGAN FIBROSIS AS A PATHOLOGICAL OBSERVATION OF LONGSTANDING DIABETES

By far, diabetes-associated nephropathy is the pathological entity that has been best characterized and studied for any organ (6). It is well established that the chronic exposure of the kidney to hyperglycemic conditions favors the development of extensive structural damage. Indeed, diabetic nephropathy (DN) is a major cause of end-stage renal disease (7). Comparable structural changes occur in the glomeruli of either type 1 or type 2 diabetic patients (8,9). Most of these changes are associated with the ECM (10). Diabetic nephropathy is associated with hypertrophy of glomerular and tubular elements as well as the thickening of their basal membrane. In addition, there is an excessive accumulation of ECM proteins in the mesangium (glomerulosclerosis), which narrows or occludes the glomerulus lumen. Another hallmark of DN is the presence of tubulointerstitial fibrosis. The severity of changes in the mesangium and tubules ultimately correlates with loss of kidney function and reduced survival rates (10). The thickening of the glomerular basal membrane is associated with increases in collagen type IV, laminin, and proteoglycans (11,12). In the mesangium, there are increases in collagen types IV, V, VI, fibronectin, and laminin, as well as proteoglycans (12–14). In latter stages of glomerulosclerosis, increases in types I and III collagens occur (13). Animal models of diabetes mirror structural changes observed in human kidneys (10). Increases in the deposition of ECM proteins can be the result of enhanced production and/or decreased degradation. Experimental data support causative roles for altered ECM turnover as assessed through changes in messenger ribonucleic acid and/or protein levels for ECM proteins, serine proteases (e.g., plasmin), matrix metalloproteinases (MMPs), or tissue inhibitors of MMPs (10).

Organs such as the peritoneum also can develop fibrosis in diabetic patients. Many diabetic patients with end-stage

---

Abbreviations and Acronyms

AGE = advanced glycosylation end products
Ang II = angiotensin II
DAG = diacylglycerol
DM = diabetes mellitus
DN = diabetic nephropathy
ECM = extracellular matrix
HG = high glucose
LV = left ventricle/ventricular
MMP = matrix metalloproteinases
PKC = protein kinase C
ROS = reactive oxygen species
TGF = transforming growth factor

---

renal disease are submitted to peritoneal dialysis (15). Over the course of time, a significant proportion of patients on peritoneal dialysis develop increased permeability of small solutes, which enhances the absorption of glucose and leads to the failure of ultrafiltration by the dissipation of the osmotic gradient (15,16). Animal models of end-stage renal disease have shown that the stimulation of profibrotic growth factor release (such as transforming growth factor [TGF]-beta-1) in the peritoneum is associated with the development of submesothelial fibrosis and it has been proposed that these events may be potentiated in diabetics (17).

The chemical modification of existing proteins also can contribute to the development of fibrosis. Part of the aging process includes the loss of elasticity of the vascular system, leading to systolic hypertension secondary to enhanced arterial stiffness. Patients with diabetes manifest increased arterial stiffness at a younger age than their nondiabetic counterparts (18). These observations have led to the concept that the mechanism(s) that underlies changes in arterial stiffness associated with aging is accelerated in diabetes. Underlying the loss of tissue elasticity is a well-understood nonenzymatic reaction between glucose and proteins known as the Maillard or browning reaction (19). The glycosylation of long-lived proteins can undergo further chemical changes, yielding very stable complex products and crosslinks known as advanced glycosylation end-products (AGEs) (Fig. 1) (19). This advanced glycation of proteins is accelerated in the presence of diabetes (18). The formation of AGEs on vessels facilitates the crosslinking of long-lived collagen molecules to each other, increases resistance to

proteolysis, and thus yields fibrosis (18). There is also loss of collagen elasticity and reductions in arterial compliance. Advanced glycosylation end products also are now recognized as playing important roles in cell signaling by interacting with specific receptors (e.g., RAGE) that link to the activation of adhesion molecules, proinflammatory cytokines, and growth factors, thus contributing to pathological entities such as atherosclerosis (20).

FIBROSIS IN THE HUMAN DIABETIC HEART

Rubler et al. (21) first recognized the existence of a diabetic cardiomyopathy in patients with congestive heart failure who had no evidence of coronary atherosclerosis. Epidemiologic evidence of an association of a specific cardiomyopathy with diabetes became evident with the Framingham study (22). Data showed a 2.4-fold greater incidence of heart failure in diabetic men and a 5.1-fold increase in women. Studies conducted in the early 1980s demonstrated changes in myocardial vasculature of diabetic subjects (5). Other studies also demonstrated evidence of left ventricular (LV) hypertrophy and fibrosis with various degrees of small vessel disease (23). Detailed pathological examinations reveal myocardial hypertrophy, interstitial fibrosis, capillary endothelial changes, and capillary basal laminae thickening (23). Microscopic alterations in small intramural coronary arteries include the narrowing of the lumen due to increased proliferation of endothelial cells, increased thickness of the arteriolar wall due to fibrosis and accumulation of mucopolysaccharides, alterations of elastic fibers, myocytolysis, and perivascular fibrosis (5,24). The increased accumulation of collagen types I and III primarily are found in the epicardial and perivascular regions, whereas type IV is found in the endocardial layer (25).

Echocardiography of diabetic patients indicates the development of LV hypertrophy (26). Analysis of data also indicates alterations in echocardiographic signals (backscatter reductions) in diabetic patients, which may reflect tissue fibrosis or other cardiomyopathic changes. Noninvasive studies have shown alterations in both systolic and diastolic function in diabetics and, to a greater extent, those with microvascular complications and/or hypertension (4). The impairment of LV diastolic function of patients with diabetes may be secondary to alterations in collagen structure, specifically increased collagen crosslinking or AGEs (18).
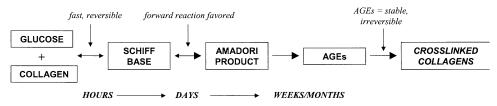

Figure 1. Production of collagen crosslinks. Glucose interacts with collagen to form a Schiff base. This reaction occurs fast and is reversible. The collagen Schiff base can then rearrange over a period of days to generate an Amadori product (i.e., glycated collagen). This step occurs faster in the forward than in the reverse direction and, thus, glycated collagen accumulates. Glycated collagen can undergo further chemical modification to yield complex compounds and crosslinks known as advanced glycosylation end-products (AGEs). Collagen AGEs are known to be more stable, virtually irreversible, and more resistant to proteolysis.

Clinical studies have yielded positive correlations between AGE serum levels and isovolumetric relaxation time and LV diameter during diastole (27).

ANIMAL MODELS OF DIABETIC CARDIOMYOPATHY

Animal models for the study of diabetes include those that mimic type 1 or type 2. An extensive review on the subject was published recently (28). Factor et al. (29) first evaluated the effects of hypertension and diabetes on rat myocardium in 1981 and showed hypertensive-diabetic rats develop cardiac hypertrophy and fibrosis. Subsequent results from Fein and Sonnenblick (3) indicated that diabetes alone did not yield morphological alterations in myocardium, but animals did exhibit weakened contraction. In a subsequent study by Factor et al. (30), increases in cellular lipids and myofibrinolysis were noted with patients with diabetes. The assessment of vascular lesions of hypertensive-diabetic rats indicated pronounced arteriolar constriction, which may have caused ischemic injury to the myocardium leading to replacement fibrosis (31). However, experimental studies in diabetic dogs and monkeys have demonstrated interstitial fibrosis and less compliant LVs (22). In addition, myocardial hydroxyproline content and associated crosslinks were significantly greater in the endocardium of diabetic pigs (32). Other studies also have examined the effects of diabetes on LV remodeling after infarction showing that diabetes decreases animal survival and exaggerates LV remodeling after infarction while increasing interstitial fibrosis and myocyte apoptosis (33,34).

THE EFFECTS OF PHARMACOTHERAPY

Pharmacologic interventions have been used experimentally in an attempt to retard or reverse the development of structural changes in the diabetic myocardium. Treating diabetic rats with the angiotensin-converting enzyme inhibitor captopril for four months prevented increases in diastolic pressure, coronary perfusion pressure, vascular resistance, and interstitial and perivascular fibrosis (35). The angiotensin-converting enzyme inhibitor enalapril can normalize glycation levels in the myocardium and reduce LV stiffness (36). The beneficial effects of enalapril may be derived at least in part by its antioxidant properties (37). Treatment of diabetic rats with the antifibrotic compound pirfenidone or the aldosterone blocker spironolactone attenuated increases in LV diastolic stiffness and reversed fibrosis without normalizing contractility (38). Evidence from clinical trials indicates that pathological cardiac remodeling can be prevented or reversed with drugs that block neurohormonal systems, including the renin angiotensin system and the sympathetic nervous system (39,40). These clinical trial results indicate that patients with diabetes benefit as much as patients without diabetes from neurohormonal-inhibition therapies (39,40). However, caution should be exercised in extrapolating pharmacologic therapy benefits demonstrated

in the general population to patients with diabetes in the absence of extensive clinical data.

The observation that AGE products may be partly responsible for the structural alterations observed in various organs, in particular blood vessels, led to the development of crosslink breaker compounds such as aminoguanidine (18). The administration of these compounds to diabetic dogs or monkeys decreases the enhanced LV stiffness that develops with diabetes and improves contractile function (41,42). The use of these compounds in rats yields the restoration of LV collagen solubility, brain natriuretic peptide serum levels, and normalized gene expression for collagen type III and the profibrotic effector connective TGF (43). In a Phase 2a study completed in January of 2003, the Distensibility Improvement and Remodeling in Diastolic Heart Failure (DIAMOND) trial results indicated that treatment with the crosslink breaker alagebrium resulted in a reduction in LV mass within a 16-week treatment period, as well as an improvement in LV diastolic filling and quality of life. The compound is currently undergoing further evaluation and testing for its potential to improve diastolic function and reduce hypertrophy.

IN VITRO MODELS OF DIABETES

Two main methods have been developed to examine effects that diabetes-like conditions have on in vitro ECM turnover. The first uses primary cell cultures isolated from normal tissues exposed to high glucose (HG) so as to simulate the hyperglycemic milieu of diabetes. The second uses cells derived from either animal models of diabetes or diabetic humans that are cultured in HG. Differences in experimental design are important in that results derived from both types of cultures may not concur. To better simulate diabetes, most investigators also rely on protocols that include the long-term cultures of cells, typically no <2 to 3 days and up to weeks. Commonly used concentrations of D-glucose are 25 to 30 mmol/l (450 to 540 mg/dl) to represent HG and 5 mmol/l (100 mg/dl) for normal glucose. It is important to note that many culture systems routinely used to examine changes in "normal" cell function inadvertently include media with HG concentrations akin to those of diabetes-like conditions (315 to 450 mg/dl, respectively). In vitro experiments also have included the use of fluctuating levels of HG as a possible treatment scheme to better simulate diabetes spikes (44).

The in vitro assessment of hyperglycemic effects has been pursued most extensively in mesangial and glomerular epithelial cells. Published observations indicate that, in cultured mesangial cells, the presence of 30 mmol/l glucose (for four weeks) yields an increase in ECM proteins such as fibronectin, laminin, and type IV collagen production (45,46). High glucose also increased mesangial cell diacylglycerol (DAG) levels and protein kinase C (PKC) (47). To determine the mechanisms involved in enhanced ECM production, Ayo et al. (48) pursued pulse-chase experiments

using mesangial cells cultured in normal and HG conditions. Under HG conditions, they observed a 40% to 50% increase in the synthesis of fibronectin, laminin, and type IV collagen with no apparent change in total protein synthesis or ECM protein degradation after one week. Studies by Pugliese et al. (49) indicate that in long-term cultures of rat glomerular mesangial cells, HG increases fibronectin, laminin, and collagen IV and VI by week 2, with no changes in total protein or cell proliferation. Interestingly, D-galactose and L-glucose treatment (which can induce nonenzymatic protein glycosylation) mimicked in part D-glucose–induced ECM overproduction, suggesting this process is partially responsible for the effects.

The inhibition of cell proliferation also has been reported to occur when mesangial cells were grown in the presence of glycated serum (serum exposed to 28 mmol/l of glucose for four days) whereas type IV collagen gene expression and secretion were stimulated (50). Cell proliferation also was inhibited by the use of culture plates precoated with glycated serum. The use of a monoclonal antibody immunoreactive with Amadori adducts in glycated albumin blocked these effects.

Fibroblasts from other organs can also increase ECM deposition when exposed to HG. Human skin fibroblasts cultured in HG increase collagen III synthesis both at mRNA and protein levels (51). Known complications of patients with DM also include retinopathy and neuropathy, in which excess ECM production can play a role in their pathogenesis. Long-term culture with HG of bovine retinal microvessel pericytes stimulated protein and collagen synthesis (52). In primary cell culture of sciatic nerves (a mixture of Schwann cells, perineurial cells, and fibroblasts) HG increased collagen I, collagen IV, and fibronectin mRNAs (53).

Few reports on the effects of HG on cardiac fibroblasts (CF) have been published. The culture of human cardiac myofibroblast in HG increases cell proliferation (54). In neonatal rat cardiac fibroblasts, HG and osmotic control media increase DNA and collagen synthesis as well as fibronectin and TGF-beta-1 gene expression (55). Recently, we assessed the effects that HG has on adult rat cardiac fibroblast protein and collagen synthesis, MMP activity, and angiotensin II (Ang II) AT1 receptor mRNA levels (56). We also determined whether co-treatment with Ang II yielded additive or synergistic effects with HG, as the additive effects of HG and angiotensin II on type IV collagen synthesis have been previously reported in mesangial cells (57). Our results indicated that HG-treated cardiac fibroblasts synthesized more protein and collagen, but the addition of Ang II did not further stimulate these effects.

Interestingly, pretreatment with losartan blocked HG-induced increases in protein and collagen synthesis. HG also decreased total MMP activity, and the effect was blocked by losartan. Notably, AT1 mRNA levels were up-regulated with HG. These results suggest that HG may promote fibrosis by increasing CF protein and collagen synthesis and decreasing MMP activity and that HG may cause these

effects via the up-regulation of AT1 receptors. As discussed in more detail to follow, the ability of HG to stimulate the production of reactive oxygen species (ROS) in cells is well established. As a result, we also determined the effects of ROS scavenger treatment on CF functions. Interestingly, although pretreatment with vitamin E blocked the effects of HG on total protein synthesis, it stimulated MMP activity without altering ECM synthesis. Thus, it is possible to speculate that vitamin E treatment may normalize HG-induced increases in ECM synthesis by enhancing its degradation.

The production/deposition of ECM induced by HG also may be organ/cell type dependent. In human gingival fibroblasts, glucose concentrations from 5 to 50 mmol/l resulted in a dose-dependent reduction of collagen synthesis. Total protein synthesis and cell number were not altered (58). In mouse proximal tubule epithelial cells, HG increases ECM and total protein synthesis (59). In human glomerular epithelial cells, HG induced the differential expression of integrins, decreased binding of the cells to type IV collagen, decreased MMP-2 gene expression, and induced up-regulation of tissue inhibitor of MMP-2 (60). In human skin fibroblasts isolated from diabetic ulcer sites cell proliferation is significantly slower versus control, and this difference persists regardless of the glucose concentration used in the media (61).

PARACRINE AND MECHANICAL FACTORS

It has been demonstrated that the exposure of ECM-producing cells to HG yields the synthesis/secretion of paracrine factors that may mediate their functional responses. For example, in cultured human mesangial cells exposed to HG, the production of Ang II was greater compared with controls and was prevented by a PKC inhibitor (62). High glucose-induced increases in TGF-beta-1, fibronectin, and type IV collagen were partially blocked by candesartan (an AT1 antagonist). Thus, HG appears to stimulate Ang II production via the activation of PKC, and Ang II partially mediates ECM synthesis (62). Human peritoneal mesothelial cells cultured in HG yield increase in basic fibroblast growth factor. Peritoneal fibroblast proliferation was noted to increase when cells were exposed to mesothelial cell HG-conditioned media, and anti- basic fibroblast growth factor neutralizing antibody blocked the effects (63). In human peritoneal fibroblasts, HG increased TGF-beta-1 and type I collagen mRNA levels in a dose-dependent manner. The use of TGF-beta-1 antibodies blocked these effects (64). In murine renal cortical fibroblasts, HG increased TGF-beta-1, -2, and -3 gene expression. High glucose and TGF-beta-1 also increased the incorporation of proline, mRNA, and protein levels for type I collagen. Pan-selective TGF-beta antibodies attenuated these effects. Therefore, HG and TGF-beta appear to elicit comparable effects on renal fibroblasts (65). Human proximal tubular cells and cortical fibroblasts that are

exposed to HG show increases in cell proliferation and secretion of TGF-beta-1 and platelet-derived growth factor. When exposed to intermittent HG, there are further increases in proliferation, TGF-beta-1 secretion, protein content, and collagen synthesis. Thus, pulsating glucose excursions may have important pathophysiologic effects on human tubulo interstitial structure (66).

Mechanical stimuli also may play roles in stimulating cell function. In isolated perfused glomeruli from diabetic rats, glomerular compliance is known to increase with time. When stretched, mesangial cells increase total collagen synthesis in both normal glucose (NG) (50%) and HG conditions (27%). However, the fraction of newly formed collagen being catabolized also increased in NG conditions, but was unchanged with HG, which results in an increase in net collagen accumulation with HG (67).

REACTIVE OXYGEN SPECIES

Results from in vitro experiments indicate that the generation of ROS is likely involved in inducing abnormal responses to HG (68). High glucose induces ROS generation in mesangial and tubular epithelial cells and up-regulates TGF-beta-1 and ECM synthesis (45,69,70). These effects also can be mimicked by H_2O_2 (71). The use of 3-O-methyl-D-glucose or L-glucose does not induce intracellular ROS, and cytochalasin B (a glucose transporter inhibitor) inhibits D-glucose–induced intracellular ROS, suggesting that HG-induced ROS depends on glucose uptake and metabolism (69). The source of HG-induced ROS generation appears to be linked to the NADPH oxidase system and mitochondrial metabolism (68). Further evidence of the involvement of ROS comes from the observation that the treatment of mesangial cells with antioxidants normalizes HG-induced increases in ECM synthesis (72). Reactive oxygen species also can activate cell signaling pathways such as PKC, mitogen-activated protein kinases, and JAK-STAT (73–75). Of particular interest is the activation of specific isoforms of PKC (e.g., PKC-beta). The use of PKC inhibitor compounds such as ruboxistaurin appears to prevent or reverse damage to organs such as the retina (76,77). The direct effects of these compounds on ECM production have not yet been evaluated, but their long-term use does improve renal function in diabetic mice (78).

SIGNALING

As can be ascertained from the topics discussed previously, the accumulation of AGEs, the activation of PKC isoforms, and HG-induced generation of ROS may explain partly how hyperglycemia mediates tissue damage and remodeling. How these events are triggered by HG is less clear. However, experimental studies have attempted to identify HG-activated cell signaling pathways associated with ECM production. In mesangial cell lines transfected with a gene expressing luciferase and murine collagen IV gene, HG stimulated luciferase activity in a dose- and time-dependent manner.

The inhibition of PKC with staurosporine or calphostin C significantly reduced the activity, suggesting that HG promotes transcriptional activity of collagen IV through PKC (79). Cultured dermal fibroblasts from non–insulin-dependent DM rats have lower cell proliferation and higher L-lactate accumulation when cultured under normal glucose conditions versus controls. When incubated in HG, fibroblasts from control and DM rats decreased total DNA content and increased L-lactate levels. A PKC inhibitor reversed HG-induced changes in both cell types, as well as in the cells from DM rats cultured under NG, suggesting that PKC may play a role as a modulator of fibroblast proliferation in patients with diabetes (80). In a follow-up study by the same group, they also demonstrated the involvement of protein kinase A and cAMP (81).

An excessive accumulation of intracellular sorbitol found in diabetic animals and in cells cultured under HG conditions has been proposed as important factor in the pathogenesis of diabetic complications (82). Aldose reductase, a key enzyme of polyol pathway, catalyzes NADPH-dependent reduction of glucose to sorbitol (sorbitol pathway). To examine the role of the polyol pathway in renal ECM accumulation, Bleyer et al. (83) incubated murine proximal tubule cells in either normal or HG concentrations in the presence or absence of the aldose reductase inhibitor sorbinil. They noted that HG increased cell sorbitol levels seven-fold and the addition of sorbinil inhibited the response. In HG cells, sorbinil also reduced mRNA levels of collagen type I and IV as well as their secretion.
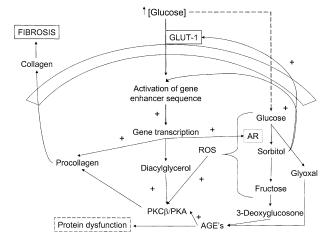

Figure 2. Sorbitol signaling pathway proposed for high-glucose–mediated enhanced collagen production in fibroblasts. Increases in intracellular glucose concentrations can activate an enhancer sequence in the collagen promoter, yielding enhanced collagen production and fibrosis. Increases in diacylglycerol can occur secondary to the altered transcription of enzymes involved in its synthesis or degradation (e.g., diacylglycerol kinase). The enhanced entry of glucose into the fibroblasts can also occur independently of transporter proteins yielding the stimulation of sorbitol and the production of 3-deoxyglucosone and glyoxal (intracellular advanced glycosylation end-product [AGEs]), both of which are direct stimulants of protein kinase C-beta. The production of aldose reductase (AR) also can be favored by an enhanced transcription of the gene. The sorbitol pathway can also increase production of reactive oxygen species (ROS), further stimulating PKC-beta. GLUT-1 = glucose transporter-1; PKA = protein kinase A.
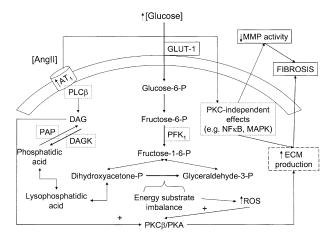
Figure 3. Diacylglycerol (DAG) signaling pathway proposed for high-glucose–mediated enhanced production of extracellular matrix (ECM) proteins in fibroblasts. High glucose can affect the manner in which fibroblasts respond to angiotensin II. Angiotensin II AT1 receptor levels increase in cells treated with high glucose. The stimulation of AT1 receptors increases the intracellular concentration of DAG. The enzyme in charge of the interconversion of this messenger DAG kinase (DAGK) favors the synthesis of other lipid mediators, which can enter the pathway for the synthesis of dihydroacetone, increase reactive oxygen species, and stimulate protein kinase C (PKC)-beta and/or protein kinase A (PKA). Dihydroacetone can also generate DAG via the action of phosphatidic acid phosphatase (PAP). There also may be protein kinase-independent effects mediated via AT1 receptors, which may alter the production and/or activity of matrix metalloproteinases (MMPs) or other ECM-related genes. Phosphofructokinase (PFK$_1$) is a rate-limiting enzyme involved in the hexosamine pathway. GLUT-1 = glucose transporter-1; MAPK = mitogen-activated protein kinase; NF-κB = nuclear factor kappa B; PLCβ = phospholipase C beta.

The topic of HG-mediated signaling and organ pathology has been extensively reviewed elsewhere (68,77,84–87). However, it is reasonable to assume that in the development of cardiac fibrosis, HG-activated signaling pathways may parallel those known to be involved in other diabetic organs. Figure 2 illustrates one such possible scenario that involves gene transcription, effects of sorbitol, aldose reductase, ROS, and AGEs. A DAG signaling pathway also can be proposed for HG-mediated enhanced production of ECM proteins (Fig. 3) (77). As we have demonstrated, Ang II AT1 receptor levels increase in fibroblasts exposed to HG conditions, and this receptor couples with the production of DAG (56). The enzyme in charge of the interconversion of this messenger, DAG kinase, allows the synthesis of other lipid mediators, which can enter the pathway for the synthesis of dihydroacetone, increasing ROS and stimulating PKC-beta and/or protein kinase A. However, dihydroacetone also can generate DAG (87). There may also be protein kinase-independent effects mediated via AT1 receptors, which may alter the production and/or activity of ECM-related genes. Among all of the well-characterized PKC-independent signaling responses associated with HG, there is emerging evidence for the involvement of the transcription factor nuclear factor kappa B and mitogen-activated protein kinases (88). Alternative pathways for HG-induced ECM overproduction have been described and include the hexosamine synthesis pathway (89). The increased

load of intracellular glucose in handled by a rate limiting enzyme phosphofructokinase (Fig. 3). When the production of phosphofructokinase is halted, excess fructose-6-phosphate allows the synthesis of glucosamine-6-phosphate an activator of PKC-beta.

CONCLUDING REMARKS

Although the first reference made to the existence of a diabetic cardiomyopathy occurred in 1972, limited progress has been made in attempting to describe the underlying molecular pathology, and in particular that related to the development of myocardial fibrosis. Most of the current knowledge about how hyperglycemia may affect the deposition of ECM proteins comes from studies performed in kidney cells. Although there may be some degree of analogy between cellular responses to HG, there can be clear differences depending on the cell type studied. In the presence of an ever growing incidence of diabetes, it will become imperative to further our understanding of the underlying pathology of myocardial fibrosis so as to develop improved pharmacological therapies.

REFERENCES

1. Zoneraich S. Unravelling the conundrums of the diabetic heart diagnosed in 1876: prelude to genetics. Can J Cardiol 1994;10:945–50.
2. Spector KS. Diabetic cardiomyopathy. Clin Cardiol 1998;21:885–7.
3. Fein FS, Sonnenblick EH. Diabetic cardiomyopathy. Cardiovasc Drugs Ther 1994;8:65–73.
4. Uusitupa MI, Mustonen JN, Airaksinen KE. Diabetic heart muscle disease. Ann Med 1990;22:377–86.
5. Gherasim L, Tasca C, Havriliuc C, Vasilescu C. A morphological quantitative study of small vessels in diabetic cardiomyopathy. Morphol Embryol (Bucur) 1985;31:191–5.
6. Jawa A, Kcomt J, Fonseca VA. Diabetic nephropathy and retinopathy. Med Clin North Am 2004;88:1001–36, xi.
7. Ritz E, Orth SR. Nephropathy in patients with type 2 diabetes mellitus. N Engl J Med 1999;341:1127–33.
8. Osterby R. Glomerular structural changes in type 1 (insulin-dependent) diabetes mellitus: causes, consequences, and prevention. Diabetologia 1992;35:803–12.
9. Osterby R, Gall MA, Schmitz A, Nielsen FS, Nyberg G, Parving HH. Glomerular structure and function in proteinuric type 2 (non-insulin-dependent) diabetic patients. Diabetologia 1993;36:1064–70.
10. Mason RM, Wahab NA. Extracellular matrix metabolism in diabetic nephropathy. J Am Soc Nephrol 2003;14:1358–73.
11. Kim Y, Kleppel MM, Butkowski R, Mauer SM, Wieslander J, Michael AF. Differential expression of basement membrane collagen chains in diabetic nephropathy. Am J Pathol 1991;138:413–20.
12. Ikeda S, Makino H, Haramoto T, Shikata K, Kumagai I, Ota Z. Changes in glomerular extracellular matrices components in diabetic nephropathy. J Diabet Complications 1991;5:186–8.
13. Makino H, Shikata K, Wieslander J, et al. Localization of fibril/microfibril and basement membrane collagens in diabetic glomerulosclerosis in type 2 diabetes. Diabet Med 1994;11:304–11.
14. Zhu D, Kim Y, Steffes MW, Groppoli TJ, Butkowski RJ, Mauer SM. Application of electron microscopic immunocytochemistry to the human kidney: distribution of type IV and type VI collagen in normal human kidney. J Histochem Cytochem 1994;42:577–84.

15. Locatelli F, Pozzoni P, Del Vecchio L. Renal replacement therapy in patients with diabetes and end-stage renal disease. J Am Soc Nephrol 2004;15 Suppl 1:S25–9.

16. Lee HB, Chung SH, Chu WS, Kim JK, Ha H. Peritoneal dialysis in diabetic patients. Am J Kidney Dis 2001;38:S200–3.

17. Gillerot G, Devuyst O. Molecular mechanisms modifying the peritoneal membrane exposed to peritoneal dialysis. Clin Nephrol 2003;60:1–6.

18. Aronson D. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. J Hypertens 2003;21:3–12.

19. Ulrich P, Cerami A. Protein glycation, diabetes, and aging. Recent Prog Horm Res 2001;56:1–21.

20. Schmidt AM, Yan SD, Wautier JL, Stern D. Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. Circ Res 1999;84:489–97.

21. Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. Am J Cardiol 1972;30:595–602.

22. Fein FS, Sonnenblick EH. Diabetic cardiomyopathy. Prog Cardiovasc Dis 1985;27:255–70.

23. Fischer VW, Barner HB, Larose LS. Pathomorphologic aspects of muscular tissue in diabetes mellitus. Hum Pathol 1984;15:1127–36.

24. Nunoda S, Genda A, Sugihara N, Nakayama A, Mizuno S, Takeda R. Quantitative approach to the histopathology of the biopsied right ventricular myocardium in patients with diabetes mellitus. Heart Vessels 1985;1:43–7.

25. Shimizu M, Umeda K, Sugihara N, et al. Collagen remodelling in myocardia of patients with diabetes. J Clin Pathol 1993;46:32–6.

26. Gregor P, Widimsky P, Rostlapil J, Cervenka V, Visek V. Echocardiographic picture in diabetes mellitus. Jpn Heart J 1984;25:969–77.

27. Berg TJ, Snorgaard O, Faber J, et al. Serum levels of advanced glycation end products are associated with left ventricular diastolic function in patients with type 1 diabetes. Diabetes Care 1999;22:1186–90.

28. Rees DA, Alcolado JC. Animal models of diabetes mellitus. Diabet Med 2005;22:359–70.

29. Factor SM, Bhan R, Minase T, Wolinsky H, Sonnenblick EH. Hypertensive-diabetic cardiomyopathy in the rat: an experimental model of human disease. Am J Pathol 1981;102:219–28.

30. Factor SM, Minase T, Bhan R, Wolinsky H, Sonnenblick EH. Hypertensive diabetic cardiomyopathy in the rat: ultrastructural features. Virchows Arch A Pathol Anat Histopathol 1983;398:305–17.

31. Factor SM, Minase T, Cho S, Fein F, Capasso JM, Sonnenblick EH. Coronary microvascular abnormalities in the hypertensive-diabetic rat. A primary cause of cardiomyopathy? Am J Pathol 1984;116:9–20.

32. Martinez DA, Guhl DJ, Stanley WC, Vailas AC. Extracellular matrix maturation in the left ventricle of normal and diabetic swine. Diabetes Res Clin Pract 2003;59:1–9.

33. Shiomi T, Tsutsui H, Ikeuchi M, et al. Streptozotocin-induced hyperglycemia exacerbates left ventricular remodeling and failure after experimental myocardial infarction. J Am Coll Cardiol 2003;42:165–72.

34. Backlund T, Palojoki E, Saraste A, et al. Sustained cardiomyocyte apoptosis and left ventricular remodelling after myocardial infarction in experimental diabetes. Diabetologia 2004;47:325–30.

35. Rosen R, Rump AF, Rosen P. The ACE-inhibitor captopril improves myocardial perfusion in spontaneously diabetic (BB) rats. Diabetologia 1995;38:509–17.

36. Avendano GF, Agarwal RK, Bashey RI, et al. Effects of glucose intolerance on myocardial function and collagen-linked glycation. Diabetes 1999;48:1443–7.

37. de Cavanagh EM, Inserra F, Toblli J, Stella I, Fraga CG, Ferder L. Enalapril attenuates oxidative stress in diabetic rats. Hypertension 2001;38:1130–6.

38. Miric G, Dallemagne C, Endre Z, Margolin S, Taylor SM, Brown L. Reversal of cardiac and renal fibrosis by pirfenidone and spironolactone in streptozotocin-diabetic rats. Br J Pharmacol 2001;133:687–94.

39. Tarantini L, Di Lenarda A, Velussi M, et al. [Diabetes mellitus, left ventricular dysfunction and congestive heart failure]. Ital Heart J Suppl 2004;5:605–15.

40. Bell DS. Heart failure: the frequent, forgotten, and often fatal complication of diabetes. Diabetes Care 2003;26:2433–41.

41. Asif M, Egan J, Vasan S, et al. An advanced glycation endproduct cross-link breaker can reverse age-related increases in myocardial stiffness. Proc Natl Acad Sci USA 2000;97:2809–13.

42. Vaitkevicius PV, Lane M, Spurgeon H, et al. A cross-link breaker has sustained effects on arterial and ventricular properties in older rhesus monkeys. Proc Natl Acad Sci USA 2001;98:1171–5.

43. Candido R, Forbes JM, Thomas MC, et al. A breaker of advanced glycation end products attenuates diabetes-induced myocardial structural changes. Circ Res 2003;92:785–92.

44. Risso A, Mercuri F, Quagliaro L, Damante G, Ceriello A. Intermittent high glucose enhances apoptosis in human umbilical vein endothelial cells in culture. Am J Physiol Endocrinol Metab 2001;281:E924–30.

45. Ayo SH, Radnik RA, Garoni JA, Glass WF 2nd, Kreisberg JI. High glucose causes an increase in extracellular matrix proteins in cultured mesangial cells. Am J Pathol 1990;136:1339–48.

46. Haneda M, Kikkawa R, Horide N, et al. Glucose enhances type IV collagen production in cultured rat glomerular mesangial cells. Diabetologia 1991;34:198–200.

47. Ayo SH, Radnik R, Garoni JA, Troyer DA, Kreisberg JI. High glucose increases diacylglycerol mass and activates protein kinase C in mesangial cell cultures. Am J Physiol 1991;261:F571–7.

48. Ayo SH, Radnik RA, Glass WF 2nd, et al. Increased extracellular matrix synthesis and mRNA in mesangial cells grown in high-glucose medium. Am J Physiol 1991;260:F185–91.

49. Pugliese G, Pricci F, Pugliese F, et al. Mechanisms of glucose-enhanced extracellular matrix accumulation in rat glomerular mesangial cells. Diabetes 1994;43:478–90.

50. Cohen MP, Ziyadeh FN. Amadori glucose adducts modulate mesangial cell growth and collagen gene expression. Kidney Int 1994;45:475–84.

51. Trevisan R, Yip J, Sarika L, Li LK, Viberti G. Enhanced collagen synthesis in cultured skin fibroblasts from insulin-dependent diabetic patients with nephropathy. J Am Soc Nephrol 1997;8:1133–9.

52. Li W, Shen S, Khatami M, Rockey JH. Stimulation of retinal capillary pericyte protein and collagen synthesis in culture by high-glucose concentration. Diabetes 1984;33:785–9.

53. Muona P, Peltonen J, Jaakkola S, Uitto J. Increased matrix gene expression by glucose in rat neural connective tissue cells in culture. Diabetes 1991;40:605–11.

54. Neumann S, Huse K, Semrau R, et al. Aldosterone and D-glucose stimulate the proliferation of human cardiac myofibroblasts in vitro. Hypertension 2002;39:756–60.

55. Tokudome T, Horio T, Yoshihara F, et al. Direct effects of high glucose and insulin on protein synthesis in cultured cardiac myocytes and DNA and collagen synthesis in cardiac fibroblasts. Metabolism 2004;53:710–5.

56. Asbun J, Manso AM, Villarreal FJ. Profibrotic influence of high glucose on cardiac fibroblast functions: effects of losartan and vitamin E. Am J Physiol Heart Circ Physiol 2005;288:H227–34.

57. Amiri F, Shaw S, Wang X, et al. Angiotensin II activation of the JAK/STAT pathway in mesangial cells is altered by high glucose. Kidney Int 2002;61:1605–16.

58. Willershausen-Zonnchen B, Lemmen C, Hamm G. Influence of high glucose concentrations on glycosaminoglycan and collagen synthesis in cultured human gingival fibroblasts. J Clin Periodontol 1991;18:190–5.

59. Ziyadeh FN, Snipes ER, Watanabe M, Alvarez RJ, Goldfarb S, Haverty TP. High glucose induces cell hypertrophy and stimulates collagen gene transcription in proximal tubule. Am J Physiol 1990;259:F704–14.

60. Kitsiou PV, Tzinia AK, Stetler-Stevenson WG, et al. Glucose-induced changes in integrins and matrix-related functions in cultured human glomerular epithelial cells. Am J Physiol Renal Physiol 2003;284:F671–9.

61. Loots MA, Lamme EN, Mekkes JR, Bos JD, Middelkoop E. Cultured fibroblasts from chronic diabetic wounds on the lower extremity (non-insulin-dependent diabetes mellitus) show disturbed proliferation. Arch Dermatol Res 1999;291:93–9.

62. Ikehara K, Tada H, Kuboki K, Inokuchi T. Role of protein kinase C-angiotensin II pathway for extracellular matrix production in cultured human mesangial cells exposed to high glucose levels. Diabetes Res Clin Pract 2003;59:25–30.

63. Ogata S, Yorioka N, Kohno N. Glucose and prednisolone alter basic fibroblast growth factor expression in peritoneal mesothelial cells and fibroblasts. J Am Soc Nephrol 2001;12:2787–96.

64. Saed GM, Diamond MP. Effect of glucose on the expression of type I collagen and transforming growth factor-beta1 in cultured human peritoneal fibroblasts. Fertil Steril 2003;79:158–63.

65. Han DC, Isono M, Hoffman BB, Ziyadeh FN. High glucose stimulates proliferation and collagen type I synthesis in renal cortical fibroblasts: mediation by autocrine activation of TGF-beta. J Am Soc Nephrol 1999;10:1891–9.

66. Jones SC, Saunders HJ, Qi W, Pollock CA. Intermittent high glucose enhances cell growth and collagen synthesis in cultured human tubulointerstitial cells. Diabetologia 1999;42:1113–9.

67. Cortes P, Zhao X, Riser BL, Narins RG. Role of glomerular mechanical strain in the pathogenesis of diabetic nephropathy. Kidney Int 1997;51:57–68.

68. Lee HB, Yu MR, Yang Y, Jiang Z, Ha H. Reactive oxygen species-regulated signaling pathways in diabetic nephropathy. J Am Soc Nephrol 2003;14:S241–5.

69. Ha H, Yu MR, Choi YJ, Kitamura M, Lee HB. Role of high glucose-induced nuclear factor-kappaB activation in monocyte chemoattractant protein-1 expression by mesangial cells. J Am Soc Nephrol 2002;13:894–902.

70. Ziyadeh FN, Sharma K, Ericksen M, Wolf G. Stimulation of collagen gene expression and protein synthesis in murine mesangial cells by high glucose is mediated by autocrine activation of transforming growth factor-beta. J Clin Invest 1994;93:536–42.

71. Iglesias-De La Cruz MC, Ruiz-Torres P, Alcami J, et al. Hydrogen peroxide increases extracellular matrix mRNA through TGF-beta in human mesangial cells. Kidney Int 2001;59:87–95.

72. Ha H, Lee SH, Kim KH. Effects of rebamipide in a model of experimental diabetes and on the synthesis of transforming growth factor-beta and fibronectin, and lipid peroxidation induced by high glucose in cultured mesangial cells. J Pharmacol Exp Ther 1997;281:1457–62.

73. Studer RK, Craven PA, DeRubertis FR. Antioxidant inhibition of protein kinase C-signaled increases in transforming growth factor-beta in mesangial cells. Metabolism 1997;46:918–25.

74. Dunlop ME, Muggli EE. Small heat shock protein alteration provides a mechanism to reduce mesangial cell contractility in diabetes and oxidative stress. Kidney Int 2000;57:464–75.

75. Wang X, Shaw S, Amiri F, Eaton DC, Marrero MB. Inhibition of the Jak/STAT signaling pathway prevents the high glucose-induced increase in TGF-beta and fibronectin synthesis in mesangial cells. Diabetes 2002;51:3505–9.

76. He Z, King GL. Protein kinase C-beta isoform inhibitors: a new treatment for diabetic cardiovascular diseases. Circulation 2004;110:7–9.

77. Way KJ, Katai N, King GL. Protein kinase C and the development of diabetic vascular complications. Diabetes Med 2001;18:945–59.

78. Koya D, Lee IK, Ishii H, Kanoh H, King GL. Prevention of glomerular dysfunction in diabetic rats by treatment with d-alpha-tocopherol. J Am Soc Nephrol 1997;8:426–35.

79. Fumo P, Kuncio GS, Ziyadeh FN. PKC and high glucose stimulate collagen alpha 1 (IV) transcriptional activity in a reporter mesangial cell line. Am J Physiol 1994;267:F632–8.

80. Hehenberger K, Hansson A, Heilborn JD, Abdel-Halim SM, Ostensson CG, Brismar K. Impaired proliferation and increased L-lactate production of dermal fibroblasts in the GK-rat, a spontaneous model of non-insulin dependent diabetes mellitus. Wound Repair Regen 1999;7:65–71.

81. Ziyadeh FN, Fumo P, Rodenberger CH, Kuncio GS, Neilson EG. Role of protein kinase C and cyclic AMP/protein kinase A in high glucose-stimulated transcriptional activation of collagen alpha 1 (IV) in glomerular mesangial cells. J Diabetes Complications 1995;9:255–61.

82. Suzen S, Buyukbingol E. Recent studies of aldose reductase enzyme inhibition for diabetic complications. Curr Med Chem 2003;10:1329–52.

83. Bleyer AJ, Fumo P, Snipes ER, Goldfarb S, Simmons DA, Ziyadeh FN. Polyol pathway mediates high glucose-induced collagen synthesis in proximal tubule. Kidney Int 1994;45:659–66.

84. Setter SM, Campbell RK, Cahoon CJ. Biochemical pathways for microvascular complications of diabetes mellitus. Ann Pharmacother 2003;37:1858–66.

85. Duby JJ, Campbell RK, Setter SM, White JR, Rasmussen KA. Diabetic neuropathy: an intensive review. Am J Health Syst Pharm 2004;61:160–73; quiz 175–6.

86. Chung SS, Ho EC, Lam KS, Chung SK. Contribution of polyol pathway to diabetes-induced oxidative stress. J Am Soc Nephrol 2003;14:S233–6.

87. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813–20.

88. Maldonado A, He L, Game BA, et al. Pre-exposure to high glucose augments lipopolysaccharide-stimulated matrix metalloproteinase-1 expression by human U937 histiocytes. J Periodontal Res 2004;39:415–23.

89. Singh LP, Gennerette D, Simmons S, Crook ED. Glucose-induced insulin resistance of phosphatidylinositol 3'-OH kinase and AKT/PKB is mediated by the hexosamine biosynthesis pathway. J Diabetes Complications 2001;15:88–96.