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REVIEW

Diabetic microvascular complications: possible targets for improved macrovascular outcomes

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Abstract: The results of recent outcome trials challenge hypotheses that tight control of both glycohemoglobin and blood pressure diminishes macrovascular events and survival among type 2 diabetic patients. Relevant questions exist regarding the adequacy of glycohemoglobin alone as a measure of diabetes control. Are we ignoring mechanisms of vasculotoxicity (profibrosis, altered angiogenesis, hypertrophy, hyperplasia, and endothelial injury) inherent in current antihyperglycemic medications? Is the polypharmacy for lowering cholesterol, triglyceride, glucose, and systolic blood pressure producing drug interactions that are too complex to be clinically identified? We review angiotensin–aldosterone mechanisms of tissue injury that magnify microvascular damage caused by hyperglycemia and hypertension. Many studies describe interruption of these mechanisms, without hemodynamic consequence, in the preservation of function in type 1 diabetes. Possible interactions between the renin–angiotensin–aldosterone system and physiologic glycemic control (through pulsatile insulin release) suggest opportunities for further clinical investigation.

Keywords: angiotensin-converting enzyme inhibitor, pulsatile insulin, diabetic nephropathy, cardiac autonomic neuropathy, podocytes, beta cells

Introduction

The Diabetes Control and Complications Trial (DCCT)[1] established that multiple injections of insulin reduce microvascular complications in type 1 diabetes (Table 1). In the Captopril Study, angiotensin-converting enzyme (ACE) inhibition was demonstrated to preserve renal function in type 1 diabetes.[2] Microvascular benefits from intensive glycemic management and ACE inhibition in type 2 diabetic patients have been reported in the UK Prospective Diabetes Study.[3,4] Until recently, aggressive control of glycemia and blood pressure in type 2 diabetic patients was felt to be effective in the reduction of cardiovascular endpoints. Now that macrovascular endpoints have been found to be unresponsive to the highest doses of medications to lower glucose[5] and blood pressure,[6] we must consider alternative research involving the preservation of renal function as an indirect way of preserving myocardial function.

Mechanisms by which kidney glomerular, interstitial, and vascular anatomy are injured include hypertension, inflammation, enhanced hemostasis, oxidative stress, diminished endothelial function, pathological angiogenesis, and accelerated fibrosis. High glucose and increased angiotensin have been shown to have additive pathological effects on renal tubules with accelerated fibrosis of the interstitium through enhanced expression of transforming growth factor (TGF)-β.[7,8] Positive endpoint responses that cannot be explained by hemodynamic variations alone have been demonstrated.

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Table 1 Advancing treatment approaches in type 1 diabetes in 1993–1995

A. Diabetes control and complications trial
Multiple insulin injections were found to be associated with a lower incidence of diabetic nephropathy, retinopathy, and neuropathy
B. Captopril trial
Angiotensin-converting enzyme inhibitor was found to decrease the rate of loss of renal function, using the time to doubling of serum creatinine
C. Pulsatile insulin study[14]
Control of high and low blood glucose in type 1 diabetic patients was found to be improved beyond use of the DCCT protocol 7 days/week by use of the DCCT protocol 6 days/week + intravenous infusions of insulin in pulses 1 day/week. Each patient was his/her own control. This study did not address long-term complications
D. Orthostatic hypotension[15]
Improved lifestyle with stabilization of locomotion through diminution of this neurological complication when pulsatile insulin was added 1 day/week to the DCCT protocol 6 days/week. Each patient was his/her own control
E. Hypertension[16]
Lower doses of antihypertensives required during 3-month rotations of the pulsatile infusion 1 day/week were added to the DCCT protocol 6 days/week

Abbreviation: DCCT, Diabetes Control and Complications Trial.

The interruption of angiotensin–aldosterone mechanisms and optimal insulinization need further study. We call for prospective investigation of nonhemodynamic angiotensin effects that may alter intracellular insulin signaling with effects on renal and myocardial physiology.

Clinical trials

Losartan or enalapril prevented the onset of retinopathy by photography, but did not alter the onset of nephropathy by histology in type 1 diabetic patients.[9] In type 2 diabetic patients, an improvement in albuminuria with angiotensin receptor blockade was associated with a reduction in markers for acceleration of inflammation and thrombosis, suggesting effects beyond blood pressure reduction.[10] In type 1 diabetic patients, pulsatile use of insulin magnified the benefits of ACE inhibition.

Improved cardiac autonomic function and reversal of left ventricular hypertrophy were associated with improved glycemia in type 1 diabetic nephropathy patients. Renal and pancreatic transplantation has also been associated with reversal of left ventricular hypertrophy.

Although most studies demonstrate the prevention of a new appearance of retinopathy in type 1 diabetic patients treated with angiotensin-active medications, there is major inconsistency in the findings of protection from retinopathy progression for both type 1 and type 2 diabetic patients treated with angiotensin-active medications.

Kidney studies

In the Renin–Angiotensin–Aldosterone System (RAAS) Study, normotensive, normoalbuminuric type 1 diabetic patients with minimal evidence of retinopathy were observed for 5 years. A renal biopsy was done at baseline and repeated at 5 years. When compared with placebo, neither enalapril nor losartan had an effect on renal biopsy histology despite the fact that blood pressures were significantly lower than with placebo.[9]

The Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria (IRMA2) Study (Table 2) found improvement in albuminuria associated with reduction in markers for inflammation, including C-reactive protein, interleukin-6 (IL-6), and fibrinogen.[10] Patients were maintained on their usual diabetes treatment, which was associated with no significant decrease in advanced glycation end products (AGE) concentration. Improvement of albuminuria without change in blood pressure over 52 weeks was found in diabetic[11] and nondiabetic[12] kidney disease patients when spironolactone was added to a regimen of ACE inhibitor or ACE inhibitor plus angiotensin receptor blocker (ARB)[12] as shown in Table 2. The latter study was associated with diminution of type 4 collagen, a marker of fibrosis. Improvement in AGE and glycohemoglobin A1c was associated with a reduction in elevated levels of fibrinogen and factor VII[13] acute-phase reactants with hemostasis effects.

Table 1 summarizes the added benefit found with pulsatile insulin infusions for the stabilization of high and low blood glucose,[14] postural hypotension,[15] and supine hypertension[16] in type 1 diabetic patients with emerging complications. Crossover studies demonstrated lower blood pressure medication requirements during pulsatile intravenous insulin infusions.[16]

A prospective randomized study of weekly pulsatile intravenous insulin infusions in type 1 diabetic patients with proteinuria and hypertension, including a control group on the multiple insulin injection (DCCT) protocol, was completed in several centers (Table 3). After 18 months, a statistically significant difference in progression of renal dysfunction was noted between the groups.[17] For the pulsatile insulin group, it would take 15 years for the creatinine clearance (Ccreat) to fall from 50–60 mL/min to 10–15 mL/min compared with 5 years utilizing the DCCT protocol without weekly pulsatile infusions. This outcome could be expected to result in quality of life benefits in the trade-off of 1 day/week spent with insulin infusions versus multiple days per week with peritoneal or hemodialysis. The mechanism for added benefit

Table 2 Human studies: effects of nonhemodynamic angiotensin mechanisms

References	Drug/patient	Primary endpoint	Comment
Persson et al[10]	Irbesartan Type 2 diabetes	Inflammatory markers decreased a. IL-6 b. C-reactive protein c. Fibrinogen	Albuminuria decreased
Flammer et al[44]	Losartan versus atenolol Type 2 diabetes and hypertension	a. Flow-mediated vasodilation decreased b. 8-Isoprostane decreased with losartan, but not atenolol	8-Isoprostane is generated from membrane phospholipid by free radicals
Kramer et al[45]	Losartan Hypertension, no diabetes	a. Platelet aggregation decreased after 8 h b. Endothelial cells (human)AT2-induced PDGF2-α + thromboxane blocked by incubation with EXP3179	Losartan metabolites: EXP3174 uses AT2 receptor EXP3179 no receptor Metabolism of losartan requires 8 h
Fortuno et al[46]	EXP3179 or losartan (but not EXP3174), irbesartan or quinapril, no diabetes	Human phagocytic mononuclear cells: a. NADPH oxidase b. Protein kinase C expression inhibited	Expression of matrix metalloproteinase inhibited
Furumatsu et al[11]	Enalapril, losartan, and spironolactone, no diabetes	Albuminuria decreased over 1 year	Urine type 4 collagen decreased
Mehdi et al[12]	Lisinopril, spironolactone diabetes a. Type 2: 80% b. Type 1: 20%	Albuminuria decreased over 1 year	

Table 3 Pulsatile insulin study: baseline[17]

A. Randomized trial of DCCT protocol (control) versus DCCT protocol 6 days/week + pulsatile insulin (infusion group) 1 day/week in type 1 diabetic patients with proteinuria, effect on progression of loss of renal function as measured by Ccreat

1. Seventy-one patients seen every week
2. Distribution: control (n = 34), infusion (n = 37)

B. ACE inhibitors preferred for blood pressure control

1. Forty-five patients
2. Distribution: control (n = 25) infusion (n = 20)
3. Distribution: no ACE inhibitors: control (n = 9) infusion (n = 17), *P* = ns

C. Blood pressures (mmHg) by 24-h ambulatory method not significantly different at baseline, 52 weeks, and 78 weeks

Baseline	Infusion group (n = 37)	Control group (n = 34)	*P* value
Systolic	133.6 ± 3.2	132.5 ± 2.6	0.79
Diastolic	77.8 ± 1.5	79.6 ± 1.7	0.44
52 weeks	Infusion group (n = 37)	Control group (n = 34)	*P* value
Systolic	136.0 ± 2.7	133.2 ± 2.6	0.46
Diastolic	76.9 ± 1.8	78.7 ± 1.9	0.50
Baseline	Infusion group (n = 23)	Control group (n = 26)	*P* value
Systolic	134.8 ± 4.7	134.5 ± 3.1	0.96
Diastolic	78.3 ± 1.8	80.4 ± 2.1	0.46
78 weeks	Infusion group (n = 23)	Control group (n = 26)	*P* value
Systolic	131.6 ± 3.8	135.1 ± 3.4	0.49
Diastolic	74.7 ± 1.8	78.8 ± 2.2	0.17

Notes: Slopes of loss of Ccreat not significantly different at 52 weeks (n = 71); significantly different at 78 weeks (n = 49); did not change when the graph was drawn from 52 to 78 weeks.

of pulsatile infusion could not be shown to involve any of the hemostatic, echocardiographic, ambulatory blood pressure, or ambulatory electrocardiographic measures that were incorporated into the protocol.[18]

In light of newer angiotensin mechanisms in the research literature, our total data set,[17] which has now become more relevant,[19] can be summarized as follows (Table 4).

a. The level of blood pressure was not lower with better preservation of renal function and was not higher with the faster loss of Ccreat in both study groups.
b. Pulsatile infusion added no benefit to multiple injections in the absence of ACE inhibitors.
c. The DCCT protocol group required significantly larger doses of ACE inhibitors (captopril, enalapril, fosinopril, lisinopril, and quinapril) compared with the pulsatile infusion group as predicted.[16]
d. The combination of ACE inhibition with pulsatile infusion was significantly better in preservation of Ccreat than ACE inhibition with multiple injections of insulin. When calculations were limited to individuals treated with ACE inhibitors, the estimated time for Ccreat to fall from 50–60 to 10–15 mL/min was ~5 years for the DCCT protocol group versus ~40 years for the DCCT + pulsatile infusion group.

Table 4 Pulsatile insulin infusion: impact of ACE inhibition

A. Normal population
1. Average rate of loss of Ccreat ~1 mL/min/year
B. Type 1 diabetic nephropathy patients
1. Prior to 2000 ~15–25 mL/min/year
2. Addition of pulsatile insulin to DCCT protocol
a. No ACE inhibition
i. Control group loss = 5.3 mL/min/year
ii. Infusion group loss = 5.2 mL/min/year
b. With ACE inhibition
i. Control group (n = 25) ~8 ± 1 mL/min/year (52 weeks = 7.1; 78 weeks = 8.9 mL/min/year)
ii. Infusion group (n = 20) ~0.8 ± 0.2 mL/min/year (52 weeks = 0.96; 78 weeks = 0.60 mL/min/year)
iii. *P* values: unpaired *t*-test: 52 weeks <0.11; 78 weeks <0.02
iv. Wilcoxon rank sum test: 52 weeks <0.20; 78 weeks <0.01
C. Mean arterial pressure for patients with highest slope of loss of Ccreat baseline, endpoint
1. DCCT protocol (control group, n = 10) 93.1 ± 2.3, 94.8 ± 3.1 mmHg
2. Pulsatile IV (infusion group, n = 10) 91.8 ± 2.0, 91.3 ± 2.5 mmHg
D. Mean arterial pressure for patients with lowest slope of loss of Ccreat baseline, endpoint
1. DCCT protocol (control group, n = 10) 103.1 ± 3.0, 109.9 ± 2.9 mmHg*
2. Pulsatile IV (infusion group, n = 10) 100.7 ± 2.1, 99.9 ± 2.8 mmHg*

Note: *$P < 0.05$.

Heart studies

The positive rationale for use of insulin therapy in type 2 diabetic patients with normal renal function has recently been reviewed.[20] Results from the United Kingdom Prospective Diabetes Study (UKPDS)[21] found both metformin and insulin to be superior to sulfonylureas in the first 10 years with insulin superior to metformin in the second 10 years, using separate endpoints for death due to myocardial infarction, complications of diabetes mellitus, or a composite of all causes. Microvascular complications were also lowest in the insulin-treated group in the 20-year follow-up.

With left ventricular mass (LVM) increase, a long-term increased risk of heart failure and other morbid cardiovascular events is observed. The prevalence of morbid events also increases as renal function decreases. LVM increases proportionately with pressure/volume increase, particularly if associated with calcification/stenosis of the cardiac valves, or the fluid overload of progressive renal dysfunction. Adverse ventricular remodeling therapy has focused on hemodynamic alteration. In our limited experience, however, lowering of glycated hemoglobin A1c concentration was associated with beneficial ventricular remodeling in diabetic nephropathy patients at equivalent blood pressures, suggesting a metabolic nonhemodynamic mechanism[22] (Table 5). Increased LVM in these patients would be seen as interstitial collagen/fibrosis deposition superimposed on myocyte hypertrophy.[23] Reversal of diabetic cardiomyopathy (a definition restricted to individuals without coronary obstructive disease) was observed after successful renal transplantation.[24] Elimination of fiber stretch may be a signal for downregulation of local angiotensin elaboration in the left ventricle, a subject of much research interest in both heart and kidney protocols.

A study of glycemia control in type 1 diabetic patients with nephropathy involved 23 patients with cardiac autonomic neuropathy (CAN). Of these, 10 were classified as early autonomic neuropathy and 13 as advanced CAN. None of the patients were receiving β-blocker medications. Glycohemoglobin fell significantly at 3, 6, and 12 months in the early CAN group, but only at 6 months for advanced CAN. There were no changes in heart rate variability (HRV) for the advanced CAN group on 24-h ambulatory EKG over the course of the study. There were statistically significant increases in several tests within the time and frequency domains for HRV, suggesting an improvement in parasympathetic function over the course of 1 year in the early CAN group.[25] Most of the patients were receiving ACE inhibitors. In this same study, an improvement in glycohemoglobin A1c was associated with a significant reduction in LVM.[22]

Table 5 Pulsatile insulin study: cardiac and autonomic neuropathic studies

A. Objective measures of autonomic nervous system function
1. Heart rate variability (HRV) not different for DCCT protocol (control group at Joslin) versus pulsatile insulin (infusion group at Joslin)
2. Combining study groups at Joslin
a. Patients with early cardiac autonomic neuropathy: Significant fall in glycohemoglobin A1c at 3, 6, and 12 months Several measures in the time and frequency domains indicated improved parasympathetic function[25]
b. Patients with advanced cardiac autonomic neuropathy: significant fall in A1c at 6 months only. No measures of HRV changed significantly for the better, indicating no improvement in parasympathetic function
c. Patient subgroup with a significant decrease in A1c had a significant reduction of left ventricular mass (LVM) on echocardiogram.[22] Patients without a significant improvement in A1c did not have a significant lowering of LVM
d. There was a significant statistical relationship between coefficient of variation of the RR interval (CVNN) and LVM[26]
B. Subjective response to questionnaire[27]
1. Peripheral nerves
a. Feet (numbness, tingling, burning, and other pain)
b. Eye (visual blurring)
c. Genital (sexual function)
2. Autonomic nervous system
a. Gastrointestinal (diarrhea)
b. Postural hypotension (imbalance)
3. Positive responses in questions relating to nerve function correlated highly with positive responses in preservation of Ccreat

Patients who did not achieve a significant improvement in A1c% also did not have a significant loss of LVM despite similar blood pressure control. Variation of HRV by the coefficient of variation of the RR interval (CVNN) test was related to loss of excess LVM.[26] A1c was related to both autonomic nerve function and LVM, so finding an association between autonomic nerve function and LVM change was expected (Table 5).

A questionnaire was used to determine subjective neurologic endpoints in the study groups. Patients who experienced relative stability of Ccreat reported significantly fewer problems with visual blurring, postural imbalance, intestinal disturbance (diarrhea), sexual dysfunction, and peripheral neuropathic sensations (numbness, tingling, burning, and other pain).[27] Although AGE may interfere with recovery from objective sensory neuropathy,[28] improvement in plasma concentration did not discriminate those patients who had subjective neurological benefits from those who did not.[27] Intermittent visual blurring may be due to dysfunction of corneal nerves that can now be studied with confocal microscopy.[29] Use of this technology has documented early regeneration of corneal nerves following pancreas transplantation.[30]

Eye studies

Diabetic Retinopathy Control Trial-Prevention (DIRECT-Prevent 1) and Diabetic Retinopathy Control Trial-Protection (DIRECT-Protect 1) studies are two randomized, double-shielded, parallel-design, placebo-controlled trials that studied the effect of the ARB candesartan in normotensive, normoalbuminuric type 1 diabetic patients without or with retinopathy, respectively. In DIRECT-Prevent 1, during 4.7 years, the incidence of new retinopathy was significantly lower in the candesartan group.[31] However, among patients with existing retinopathy (DIRECT-Protect 1), candesartan did not have a beneficial effect on progression,[31] unlike results in the EURODIAB-controlled trial of lisinopril in insulin-dependent diabetes (EUCLID) in which lisinopril decreased retinopathy progression in nonhypertensive patients who had type 1 diabetes with little or no nephropathy.[32] With regard to type 2 diabetic patients with mild to moderate retinopathy studied in the DIRECT-Protect 2 trial, candesartan significantly promoted the regression of retinopathy,[33] as opposed to findings in the ADVANCE study in which perindopril–indapamide-based blood pressure lowering or intensive glucose control did not significantly reduce the incidence and progression of retinopathy.[34] Although most studies demonstrate the prevention of the new appearance of retinopathy in type 1 diabetic patients treated with angiotensin-active medications, there is major inconsistency in findings of protection from retinopathy progression for both type 1 and type 2 diabetic patients treated with angiotensin-active medications.

ACE inhibition and blockade of angiotensin receptor have been studied in multiple centers with an emphasis on small vessel complications of retinal and renal circulations. The effect of blood pressure lowering was demonstrated in the UKPDS study in which there was not much difference in retinopathy outcomes between an ACE inhibitor (captopril) and a β-blocker (atenolol), as long as similar blood pressure control was obtained (average attained BP 144/82, mean pressure 108 mmHg), suggesting that the origin of retinal benefits in newly diagnosed type 2 diabetic patients was hemodynamic.[35] When compared with placebo, progression of retinopathy in the RAAS study was reduced by 65% with enalapril and by 70% with losartan. Blood pressures were significantly lower in the enalapril and losartan groups when compared with placebo.[9] Confirmation of the relationship between microvascular complications,

markers of inflammation, and glycemia control can now be achieved through the use of computer-assisted intravitreal microscopy.[36] Future studies with technology that is capable of quantifying macular edema[37] by optical coherence tomography and retinal neurodegeneration[38] may be useful in the evaluation of treatments.

Use of the standard grading system established by the Early Treatment Diabetic Retinopathy Study (ETDRS)[39] at the central reading center (University of Wisconsin, Madison, WI, USA) provided an adequate measure of objectivity in evaluating a subset (n = 57) of the Pulsatile Intravenous Insulin Treatment Study.[17] There were no statistically significant differences in the two insulin study groups with respect to changes in retinal grading, despite significant differences in objective renal and subjective neurologic function over the course of the study.[40] Baseline blood pressure, glycohemoglobin A1c, and use of ACE inhibitors were not statistically significantly different. This study was not powered to define an effect of pulsatile intravenous insulin on the progression of retinopathy. In this small study, when aggressive lowering of glycemia in the total group was analyzed, it appeared that progression of retinopathy was associated with a higher degree of variability of A1c despite lower levels of A1c, although not meeting statistical significance.[41] An effect of high degrees of A1c variability has been reported to adversely affect incidence and progression of nephropathy/retinopathy in type 1 diabetes;[42,43] however, in both instances, variability was highest when A1c was highest.

Research studies

Research studies in humans indicate that the protective effects on microvasculature from the ARB losartan are related to its minor metabolite, which does not block the angiotensin receptor. Instead, EXP3179 has anti-inflammatory, antiplatelet aggregation and antioxidant properties not found in other ACE inhibitors or ARBs. In animal models, angiotensin receptor blockade and ACE inhibition have been found to have antifibrotic effects, related in part to their effect on profibrotic local RAAS in the kidney, heart, and eyes.

Human studies

A pathogenetic role of angiotensin 2 (AT2) in microvascular complications in which blood pressure was not the central issue came from a study of 13 type 2 diabetic patients treated in a randomized fashion with either atenolol or losartan.[44] Flow-mediated dilation (endothelial-dependent) via hyperemia increased significantly following losartan compared with atenolol ($P = 0.01$) despite similar blood pressure control. Levels of 8-isoprostane, a marker for oxidative stress, did not change with atenolol but decreased significantly with losartan. The rise in flow-mediated dilation versus the fall in concentration of 8-isoprostane was statistically significant (Table 6).

Losartan, an angiotensin receptor 1 (AT1) blocker, functions through its major metabolite, known as EXP3174. Its minor metabolite, EXP3179, has important physiologic effects not involving blockade of the AT1 receptor. In a study involving 28 subjects with normal renal function, losartan treatment was associated with a significant decrease in platelet aggregation and concentrations of prostaglandin F2-α (PGF2α). The same researchers treated human endothelial cells ex vivo with EXP3179, which has molecular homology with indomethacin, prior to treatment with the proinflammatory agents (AT2, lipopolysaccharide), demonstrating inhibition of cyclooxygenase and arachidonic acid stimulation of platelet aggregation.[45] Mononuclear cells from hypertensive subjects (n = 153) that were evaluated ex vivo through the use of EXP3179[46] demonstrated inhibition of superoxide generation by nicotine adenine dinucleotide phosphate (NADPH) oxidase. Levels of plasma matrix metalloproteinase were also suppressed. This antioxidant response could not be demonstrated with an ACE inhibitor (quinapril), AT1 blockers (irbesartan and losartan), or EXP3174 (Table 6).

Reported functions of EXP3179 include cyclooxygenase blockade (anti-inflammation), nitric oxide synthase stimulation (vasodilation), tumor necrosis factor inhibition (antiapoptosis), peroxisome proliferator-activated agonist response (protects proximal tubule from toxicity of fatty acids in nephrotic syndrome), and decreased platelet aggregation by collagen inhibition (diminishes thrombosis).

Animal models

Fibrosis of the kidney and heart has been studied in experimental animals with or without diabetes. Anti-angiotensin–aldosterone treatments have been found to interrupt fibrosis. In some instances, the impact occurred without change in blood pressure. The diabetic Akita mouse model was used to test ACE2, a homolog for ACE, and was found to be associated with a decrease in AT2 levels in the plasma and renal cortex.[47] Urinary albumin and 24-h albumin excretion were significantly lower compared with untreated controls. Glomerular hypertrophy, basement membrane thickening, and mesangial matrix expansion with collagen and smooth muscle actin were significantly less prominent than observed in controls. Renal cortical expression of nicotine adenine dinucleotide (NAD) phosphate oxidase,

nitric oxide synthase oxidase, and protein kinase C was significantly lower in treated animals. These findings provide evidence that blockade of signals of inflammation and oxidative stress diminished renal interstitial and mesangial fibrosis in the Akita diabetic mouse (Tables 6 and 7).

Demonstration of the effect of AT2 on the cardiac fibroblast was undertaken in a newborn Wistar rat model (nondiabetic). Genes for fibronectin and collagen increased within 45 min, but TGF as a marker for extracellular matrix remodeling did not rise until after 60 min

Table 6 Treatment of angiotensin signaling in microangiopathic remodeling

	Inflammation	Hemostasis	Oxidative stress	Vasodilation	Angiogenesis	Fibrosis
A. Endothelial function						
1. Human study						
a. Losartan: Flammer et al[44]		+	+	+		
Type 2 diabetes						
b. Losartan: Kramer et al[45]		+				
No diabetes						
c. EXP3179: Fortuno et al[46]			+			
No diabetes						
2. Animal study						
a. EXP3179: Watanabe et al[63]				+	+	
No diabetes						
b. Valsartan: Michel et al[64]					+	
Spironolactone					+	
No diabetes						
B. Retinal function						
1. Animal study						
a. Candesartan: Kim et al[60]			+		+	
b. Fosinopril: Zheng et al[58]					+	
c. Enalapril: Kim et al[57]					+	
d. Candesartan: Fukumoto et al[59]	+		+		+	
e. Valsartan: Wilkinson-Berka et al[61]					+	
Spironolactone	+				+	
C. Renal function						
1. Human study						
a. Irbesartan: Persson et al[10]	+					
b. Protein kinase C inhibitors:						
Gruden et al[56]					+	
Tyrosine kinase inhibitors						
Human mesangial cells: no diabetes						
c. Enalapril, losartan, and spironolactone:						
Furumatsu et al[11]						+
Nondiabetic						
2. Animal study						
a. ACE2: Qudit et al[47]	+		+			+
Akita diabetic mouse						
b. Quinapril: Blanco et al[83]	+					+
Zucker obese rat						
D. Cardiac function						
1. Animal study						
a. Enalapril: Ma et al[49]	+	+				
Losartan	+	+				
Sprague–Dawley rat						
b. Trandolapril: Onozato et al[50]			+			+
Eplerenone			+			+
Dahl salt-sensitive rat						
c. Quinapril: Nemeth et al[51]						+
Spironolactone						+

Note: + indicates a favorable response in returning marker toward control level.

Table 7 Studies that specifically mention that blood pressure was not changed or in which there was no difference between study groups when anti-angiotensin treatments reversed mechanisms of diabetic microvascular complications

A. Alloxan diabetic dog
1. Avendano et al:[55] both aminoguanidine and enalapril prevented ventricular stiffness associated with pathologic glycation of collagen over 6 months. There were no significant differences in aortic pressure, ejection fraction, or heart rate compared with controls despite the increased pressure/volume relationship.
The concentration of ventricular collagen increased in the alloxan diabetic animals whether they were treated with enalapril or aminoguanidine or not treated
B. Akita diabetic mouse
1. Qudit et al:[47] human recombinant angiotensin-converting enzyme 2 (ACE2), carboxypeptidase that transforms AT2 into A 1–7 without changing the 'slightly elevated' blood pressure of this animal, which has high blood glucose
2. Factors that did change after 4 weeks of treatment included improvement in increased
a. Protein kinase C
b. Nitric oxide synthase oxidase
c. NADPH oxidase
d. Albuminuria
e. Thickening of glomerular basement membrane
f. Enlargement of glomerular mesangium
g. Genes for collagen and actin
C. Zucker obese rat
1. Blanco et al[83]
2. Quinapril
3. Endpoint
a. Proteinuria
b. Glomerular histology (nephrosclerosis)
c. Interstitial histology (infiltrate)

of AT2 superimposed upon hypoxia.[48] The investigators concluded that the combined influence of AT2 and hypoxia may promote remodeling of myocardial interstitial matrix even in the absence of diabetes.

Three additional animal models for fibrosis (glomerulosclerosis) have concentrated on relevant mechanisms although are unable to eliminate hypertension as a factor (Table 6). Two of the studies used 5/6 nephrectomy in the rat, and one employed the Dahl salt-sensitive hypertensive rat. Mechanisms for fibrosis included increased plasminogen activator inhibitor,[49] increased TGF-β (TGFB) + NADPH oxidase,[50] and TGFB + collagen type 4.[51] In these three animal models, treatment included enalapril, losartan, or both;[49] trandolapril, eplerenone, or both;[48] and quinapril, spironolactone, or both.[51] Glomerulosclerosis was reduced toward baseline with all of these therapies along with reversal of increased profibrosis mechanisms. Such studies are encouraging in that use of anti-angiotensin–aldosterone medications may prevent irreversible remodeling of the left ventricle. Chronic kidney disease models associated with deficiency of vitamin D[52] or vitamin D receptor[53] demonstrate increased interstitial inflammation/fibrosis of the kidney[52,53] or heart.[54] Either losartan[53] or 1,25-dihydroxyvitamin D[54] may impede interstitial fibrosis of the kidney[53] or heart[54] in chronic kidney disease models.

When alloxan diabetic dogs were observed for 6 months, with no significant differences in aortic pressure, heart rate, or ejection fraction compared with normal controls, an increase in myocardial collagen was found (Tables 7). This collagen was linked to AGE and associated with decreased end-diastolic volume and increased end-diastolic pressure (and could be prevented by either aminoguanidine or enalapril), again suggesting a biochemical nonhemodynamic target.[55]

A local RAAS exists in both the kidney and heart. Tension to the glomerular mesangium increases expression of protein kinase C and vascular endothelial growth factor (VEGF), resulting in fibrosis of the kidney,[57] eye, and heart.[23]

Mechanisms of microangiopathic remodeling as they relate to AT2 signaling in the retina are summarized in Table 6. A combination of reverse transcriptase polymerase chain reaction, immunohistochemistry, and Western blotting demonstrated increased expression of VEGF in the retina of streptozotocin diabetic (STZ) rats. Eight weeks of treatment with enalapril (10 mg/kg) prevented an increase in retinal VEGF expression.[56] Increased VEGF expression both in the retina of STZ rats and in bovine retinal endothelial cells (BREC) exposed to hyperglycemia has also been demonstrated. This increased VEGF expression was attenuated by an ACE inhibitor that is associated with a diminution in histologic markers for retinal vascular damage.[58] This study showed the protective effect of perindopril on mitochondrial dysfunction-induced reactive oxygen species (ROS).[57] The Tori rat, a model of spontaneous type 2 diabetes, was used to demonstrate that retinal expression of genes for both VEGF and NADPH oxidase were promoted by AT2, which was reduced by candesartan.[59] This RAAS-ROS connection via AT2 infusion was associated with increased expression of VEGF and a subunit of the ROS-generating NADPH oxidase;[59] these increases in expression (Table 6). were attenuated by treatment with the ARB candesartan. It was found that retinopathy in the STZ diabetic animal model was associated with vascular leakage and increased VEGF expression (Table 6). Treatment with perindopril was able to reverse all of these abnormalities and restore the integrity of tight junction proteins that occlude the spaces between cells.[60]

An RAAS process was associated with inflammation, angiogenesis, and enhanced expression of NADPH oxidase in oxygen-induced retinopathy. Spironolactone and valsartan both prevented inflammation and angiogenesis.[61] Because aldosterone inhibits nitric oxide synthase, spironolactone may have been helpful in promoting the generation of nitric oxide.[62]

The post-insulin receptor signal is conveyed to mitochondria via phosphoinositol 3-phosphate (PI3p), Akt, and adenosine monophosphate (AMP) with inhibition by AT2. This PI3p/Akt signal system operates in bovine aortic endothelial cells. Studies using losartan have shown that metabolite EXP3179 inhibits the angiotensin response, protects the receptor for VEGF, and therefore diminishes cellular death through apoptosis.[63] In the mouse limb ischemic model, induction of neovascularization and VEGF protein are demonstrated in aldosterone-treated animals and inhibited equally by spironolactone, valsartan, and antibody to VEGF[64] (Table 6). Because aldosterone is instrumental in neovascularization of both the ischemic limb and the retina exposed to excess oxygen, the interactions of angiotensin/aldosterone, VEGF, and the insulin signal require further elucidation. Further research could be done on whether the new class of renin inhibitors might also exhibit nonhemodynamic synergy with pulsatile insulin release with microvascular benefits.[65]

Type 2 diabetes: glomerular podocyte and pancreatic β-cell undergo similar dysfunction

Ultrastructural studies of the podocyte and pancreatic β-cell reveal similarities in cytoskeletal proteins, such as nephrin, that are related to cell trafficking as well as to leveraging proteins in cardiac myocytes. Inflammatory effects of obesity interfere with efficient functioning of those proteins and thus interfere with the podocyte slit diaphragm and insulin release. Type 2 diabetes is characterized by increased size and disordering of the amplitude of pulsatile insulin release from β-cells and eventually by decreased size of the pulse, and may be related to a local angiotensin–aldosterone effect on somatostatin. The interruption of adequate delivery of glucose to the medullary thick ascending limb leads to injury from excess angiotensin–aldosterone. Future research may show that correction of excess angiotensin–aldosterone may help to reverse these effects.

Developments in researching the ultrastructure of the kidney epithelial cell podocyte, the pancreatic islet β-cell, and certain muscular structures have identified similar proteins through which the cytoskeleton provides leverage for timely functions. Resisting the excretion of albumin, enhancing the secretion of insulin, or relaxing a muscle appropriately after contraction requires proteins, some of which are structurally similar, that can function through attachment to the cytoskeletal protein actin. Electron microscopy of the β-cell has identified an actin latticework that is available for attachment.[66]

The cytoskeleton is no longer viewed as a capsule to prevent entry of bacteria and viruses. Actin filaments form polymers that have been described as a polygon on electron microscopy.[67] Much is known about the β-cell, which mainly employs nephrin and syntaxin interaction with actin. The podocyte utilizes large numbers of cooperating proteins, among which are nephrin, NCK protein, podocin, and synaptopodin. When the extracellular portion of nephrin engages neighboring foot processes,[68] the intracellular portion engages actin to promote leverage (Table 8).

In both the podocyte and the β-cell, coordinated function is most critical after the intake of food. Efficient function of the nephron and the islets of Langerhans requires opening and closing of transit pathways. The β-cell has a cytoplasmic latticework[66] 'cell web' that conducts vesicles of insulin to the outer cell membrane. The podocyte has efficient glucose absorption once the glucose transport (GLUT) apparatus has been conducted to the outer cell membrane. Both podocytes and β-cells have a vesicular-binding protein, VAMP (vesicle-associated membrane protein), that associates with moving components and then separates as movement ends with docking at the plasma membrane. In the podocyte, nephrin and VAMP associate with GLUT protein vesicles that are then able to move to the plasma membrane with a signal from insulin.[69] In the β-cell, nephrin and VAMP associate with insulin vesicles[70] that are then able to move to the plasma membrane with the help of syntaxin[71] and a signal from glucose (Table 8).

The effects of diabetes or obesity may inhibit the impact of nephrin and adiponectin. AGEs impede podocyte function by cross-linking of filaments of actin and associated leverage proteins, making it difficult to keep the foot processes of adjacent podocytes aligned. The receptor for AGE is colocalized with synaptopodin, one of the leverage proteins responsible for the retention of albumin.[72] AGEs also accelerate apoptosis of β-cells and podocytes by elaboration of an apoptosis effector protein.[73]

Podocyte chemistry has remote relationships to cardiac physiology. Nephrin is coexpressed with myocardin, a protein of both cardiac and smooth muscle origin. Other podocyte proteins (smoothelin and calponin) relate strictly to a smooth muscle origin. Through smoothelin, the podocyte responds to AT2 by contraction in an actin-dependent manner[74] (Table 8). Insulin resistance is associated with

Table 8 Nephrin functions in several tissues (kidney, pancreas, and possibly central nervous system)

Podocyte of kidney	Pancreas β-cells
Anchors adjacent foot processes by extracellular domain through attachment to actin cytoskeleton by its intracellular domain[68]	Anchors intracellular filaments of actin cytoskeleton[66,67]
Requires assistance of other proteins (NCK, podocin, and syn protein family). Receptor for advanced glycated end products colocated with synaptopodin[72]	Requires assistance of syntaxin[71] intracellularly in a way more closely cooperative than the functions of podocin and synaptopodin, where the leverage effect occurs extracellularly
Cooperates in translocation of glucose transporter protein vesicles across cytoplasm to dock at plasma membrane.[69] Accompanied by VAMP, a protein that moves with other proteins that have taken the form of vesicles (granules). Process is critical for intake of fuel; insulin and adiponectin have a signaling role	Promotes translocation of insulin vesicles across cytoplasmic 'cell web' to dock at plasma membrane. Accompanied by VAMP. Process critical for timely insulin secretion[70]
Inhibits nuclear factor-κβ (NF-κβ).[82] Animal model of mutant nephrin[79,80] adiponectin knockout[81] have elevated NF-κβ, proteinuria, glomerular pathology corrected with replacement of nephrin	AGEs cause apoptosis. AGEs also can cross-link actin filament to alter the timing and amplitude of insulin secretion[73]
Coexpressed with myocardin, a protein of both cardiac and smooth muscle origin. Other podocyte proteins (smoothelin and calponin) relate strictly to a smooth muscle origin. Through smoothelin, podocyte responds to angiotensin 2 by contraction in actin-dependent manner.[74] Podocytes have similarities to pericytes of the central nervous system and retina	Angiotensin 2 provokes apoptosis through NADPH oxidase; prevented by telmisartan[75]

inhibition of AMP kinase through increased protein concentration of NOX4, an oxidase of NAD. Angiotensin-induced oxidative stress in heart failure may respond to the use of anti-angiotensin medications, such as receptor blockers. Telmisartan inhibits the oxidase of NADPH and promotes AMP kinase, which leads to more efficient generation of adenosine triphosphate (ATP). This same sequence occurs in the β-cell, which would otherwise experience a reduction in glucose-stimulated insulin secretion,[75] and might also be at risk for apoptosis.[76] Metformin enhances AMP kinase, which is protective in experimental models of β-cell apoptosis[77] and heart failure.[78]

Nephrin suppresses the inflammation cascade by inhibiting nuclear factor-κβ (NF-κβ), as does adiponectin, the adipokine most active in promoting the postreceptor insulin signal. Animal models of mutant nephrin[79,80] or adiponectin knockout[81] demonstrate that proteinuria is found to be reversible with restoration of functional nephrin or adiponectin (Table 8). Adiponectin inhibits NF-κβ through a cyclic AMP-dependent pathway,[82] the last major point in the insulin signal before activation of mitochondrial glucose oxidation[80] (Table 8). The obese diabetic Zucker rat develops glomerulosclerosis and interstitial infiltrate. Glomerular nephrin is depleted. Quinapril, but not diltiazem, reversed all of these abnormalities without a change in blood pressure[83] (Table 6).

Insulinization utilizing pumps has been an immense step forward in glycemia control. Nevertheless, there is no method of physiological infusion of insulin in routine use. Insulin is secreted in pulses every 10 ± 2 min. It has been considered that the first phase of insulin secretion is the release of older granules that are already docked at the plasma membrane. The second phase of insulin secretion involves younger insulin vesicles bound to their intracytoplasmic protein carriers (nephrin and syntaxin) co-operating in propulsion through the transit pathway toward the plasma membrane (Table 8). Recent cellular research has suggested that under certain experimental conditions, the younger insulin granules from healthy human β-cells may be the first to be released in response to glucose.[84] Both oral intake and intravenous infusion of 30 g of glucose increases the rate of pulsation by 40%, but the incretin effect with oral intake results in a 70% greater mass of insulin secreted in these same pulsations.[85]

The onset of type 2 diabetes is characterized by increased amplitude and disordering of the rhythm of insulin pulses. In later stages, as the size of the pulse decreases, the patient becomes insulin dependent. Several drugs increase the amplitude but do not change the rhythm of pulses: sulfonylurea,[86] glucagon-like peptide,[87] and sodium salicylate.[88] Decreases in pulse amplitude of insulin secretion may occur as a result of catechol stimulation. A link between type 2 diabetes and pheochromocytoma has been described in patients with a genetic mutation, resulting in an increased number of α-2 adrenoreceptors with a decreased number of insulin granules docked at the β-cell plasma membrane.[89] This could explain the observation of hyperglycemia during increased catecholamine expression as being related to increased glycogenolysis and decreased secretion of insulin pulses.[90]

Metabolic efficiency is improved by pulsatile hormonal delivery when compared with steady infusion[91,92] (Table 9). The islets of Langerhans secrete glucagon,[91] insulin,[92] and somatostatin[91] in a pulsatile fashion from the α-, β-, and γ-cells. There is general agreement that somatostatin acts as a governor of these cells through receptors on α- and β-cells,[93] preventing overwork to the point of exhaustion. β-cells also have local AT2-generating systems[94] through angiotensin-converting enzyme with receptors (AT1 and AT2) in place. Action on the β-cell angiotensin axis can be expected to affect metabolism of somatostatin, insulin, and glucagon. Studies of insulin secretion following infusion of AT2 in healthy volunteers have shown that pressor doses diminished levels of basal and glucose-stimulated oscillations. The regularity of insulin oscillation was not changed by AT2 infusion.[95] We suspect that future studies will demonstrate that inhibition of excess angiotensin–aldosterone has widespread benefits in hormonal balance that may not be corrected simply with physiological insulinization.

Preservation of function in chronic glomerular disorders depends on remodeling of the interstitium. Disorders of blood supply or oxygen transfer, such as diabetes or sickle cell anemia, are associated with medullary papillary necrosis while the cortex is spared. The cortex can utilize fuels generated under stress,[96,97] including ketones (β-hydroxybutyrate), fatty acids (palmitate), glycerol, triglycerides, glutamine, lactate, mannose, and fructose.[97]

Medullary energy production depends on anaerobic glycolysis in the thick ascending limb of the distal tubule where mitochondria exists. Energy for sodium/chloride transport against an interstitial gradient for water resorption in the collecting ducts depletes medullary reserves.[98] As the renal medulla operates at lower levels of oxygen concentration/consumption,[99,100] hematocrit,[101] and blood flow[101] than the cortex, pulsatile insulin delivery may be critical. These observations pinpoint the renal medullary interstitium as a susceptible focus of injury from excess angiotensin–aldosterone when imperfect insulin supply occurs.

Individuals with type 1 diabetes are not exempt from the growing epidemic of obesity, which would be expected to add insulin resistance to insulin deficiency. In type 1 diabetic nephropathy patients treated with pulsatile insulin infusions, we have observed an increase in respiratory quotient (RQ) in every instance, indicating a direction of fuel oxidation away from β-oxidation of fatty acids to aerobic oxidation of glucose in mitochondria.[102] In several patients, transitory elevations of RQ to >1.0 may have been the result of excess fuel with synthesis of fatty acid, leading to deposition of triglyceride, because diacylglycerol is also an endpoint of insulin action. Deposition of triglyceride may occur in the liver, adipose, muscle (skeletal and cardiac), and pancreas (β-cell). We have no experience with type 2 diabetic nephropathy patients undergoing experimental use of pulsatile insulin infusion.

From these biological observations it can be speculated that glomerular and β-cells are injured in similar ways by mechanisms seen in uncontrolled hyperglycemia. Therefore, treatments that eliminate these injury patterns may be expected to preserve the function of both the nephron and the islets of Langerhans. The function of the myocardium tends to improve in situations that are favorable to either the pancreas or the kidney. When the function of the myocardium has been improved, there is improvement in renal function.

Table 9 Pulsatile insulin secretion

A. Insulin secretion
1. Insulin is secreted ~10 times/h[85]
2. Hormones secreted in oscillations are more efficient than when equimolar amounts are tested by continuous infusion[91–93]
B. Islets of Langerhans
1. Glucagon and somatostatin secreted together at the same pace[93]
2. Insulin has a different cadence
C. Type 2 diabetes
1. Rhythm of insulin secretion disordered
2. Amplitude increased at first, then after several years begins to decrease due to AT2 generated in islets[94]
D. Drugs that increase amplitude, but do not change rate of oscillations
1. Sulfonylurea[86]
2. Glucagon-like peptide[87]
3. Sodium salicylate[88]
E. Drugs that decrease amplitude, but do not change secretion rate
1. Thiazide diuretic[88]
2. α-Adrenergic agonist[89,90]

Future potential interventions to control cellular metabolic derangement

Studies of the use of TNF-α inhibitors in rheumatologic disease suggest clinical (reduction of cardiac events) and laboratory evidence (improved HDL antioxidant capacity and endothelial responsiveness) for cardiovascular benefit.[103] A role of both TNF-α and IL-1 has been demonstrated in postinfarction human heart failure and isolated rat cardiac fibroblast. In both instances the process is augmented by angiotensin.[104–106]

Demonstration that RAAS activation, mechanical stretch, and myocardial injury stimulate production of TNF-α,[107] hydroxyl radicals, IL-1β, and NF-κβ has led to studies that

demonstrate the possibility that the benefit of medications such as carvedilol[108,109] and statins[110] may be related not only to their effect on heart rate, blood pressure, and lipids but also to regulation of oxidative stress. Animal studies support TNF-α inhibition as a therapy for the reduction of oxidative stress, myocardial mitochondrial dysfunction, and apoptosis.[111,112] Laboratory and clinical evidence additionally links TNF-α and oxidative stress to postmyocardial infarction progression to heart failure.[113] Thiazolidinediones may enhance TNF-α induction of IL-1, and this may explain problems within this class of drugs.[104]

There are no clinical outcome studies involving inhibition of TNF-α in patients with diabetic nephropathy. The effect of both AT2[114] and TNF-α[115] on sodium potassium ATPase of the medullary thick ascending limb of the loop of Henle is to inhibit resorption of sodium and chloride. We have referred to this site in the renal medulla, which is critical for water preservation, as a potential site for injury in uncontrolled glycemia with insulin deficiency/resistance.[98] The oxidative stress that attacks the most vulnerable position in the renal medulla, where energy is required for transport of sodium chloride, has been shown to be overcome by candesartan/valsartan in type 2 diabetes.[116] Because this therapeutic process was associated with inhibition of expression of IL-6, there is a suggestion that future studies should involve a combination of therapy of ARBs with inhibitors of IL or TNF-α. A note of caution is required, however, due to reports of the development of immune glomerulonephritis in rheumatoid arthritis patients treated with etanercept, adalimumab, or infliximab.[117]

The primary focus for the preservation of cardiovascular, renal, and retinal integrity in diabetes has been related to RAAS activation, blood pressure, and glycemic control. Recent studies have demonstrated relationships between these factors and cellular signaling that may be additional targets for intervention. The goal is to alter rates of programmed cell death (apoptosis), oxidative stress, thrombosis, inflammation, and fibrosis. Given the impact of successes in the rheumatologic therapies, future research using these additional cellular targets may be worthwhile.

Conclusion

We have reviewed angiotensin–aldosterone mechanisms of tissue injury that magnify microvascular damage caused by hyperglycemia and hypertension. Many studies describe interruption of these mechanisms, without hemodynamic consequence, in the preservation of function in type 1 diabetes. Possible interactions between the RAAS and physiologic glycemic control (through pulsatile insulin release) suggest opportunities for clinical research. Until we have better markers of risk for microvascular complications, therapy must be directed at minimizing variability of hemoglobin A1c.[118]

The results of recent outcome trials challenge hypotheses that tight control of both glycohemoglobin and blood pressure diminishes macrovascular events and survival among type 2 diabetic patients. These results raise relevant questions. Is glycohemoglobin an adequate measure of diabetes control? Are we ignoring mechanisms of vasculotoxicity (profibrosis, altered angiogenesis, hypertrophy, hyperplasia, and endothelial injury) inherent in current antihyperglycemic medications? Is the polypharmacy for lowering cholesterol, triglyceride, glucose, and systolic blood pressure producing drug interactions that are too complex to be clinically identified? Answers to these questions will most certainly improve our understanding of disease mechanisms and further refine therapies.

On the basis of our review of the literature, we suggest that these nonhemodynamic effects on renal, cardiac, and ocular microvasculature are as important, if not more so, as the hemodynamic effects of ACE inhibition and AT2 receptor blockade. Although the macrovascular effects of uncontrolled hypertension and diabetes are clear, we are only now beginning to understand the microvascular complications. Answers to these questions will most certainly improve our understanding of disease mechanisms and allows us to further refine therapies, such as the interruption of local RAAS and use of pulsatile insulin to reduce proinflammatory and profibrotic forces. These therapies, in turn, will help to improve the lives of our patients.

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Disclosure

The authors report no conflicts of interest in this work.

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