Editorial

Platelets, Antiplatelet Therapy, and Diabetic Nephropathy

Chronic renal insufficiency develops in approximately 40% of patients with type 1 insulin-dependent diabetes mellitus.[1] Nephropathy appears from 15 to 30 years after the onset of diabetes, is signaled by dipstick-positive proteinuria, and is commonly antedated by diabetic angiopathy, especially diabetic retinopathy.[1] After the appearance of proteinuria, renal function progressively deteriorates, and within 5 years (as indicated by earlier studies[2]) or 10 years (as cited in a more recent publication[3]), end-stage diabetic renal disease is reached. During the course of diabetic nephropathy, patients exhibit unremitting proteinuria that frequently is in the nephrotic range, are commonly hypertensive, and are subject to a variety of symptoms that arise from such extrarenal complications of diabetes as retinopathy, neuropathy, gastrointestinal autonomic dysfunction, and coronary insufficiency.[1-3] The added burden of renal insufficiency and end-stage renal disease contributes substantially to morbidity and mortality in patients with diabetes mellitus.[4]

In an effort to retard the progressive decline in renal function and to delay the onset of end-stage renal disease, several therapeutic approaches have been proposed in recent years. Effective control of even mild to moderate hypertension in patients with diabetic nephropathy leads to diminished rates of protein excretion and a decreased rate of loss of renal function.[5] For example, in a study by Parving and associates,[5] reduction of mean arterial pressure from 144/98 mm Hg to 128/84 mm Hg with regimens that consisted of β-blockers, hydralazine, and diuretics reduced the rate of decline of the glomerular filtration rate from 0.91 to 0.39 mm/min per month and delayed the estimated onset of end-stage renal disease for several years. Converting enzyme inhibitors may exert a protective effect in diabetic nephropathy by mechanisms that are additional to the antihypertensive effect of these agents. Administration of captopril for several weeks to patients with diabetes who have advanced nephropathy and heavy proteinuria decreases rates of protein excretion before an effect of captopril on systemic arterial pressure is observed.[6] Treatment with captopril extended over 2 years diminishes rates of decline of renal function from 10.3 to 2.4 ml/min per year while reducing mean arterial pressure by only 5 mm Hg.[7] Restriction of dietary protein may also prove to be an effective means of attenuating the loss of renal function in patients with diabetic nephropathy.[8] In seven patients who were maintained on a 40-g high biologic value protein diet and an effective antihypertensive regimen for a 12-month period, glomerular filtration rate, as measured by creatinine clearance, was unchanged whereas proteinuria was dramatically reduced.[8] Even short-term restriction of dietary protein for 10 days confers beneficial effects on patients with diabetic glomerular injury, as indicated by an amelioration in the glomerular size selective defect with concomitant reduction in rates of protein excretion.[9]

The study by Donadio and associates reported in this issue of the *Proceedings* (pages 3 to 15) introduces another therapeutic approach to retard the progression of diabetic nephropathy. These investigators report that in a selected population of 28 patients with diabetes and established nephropathy, maintained for several years on an antiplatelet regimen that consisted of dipyridamole and aspirin, 7 demonstrated appreciably decreased rates of protein excretion and minimal decline in glomerular filtration rate, whereas the other 21 patients had progressive loss of renal function, including 11 who had end-stage renal disease. Although the glomerular filtration rate before antiplatelet therapy was higher in the patients who failed to exhibit progressive loss of renal function, a substantial fraction of the patients with progressive disease exhibited pretreatment glomerular filtration rates in the range observed in the stable patients. Basal rates of protein excretion, although tending to be lower in the patients responsive to anti-

Address reprint requests to Dr. K. A. Nath, Section of Renal Diseases, Box 736 UMHC, University of Minnesota, 516 Delaware Street S.E., Minneapolis, MN 55455.

platelet therapy, were not significantly different from those in patients with progressive disease. Thus, the beneficial effects of antiplatelet therapy in these seven patients are unlikely to be attributable to less functional impairment before the initiation of therapy. Furthermore, such beneficial effects did not arise from more effective control of systemic hypertension or hyperglycemia because systolic and diastolic blood pressures as well as mean fasting plasma glucose and hemoglobin A_{1c} were not significantly different between the responders and the nonresponders. Finally, the patients who failed to have significant deterioration in renal function with this therapeutic regimen are also unlikely to be that subset of patients with diabetic nephropathy who exhibit relatively slow rates of decline of renal function. First, after institution of this regimen, these patients demonstrated a reduction in the rate of decline of renal function, as measured by the reciprocal of the serum creatinine plot with time. Such a reduction in the slope of the reciprocal creatinine plots rarely occurs spontaneously in the course of diabetic nephropathy. Second, these seven patients also had a reduction in rates of protein excretion to one-third the basal values when maintained on antiplatelet therapy. Thus, the beneficial effect observed in 25% of the patient population of this study, albeit uncontrolled, cannot be attributed to better preserved pretreatment renal function, stricter glycemic or blood pressure control, or spontaneous reduction in the rate of decline of renal function.

The findings in the short-term studies reported by Donadio and colleagues that the regimen of dipyridamole and aspirin led to increased platelet survival and decreased urinary excretion of thromboxanes, an aggregative prostanoid derived partly from platelets, suggest that the efficacy of this regimen may stem from its inhibitory effects on platelet activation. There is a growing consensus that patients with diabetes mellitus have an inordinate activation of platelets,[10] and the attendant release of platelet products exerts damaging effects on vessel walls and microcirculatory beds including those of the kidney.[11-13] Banga and Sixma[13] recently summarized the evidence that suggests that diabetes mellitus is a "hypercoagulable state" with ongoing platelet activation. Platelet survival is decreased, and circulating levels of β-thromboglobulin and platelet cofactor 4, markers of platelet release, are elevated. Imbalances in coagulation factors and fibrinolytic mechanisms predisposing toward clotting have also been noted.[13] Perhaps the most telling observation is the increased sensitivity of platelets to aggregative stimuli.[14] Platelets harvested from patients with diabetes display increased aggregation after exposure to adenine diphosphate, thrombin, and collagen, and such amplified aggregation can be attenuated by inhibitors of cyclo-oxygenase enzyme activities such as aspirin. The propensity for increased platelet aggregation in patients with diabetes is multifactorial in origin, the factors encompassing such mechanisms as increased synthesis of thromboxane by platelets,[15] diminished sensitivity of platelets to the antiaggregative prostanoid, prostaglandin I_2, and diminished production of prostaglandin I_2 by blood vessel walls.[10,13,14] Other abnormalities that suggest perturbed platelet-endothelial interaction and platelet activation include elevated levels of von Willebrand factor and fibronectin,[13] proteins that promote platelet adhesion to the vessel wall. Abnormalities in erythrocytes such as decreased deformability and abnormal rheologic properties have also been implicated in enhanced platelet-endothelial interaction.[13,16]

Ongoing platelet activation and aggregation lead to release of platelet contents. These constituents, summarized in Table 1, exert a range of mitogenic, inflammatory, and vasoactive effects. The pathogenesis of atherosclerosis serves as a paradigm for the participation of platelets and its products in tissue injury.[17,18] Of special interest is the α-granule protein, platelet-derived growth factor (PDGF), a cationic protein of approximately 30,000 molecular weight. This mitogen stimulates the proliferation and migration of smooth muscle cells and fibroblasts and is chemotactic to circulating monocytes. PDGF has been assigned a pivotal role in orchestrating the cellular responses that culminate in the proliferative and degenerative changes seen in the walls of atherosclerotic blood vessels.[18] According to the "response to injury" hypothesis of Ross,[18] endothelial damage and exposure of subendothelial structures incite platelet adherence, activation, and the discharge of platelet contents. PDGF stimulates the proliferation of smooth muscle cells and migration of these cells from the media into the intima, promotes intracellular accumulation of lipid, and stimulates fibroblastic

Table 1.—**Summary of Platelet Contents**

Source	Substance	Biologic actions
α-Granule	Platelet-derived growth factor	Mitogenic, chemotactic, cationic protein, vasoconstrictive, contracts mesangial cells
	Platelet cofactor 4	Cationic protein
Lipid-derived	Thromboxane A_2	Platelet aggregation, vasoconstrictive
	Platelet-activating factor	Platelet activation and aggregation, leukocyte aggregation, chemotactic, increases vascular permeability, vasoactive
Lysosomes	Heparatinase	Enzymic degradation of heparan sulfate
Dense bodies	Adenosine diphosphate	Vasoactive
	5-Hydroxytryptamine	Vasoactive, increases vascular permeability
	Ca^{2+}	

activity and the deposition of collagen. Diabetic macrovascular disease is viewed as essentially a process similar to atherosclerosis in patients without diabetes.[13] Continuous in vivo platelet activation and persistent release of PDGF into the vessel wall, as might be expected in patients with diabetes, may account for the notable propensity for macrovascular disease. Additionally, extracts from platelets of patients with diabetes exert greater mitogenic effects on aortic smooth muscle cells in comparison with an identically prepared extract from normal platelets.[19] Thus, greater availability of PDGF in platelets and also enhanced release of PDGF may underlie the increased incidence of vascular disease in patients with diabetes in comparison with normal subjects.

A similar sequence of events may contribute to the pathogenesis of diabetic nephropathy. Early diabetic kidney lesions consist of basement membrane thickening and mesangial expansion. Mesangial cells are the glomerular analogue of vascular smooth muscle cells in that both cellular types are contractile in nature and mesenchymal in origin. Early mesangial expansion in diabetic kidney disease later evolves to diffuse or nodular diabetic glomerulosclerosis. PDGF is a potent mitogen to the glomerular mesangium. PDGF binds to human mesangial cells in culture and stimulates synthesis of DNA and growth of mesangial cells.[20] Heightened platelet activity may thus provide a trophic stimulus to the mesangial area that promotes mesangial expansion and subsequent sclerotic changes. PDGF is also vasoconstrictive and induces contraction of mesangial cells in culture, a property also shared by thromboxanes.[21] Thus, by influencing the contractile state of the glomerular mesangium, PDGF may decrease the glomerular ultrafiltration coefficient and thus glomerular filtration rate. Finally, PDGF is a cationic protein, exhibiting a positive charge at physiologic pH. Heightened release of PDGF, and other cationic platelet proteins as platelet cofactor 4, may bind to and neutralize a fixed negative charge of the glomerular filtration barrier; thereby, the selectivity of the glomerular charge is impaired and macromolecules can leak into the urinary space. Thus, the effectiveness of antiplatelet therapy in preserving glomerular filtration rate and decreasing rates of protein excretion, at least in some subsets of patients with diabetic nephropathy, could conceivably arise from decreased activation of platelets and attenuation of the effects of PDGF and other platelet contents on mesangial expansion, mesangial contractility, and glomerular selectivity. Interestingly, as demonstrated in previous studies by Donadio and colleagues,[22] antiplatelet therapy also preserves glomerular filtration rate in membranoproliferative glomerulonephritis, a disease that, like diabetic glomerulopathy, exhibits early mesangial expansion.

Platelet activation may also contribute to the microalbuminuria observed in the preclinical phase of diabetic nephropathy.[23] Subjects with diabetes who are dipstick negative on urinalysis but excrete more than 20 μg/min of albumin exhibit so-called microalbuminuria. Thirty such patients were subjected to 16 weeks of treatment with UK-38,485, a specific inhibitor of thromboxane synthetase in platelets and other tissues. Rates of excretion of albumin substantially diminished with such therapy and rebounded to pretreatment levels after discontinuation of use of the drug. No significant change occurred in rates of excretion of albumin in subjects treated with placebo. Reduction in rates of excretion of albumin in conjunction with inhibition of synthesis of thromboxane could not be ascribed to reduction

in systemic arterial pressure, nor were differences in glycosylated hemoglobin or mean blood pressure detected between the two groups. These findings raise the intriguing possibility that microalbuminuria in patients with diabetes is dependent on increased generation of thromboxane, possibly arising from increased platelet activity. The mechanism by which increased synthesis of thromboxane provokes proteinuria is currently unknown, but such a correlation between rates of generation of thromboxane and excretion of protein has been observed in multiple models of experimental kidney disease, including nephrotoxic serum nephritis, minimal-change nephropathy, the remnant kidney model, and murine lupus nephritis.[24] Because a view is emerging that microalbuminuria might predict that subset of patients with diabetes subsequently destined to have overt diabetic nephropathy,[25] inordinate platelet activity may be at least one determinant for the subsequent manifestation of kidney disease in patients with diabetes mellitus.

Alterations in glomerular hemodynamic function, per se, are germane to the mechanisms underlying platelet activation in the glomerular microcirculation. Before the onset of clinical diabetic nephropathy, a substantial number of patients with diabetes exhibit elevated glomerular filtration rate and renal plasma flow.[26] As reported in studies by Hostetter and associates,[27] animals with experimentally induced diabetes also demonstrate hyperfiltration and hyperperfusion, both at a whole-kidney and at the single-nephron level. Such augmentation in single-nephron glomerular filtration rate is due to increments in the glomerular capillary hydraulic pressure gradient and single-nephron plasma flow rates.[27] Experimental maneuvers such as dietary protein restriction[28] and converting enzyme inhibition[29] decrease intraglomerular hypertension in experimental diabetes and reduce rates of protein excretion and glomerular structural lesions. These findings have given rise to the hypothesis that elevation in intraglomerular pressures and plasma flow rates incite glomerular damage, and as glomeruli become obsolescent, adaptive hemodynamic changes in surviving glomeruli maintain ongoing glomerular damage.[30] Such hemodynamic alterations may trigger platelet activation. Exposure of the glomerular endothelial surface to sustained elevations in glomerular pressures and flows in all likelihood provokes endothelial damage with concomitant loss of the nonthrombogenic nature of the endothelium. Platelet adherence, platelet activation, and discharge of platelet granules ensue. Antiplatelet agents may exert their effects by diminishing platelet hypersensitivity to such aggregative surfaces. Additionally, antiplatelet agents may directly influence glomerular hemodynamics. Dipyridamole decreases the filtration fraction in assorted human glomerulopathies, an effect that suggests efferent arteriolar dilatation and reduction in the transcapillary hydraulic pressure gradient.[31] Acute inhibition of cyclooxygenase activity in experimental diabetes leads to reductions in single-nephron plasma flow rates and the glomerular hydraulic pressure gradient,[32] whereas chronic inhibition of prostanoid synthesis with aspirin prevents the hyperfiltration of early experimental diabetes and diminishes glomerular structural lesions.[33] Thus, antiplatelet therapy may derive efficacy either through direct effects on glomerular hemodynamics or by attenuating the responsivity of platelets to hemodynamically mediated endothelial damage.

Biochemical transformations occurring in the glomerular basement membrane may also predispose toward platelet aggregability. Glomerular basement membranes as well as membranes in extrarenal sites undergo thickening and chemical alterations before the onset of clinical nephropathy.[34,35] One of these biochemical transformations is nonenzymatic glycosylation that involves interaction between carbohydrate and terminal amino groups of proteins or epsilon amino groups of lysine residues.[34,35] This reaction is influenced by the severity of glycemia and affects proteins with both short and long turnover rates. Such glycosylation of proteins alters both structural and functional properties.[35] Relevant to platelet behavior is the recent demonstration that collagen extracted from the placenta of patients with diabetes displays increased ketoamine-linked glucose, a marker of nonenzymatic glycosylation, and such collagen exhibits increased platelet-aggregating ability.[36] If such properties are also displayed by nonenzymatic glycosylated glomerular proteins, this finding may represent another pathway for platelet activation. Thus, biochemical and hemodynamic alterations in the kidneys of patients with diabetes mellitus provide aggregative stimuli to platelets already rendered hyperresponsive by the diabetic milieu.

The importance of the study by Donadio and co-workers is twofold. First, it provides evidence that implicates platelet participation in the pathogenesis of diabetic nephropathy. Second, it introduces a new therapeutic modality for the management of patients with renal disease. This regimen is simple, comparatively inexpensive, and without major side effects; however, data on the effect of this therapy on diabetic retinopathy and the incidence of retinal bleeding are needed. The promising results in this study should serve as a stimulus for a controlled, prospective trial of antiplatelet therapy in diabetic nephropathy.

Karl A. Nath, M.B.,Ch.B.
Section of Renal Diseases
University of Minnesota
Minneapolis, Minnesota

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