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FIBRINOLYSIS AND DIABETES

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1. ABSTRACT

Diabetes is characterized by impaired fibrinolysis. This phenomenon reflects augmented concentrations of plasminogen activator inhibitor type-1 in tissues and in blood. The derangement appears to depend in part on elevated concentrations of free fatty acids, triglycerides, and insulin in association with the insulin resistance syndrome. Impaired fibrinolysis may exacerbate already existing coronary artery disease and potentiate its evolution. Several measures are available to favorably modify fibrinolytic system capacity. They include inhibition of the renin angiotensin system, attenuation of dyslipidemia, and enhancement of insulin sensitivity. Accordingly, normalization of the derangement in fibrinolysis typical of diabetes is an important and achievable therapeutic objective.

2. INTRODUCTION

Among the 17,000,000 Americans estimated to have diabetes, 90% of whom have type 2 diabetes, morbidity and mortality attributable to coronary artery disease is several-fold greater in both men and women compared with that in people without diabetes. Even when normalized for the presence of other covariate risk factors such as hypercholesterolemia or hypertension, the disparity persists. Glycemic control has had relatively little impact on reduction of risk associated with macroangiopathy in contrast to the dramatic positive impact it has in reducing risk attributable to microangiopathy. Thus, factors other than hyperglycemia and its associated metabolic abnormalities have been implicated (1). One, and the focus of this review, is an abnormality in fibrinolysis. Impaired fibrinolysis has been strongly implicated as a determinant of accelerated coronary artery disease and precipitation of acute coronary syndromes. Because of the acceleration of coronary artery disease associated with type 2 diabetes, reference to diabetes in this review will be to type 2 diabetes unless specified otherwise. The increased cardiovascular risk seen with type 2 diabetes appears to be evident in patients with syndromes of insulin resistance without frank diabetes as well.

3. THE INSULIN RESISTANCE SYNDROME

Diverse disorders are associated with syndromes of insulin resistance. Such syndromes are manifested by obesity, hypertension, hypertriglyceridemia, elevated VLDL and small dense forms of LDL and VLDL. Abnormalities of fibrinolysis associated with elevated concentrations in blood of plasminogen activator inhibitor type-1 (PAI-1) occur as well. It is only when compensatory hyperinsulinemia is insufficient to overcome insulin resistance that frank diabetes ensues. Pancreatic beta-cell "exhaustion," thought to be attributable to a genetic predisposition possibly independent of factors underlying insulin resistance itself is what precipitates the diabetes. Thus, people with type 2 diabetes have had syndromes of insulin resistance for years to decades before developing hyperglycemia. During that time coronary vascular disease progresses in an often occult fashion under conditions in which metabolic disturbances such as hyperglycemia and its concomitants are not detectable. Common antecedents may underlie both insulin resistance and macroangiopathy. Altered coordinate expression of genes may influence both. However, a considerable body of information supports the likelihood that insulin resistance and the compensatory hyperproinsulinemia and hyerinsulinemia directly alter fibrinolytic system capacity. Through this mechanism and others they may contribute to acceleration of macrovasculopathy (5,6).

4. VASCULOPATHY ASSOCIATED WITH TYPE 2 DIABETES

Both type 1 and type 2 diabetes are associated with microangiopathy. Attenuation of progression of nephropathy, neuropathy, and retinopathy requires stringent control of hyperglycemia regardless of the nature of

Table 1. Factors Predisposing to Thrombosis and Factors Attenuating Fibrinolysis in Patients with Diabetes

Factors predisposing to thrombosis
• Platelet hyperaggregability
• Decreased platelet cAMP
• Decreased platelet cGMP
• Increased thromboxane synthesis
• Elevated concentrations of procoagulants
• Increased fibrinogen
• Increased von Willebrand factor and procoagulant activity
• Increased thrombin activity (suggested by increased fibrinopeptide A)
• Decreased concentration and activity of antithrombotic factors
• Diminished activity of antithrombin III
• Diminished sulfation of endogenous heparin
Factors attenuating fibrinolysis
• Decreased t-PA activity
• Increased PAI-1 synthesis and activity (directly increased by insulin and proinsulin)
• Decreased concentrations of alpha$_2$-antiplasmin

cAMP=cyclic AMP; cGMP=cyclic GMP; PAI-1=plasminogen activator inhibitor type-1; t-PA=tissue-type plasminogen activator

diabetes responsible. However, the favorable impact of glycemic control on attenuation of progression of vasculopathy is much less evident with respect to coronary artery disease and other macroangiopathic manifestations compared with microangiopathy and its sequelae. Microangiopathy appears to be linked to increased glyco-oxidation of proteins and nucleic acids, products of aldose reductase reactions, accumulation of advanced glycation end products (AGEs), and other direct or indirect consequences of hyperglycemia and its metabolic concomitants (7-9). Thus, as shown in the landmark diabetes control and complications trial, stringent glycemic control diminishes progression of microangiopathy and its consequences (10). Macroangiopathy is the dominant phenomenon in people with type 2 diabetes. Their diabetes occurs relatively late in life following prolonged intervals of insulin resistance. Macroangiopathy is probably retarded by stringent glycemic control though to a much lesser extent than microangiopathy.

It is not yet clear whether treatment with insulin or insulin secretogogues reduces or even perhaps paradoxically increases the risk of cardiovascular disease. Ironically, in some populations with profound impairment of glucose tolerance, cardiovascular risk is not profoundly augmented (11,12). In view of these considerations, vigorous efforts have been undertaken to identify pathophysiological links between insulin resistance and acceleration of coronary artery disease and macroangiopathy in general. Identification of such links would provide promising targets for prevention, retardation, and attenuation of progression of coronary artery disease and macroangiopathy.

5. THE FIBRINOLYTIC SYSTEM IN BLOOD

Numerous abnormalities have been identified that induce a procoagulant and an antifibrinolytic state in people with type 2 diabetes and syndromes of insulin resistance in general (Table 1). The fibrinolytic system in blood operates through activating a zymogen, plasminogen, which is biologically inert, to form plasmin, a serine protease capable of cleaving fibrin and thereby dissolving clots. Activation of plasminogen results from cleavage at a specific site by plasminogen activators. Tissue-type plasminogen activator (t-PA) is the predominant plasminogen activator in blood. Urokinase-like plasminogen activator (u-PA) is the predominant plasminogen activator in tissues. Activation of plasminogen by plasminogen activators is inhibited by PAI-1. Such inhibition of unleashed proteolysis is essential to maintain homeostasis and preclude induction of a bleeding diathesis when plasminogen activation is initiated physiologically (11).

Specificity of activation of fibrinolysis in blood results from the avid binding of t-PA and of plasminogen in juxtaposition on lysine binding sites of fibrin. In the absence of a fibrin surface or similarly constituted entity, the biological activity of t-PA is markedly diminished compared with that reflected by plasminogen activation on the surfaces and within the interstices of clots. In addition to PAI-1 in blood, PAI-1 in platelet granules can be released when platelets are activated thereby inhibiting fibrin dissolution at sites of active clot accumulation.

In addition to inhibition of fibrinolysis (please see below) many abnormalities consistent with a procoagulant state are seen in association with diabetes. They include elevated concentrations of fibrinogen in blood, increased concentrations of coagulation factor VII, activation of prothrombin reflected by elevated concentrations of markers of its activation such as prothrombin fragment 1.2, thrombin-antithrombin complexes, and elevated concentrations in blood of fibrinopeptide A (FPA) a marker of activity of thrombin in converting fibrinogen to fibrin (14).

6. THE ROLE OF ENDOTHELIUM

Endothelial cells exhibit barrier and selective transport functions on the luminal surfaces of vessel walls. They facilitate assembly of pivotal components of the coagulation cascade such as prothrombinase derived through both the tissue factor and the intrinsic pathways of coagulation. They elaborate t-PA, PAI-1, and prostacyclin (a powerful platelet inhibitor). They also elaborate procoagulant factors such as von Willebrand factor, cytokines, adhesive proteins, integrins, selectins, and growth factors. All of these factors can influence the activities of diverse systems including the fibrinolytic system and the evolution of vascular lesions.

7. PLATELET-MEDIATED EFFECTS

Activation of platelets occurs in association with both type 1 and type 2 diabetes. Emersion of platelets from

normal subjects in plasma from patients with diabetes alters platelet function (15-17). Emersion of platelets from patients with diabetes in plasma from normal subjects normalizes platelet function to some extent. These observations have implicated alterations in platelet membrane fluidity directly related to hyperglycemia and other metabolic changes as the cause of increased platelet reactivity. Glycation of both fibrinogen and platelet membrane receptor GP IIb/IIIa exert directionally similar effects on inhibition of platelet activation by anti-GP IIb/IIIa agents (18). Insulin can increase elaboration of PAI-1 from washed platelets. Adhesion molecule expression on platelet surface membranes indicative of increased activation persists in platelets from people with diabetes despite near normalization of hyperglycemia (15,19 [15 reviewed in 19]). Thus, abnormalities of platelet function may contribute to the impairment of fibrinolysis typical of diabetes.

8. IMPAIRED FIBRINOLYSIS WITH DIABETES

Juhan-Vague and her collaborators demonstrated decades ago that obesity, insulin resistance, diabetes, and elevated triglycerides were associated with impairment of fibrinolysis (20,21). They implicated augmentation of concentrations of PAI-1 as an operative factor. We found that the response to transitory venous occlusion, a physiological stimulus of elaboration of PAI-1 locally, was inhibited in obese subjects and in people with type 2 diabetes in association with increased activity of PAI-1 in plasma (22). These changes correlated closely with increased concentrations of immunoreactive insulin and C-peptide in plasma as well. These observations are consistent with augmented synthesis of PAI-1 by insulin and insulin-like growth factor type 1 (IGF1) that we observed when a human hepatoma cell line, Hep G2 cells, was exposed to insulin or IGF1, which binds weakly to the insulin receptor (23) in vitro (24). It is well known that insulin resistance is associated with increased ratios of the concentration of proinsulin to the concentration of insulin in blood indicative of a compensatory increased elaboration of precursors of insulin as well as insulin by highly stimulated pancreatic beta cells under conditions of insulin resistance in the periphery. Proinsulin as well as insulin can augment the synthesis of PAI-1 in vitro and in vivo (25), presumably by binding, albeit with much lower affinity than insulin, to the insulin receptor. Thus, Hep G2 cells exposed to proinsulin exhibited increases in elaboration of PAI-1 into conditioned media whereas exposure to C-peptide did not. Because proinsulin is cleared so much more slowly than insulin from blood in vivo, the impact of proinsulin on elaboration of PAI-1 in association with type 2 diabetes may be quite substantial. In fact, in euglycemic conscious rabbits, effects of equimolar proinsulin and insulin were comparable with respect to induction of increased concentrations of PAI-1 mRNA in tissues indicative of increased gene expression (26). The increases in PAI-1 expression induced by insulin and proinsulin in vivo are not confined to changes in concentrations of PAI-1 in blood. Increased expression in aorta and arterial endothelium in general is evident (27). Atheroma from coronary arteries harvested by atherectomy from patients with type 2 diabetes and compared with those from nondiabetic age and gender matched subjects exhibit markedly increased concentrations of PAI-1 and markedly diminished concentrations of plasminogen activators (28). Mechanisms responsible for induction of PAI-1 synthesis by insulin appear to include stabilization of PAI-1 mRNA (29). Thus, insulin prolongs the half life of the 3.2-kb PAI-1 mRNA species by 2.7-fold in Hep G2 cells exposed to concentrations of insulin comparable to those encountered in association with diabetes.

In addition to effects of insulin and proinsulin on PAI-1 gene expression, diabetes may augment synthesis of PAI-1 through several other mechanisms. Thus, in association with any given concentration of insulin, increasing concentrations of glucose augment synthesis of PAI-1 by arterial endothelial cells just as is the case when proinsulin augments expression of PAI-1 in such cells (30). During the course of atherogenesis, decreased luminal fibrinolytic system capacity is evident as judged from comparisons of concentrations of plasminogen activators with respect to concentrations of PAI-1 normalized for extracted tissue protein. Thus, there is an increased fibrinolytic system capacity in walls from human arterial segments obtained from normal subjects, subjects harvesting fatty streaks (very early atherosclerotic lesions), those manifesting moderate atherosclerosis, and those manifesting severe atherosclerosis with mural thickening greater than 70% and luminal obstruction (31). Such changes in expression of fibrinolytic system components in vessel walls may contribute directly to the nature of atherosclerotic lesions evolving in patients with diabetes (please see below).

From a mechanistic and potentially therapeutic perspective, it is important to recognize that several phenomena may contribute to increased synthesis and expression of PAI-1 in diabetes. Thus, the increased concentrations in blood of free fatty acids (FFA) may account for synergistic augmentation of expression of PAI-1 induced by insulin alone or insulin in association with very low-density lipoproteins (VLDL) (32). Maximal induction of synthesis of PAI-1 in Hep G2 cells occurs with concentrations of medium and long-chain FFA consistent with concentrations present in vivo in plasma in people with type 2 diabetes. The extent of saturation does not appear to be a factor. Longer chain FFA exhibits maximal effects at lower concentrations. Thus, normalization of elevated concentrations of FFA by enhancement of glycemic control may normalize fibrinolytic system activity to some extent in type 2 diabetes. This observation is consistent with the favorable effects induced by sulfonylureas, metformin, and the two in combination in normalizing fibrinolytic system activity in type 2 diabetes that is poorly controlled at the time of onset of treatment (33).

The potential contribution of adipocytes to augmented activity of PAI-1 in diabetes has been recognized for many years. It may account, in part, for the association between obesity and impaired fibrinolysis (34-36). The PAI-1 may be released directly from an increased

mass of adipose tissue, particularly visceral fat. Altered concentrations of cytokines such as transforming growth factor-beta (TGF-beta from platelet alpha granules and tumor necrosis factor-alpha (TNF-alpha) that can act alone and synergistically in combination with insulin to augment PAI-1 expression in adipocytes may contribute (37). Because of the possibility that insulin resistance is, itself, a reflection of activity of TNF-alpha released from adipose tissue, the potential role of TNF-alpha as an agonist of increased PAI-1 expression merits particular consideration.

The potential therapeutic importance of targeting adipose tissue and lipid-mediated effects on PAI-1 gene expression is underscored by several observations including the modulation of induction of PAI-1 expression in endothelial cells by basic fibroblast growth factor in response to fibric acid (38). Fenofibric acid, an agonist for peroxisome proliferator-activated receptor-alpha (PPAR-alpha) inhibits both basal and basic fibroblast growth factor-stimulated PAI-1 expression in endothelial cells. Pharmacologic concentrations of gemfibrozil decrease basal PAI-1 secretion by Hep G2 cells and attenuate augmentation of PAI-1 synthesis induced by several growth factors including endothelial cell growth factor (EGF), TGF-beta, and growth factor-rich autologous platelet lysates infused into rabbits (39). Fibrates also inhibit the augmented PAI-1 expression manifested by human arterial smooth muscle cells exposed to growth factors (41). Niacin, an antihyperlipidemic agent with an entirely different mode of action, decreases PAI-1 expression as well as judged from responses of Hep G2 cells to selected concentrations of niacin and diverse mediators of augmented PAI-1 synthesis in vitro (40). Of interest, insulin sensitizers such as troglitazone diminishes augmented concentrations of PAI-1 in blood evident otherwise in association with type 2 diabetes, obesity, or insulin resistance seen with the polycystic ovarian syndrome (42). The effect appears to be largely dependent on normalization of insulin resistance in vivo rather than a direct effect of the glitazone on PAI-1 expression as judged from comparative studies of effects of glitazones on PAI-1 expression in arterial smooth muscle cells and Hep G2 cells (43).

Another factor likely to influence PAI-1 expression in diabetes is the renin-angiotensin system. It has been long recognized that type 2 diabetes may be associated with a paradoxical condition of hyperkalemia and hyporeninemia. The renin-angiotensin system (RAS) is activated locally within the kidney in people with type 2 diabetes. This accounts for locally increased activity of angiotensin within the kidney, diminution of RAS activity in the periphery, and a potentiation of microalbuminuria attributable to efferent arteriolar constriction. In fact, the American Diabetes Association has recommended consideration of the use of angiotensin receptor blockers (ARBs) alone or in combination with angiotensin converting enzyme inhibitors (ACE inhibitors) to attenuate microalbuminuria even in the absence of hypertension. Such therapy must be accompanied by careful monitoring of potassium status but may be useful for nephro protection. From the perspective of this review, it is important to note that ACE inhibition attenuates hypofibrinolysis not only in blood but also in tissues including the heart (44). This observation is consistent with earlier work demonstrating that PAI-1 synthesis is augmented by angiotensin II and that the effect is modulated by interactions with the AT1 receptor (45).

Other cytokines including interleukin 1 and 6 and oxygen-centered free radicals have been implicated as agonists for PAI-1 synthesis (46). Because of the association of inflammation with type 2 diabetes as reflected by augmented concentrations in blood of markers of inflammation, targeting the increased production of these entities may ultimately be useful in modulating the impairment in fibrinolysis typical of type 2 diabetes.

To recapitulate, fibrinolytic system capacity is impaired in association with type 2 diabetes. The impairment is a consequence of augmented concentrations of PAI-1 in blood. Their presence results in attenuation of the capacity of the fibrinolytic system to induce clot lysis. Impaired fibrinolysis is a known risk factor for acceleration of coronary artery disease. It may operate by potentiating the persistence of microthrombi and their clot associated mitogens thereby exacerbating progression of atherosclerosis. Alternatively, as discussed below, it may accelerate formation of lipid-laden plaques vulnerable to rupture as a result of its impact on cellular migration, proliferation, and apoptosis within the vessel wall itself. The altered fibrinolytic system capacity is evident not only in blood but also in tissues. Its consequences on the evolution of atherosclerotic lesions may depend on both. A major factor responsible for the impairment of fibrinolysis in type 2 diabetes is insulin resistance. This phenomenon, its progenitors, its metabolic consequences including hyperlipidemia, elevated concentrations of FFA, increased concentrations of VLDL and triglycerides, and direct effects of compensatory hyperinsulinemia and hyperproinsulinemia on synthesis of PAI-1 appear to be prominent causes of the impairment in fibrinolysis. Induction of hyperinsulinemia in association with hyperglycemia and hypertriglyceridemia increases concentrations of PAI-1 in blood in normal human subjects (47). Amelioration of inhibition of fibrinolysis in elderly obese subjects can be accomplished by moderate caloric restriction (48), which will of course reduce obesity and tend to normalize insulin resistance. The use of insulin sensitizers not only enhances glycemic control but also normalizes fibrinolytic system capacity otherwise deranged in association with syndromes of insulin resistance including type 2 diabetes (49).

9. THE PROTEO(FIBRINO)LYTIC SYSTEM WITHIN VESSEL WALLS

Intramural proteolysis within vessel walls is mediated in part by proteins that are the same as those that mediate fibrinolysis in blood. We refer to this system as the proteo(fibrino)lytic system. It is well known that cellular elements within the walls of vessels migrate in response to diverse stimuli. Such migration is mediated by increased expression on cell surfaces of u-PA bound to its

receptor. The surface expression of plasminogen activators facilitates activation of plasminogen within the extracellular matrix of vessel walls, plasmin-dependent activation of matrix metalloproteinases, degradation of matrix facilitating migration of cells, and population of nascent atheroma with vascular smooth muscle cells migrating from the tunica media into the neointima (reviewed in 13). Inhibition of cell surface proteo(fibrino)lysis is likely to result from augmented concentrations of PAI-1 within the vessel walls in people with type 2 diabetes (6). The anticipated consequence is accumulation of extracellular matrix, limitation and inhibition of migration of vascular smooth muscle cells into the neointima, altered evolution of atherosclerotic lesions stimulated by the hyperlipidemia, hyperglycemia, insulin resistance, oxygen-centered free radicals, dyslipidemia and hypertension typical of type 2 diabetes. Such changes can be quantified with immunohistochemical methods (50) even in lesions in which the extent of obstruction to blood flow is minimal or absent. In rodents, overexpression of PAI-1 in vessel walls attenuates the response to vessel wall mechnical injury (51) even though it increases persistence of thrombosis. The latter generates fibrin, known to facilitate neointimal formation (52). In view of these considerations, it appears likely that the presence of insulin resistance and augmented expression of PAI-1 may potentiate the formation of atherosclerotic plaques particularly prone to rupture and precipitate acute coronary syndromes in people with type 2 diabetes.

The clinical importance of plaques prone to rupture has been well established. Following the seminal work of Falk and Davies (53,54), it has become increasingly clear that morbidity and mortality associated with coronary artery disease is often not explicable by high grade obstruction anteceding an acute event. Instead, precipitating factors are often abluminal lesions (55), particularly prone to rupture, that are detectable by intravascular ultrasound, magnetic resonance imaging and possibly other modalities such as positron emission tomography, optimal coherence tomography, and thermal detection. Type 2 diabetes is associated with development of lesions prone to rupture as judged from their characteristic composition evaluated histologically and by intravascular ultrasound (S. Nissen, personal communication) (56,57). To the extent that increased expression of PAI-1 alters the proteo(fibrino)lytic system's activity within vessel walls and to the extent that the alteration contributes to compositional changes predisposing plaques to rupture, the augmented expression of PAI-1 becomes a particularly attractive target for prophylactic and therapeutic intervention designed to reduce morbidity and mortality from coronary artery disease.

This hypothesis is being tested in the NIH sponsored the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) multicenter trial. Two therapeutic modalities directed toward the treatment of diabetes in the population of patients with coronary artery disease associated with mild or no symptoms are to be evaluated. One is pharmacologic therapy designed to induce glycemic and metabolic control primarily by providing endogenous or exogenous insulin. The other is pharmacologic therapy designed to induce comparable glycemic and metabolic control primarily by attenuating insulin resistance. The impact of the two types of induction of metabolic control on the evolution of coronary arterial lesions and the frequency and severity of their sequelae is being followed prospectively for 5 years (58). Thus, the trial is evaluating the extent to which early intervention targeting coronary arterial lesions capable of inducing ischemia modifies outcome in patients with diabetes. In addition, it should help to determine the extent to which specific therapeutic approaches to ameliorating insulin resistance normalize fibrinolysis and the extent to which such normalization is associated with improved outcome indicative of reduction of the evolution of plaques vulnerable to rupture (58).

10. CLINICAL IMPLICATIONS

People with type 2 diabetes require stringent glycemic control. In addition, they may benefit from amelioration of insulin resistance because of the likelihood that it, in addition to metabolic derangements, contributes to impairment of fibrinolytic system capacity in blood and in tissues. The impaired fibrinolytic system capacity may potentiate the likelihood of thrombotic coronary occlusion and hence acute coronary syndromes engrafted upon vulnerable plaques prone to rupture. The evolution of such plaques may be potentiated by impairment of the proteo(fibrino)lytic system in vessel walls in parallel with the impairment of the fibrinolytic system in blood. Impairment of both appears to reflect primarily altered expression of PAI-1. It is essential to achieve glycemic control with the use of favorable lifestyle modifications, caloric restriction, and judicious use of the diverse classes of pharmacologic agents available for this purpose presently. In addition, attention should be given to minimizing insulin resistance with the use of insulin sensitizers. A variety of pharmacologic agents likely to be useful in diabetes may be helpful also with respect to the altered activity of the fibrinolytic system almost invariably present. Thus, aspirin, used to exert antiplatelet and anti-inflammatory effects, and platelet GP IIb/IIIa receptor blockers, used to potentiate benefit conferred by coronary stenting and percutaneous coronary intervention (PCI) may diminish inhibition of fibrinolysis. The reduction of concentrations in blood of acute phase reactants may in turn diminish expression of PAI-1. Statins, used to ameliorate hypercholesterolemia, as well as other lipid-lowering agents, have been found, unexpectedly, to be anti-inflammatory as well. They, too, are, therefore, potentially capable of contributing to normalization of fibrinolytic system capacity in people with type 2 diabetes. The use of ARBs and ACE inhibitors both for management of hypertension and to induce protective effects on the kidney manifested by reduced progression of microalbuminuria is likely to contribute to normalization of impaired fibrinolytic system capacity as well. Both angiotensin II and other products of catabolism of angiotensin are agonists of PAI-1 gene expression. As pharmacologic means are developed to modulate the impaired fibrinolytic

system capacity directly, they may well find a place in the therapeutic armamentarium.

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