Changes in Blood Coagulation, Platelet Function, and Plasminogen-Plasmin System in Diabetes

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The increased risk of thromboembolism in people with diabetes mellitus is in part due to changes in the hemostatic mechanism including abnormal platelet function leading to platelet activation, increase in several coagulation factors, decrease in natural anticoagulants, and impaired fibrinolytic activity. Both microangiopathy and atherosclerosis in people with diabetes will enhance the thrombotic potential of these abnormal hemostatic changes. The recent recognition of a role of the components of the plasminogen-plasmin system in many biologic functions at the cellular level has led to studies showing that the angiopathic complications of diabetes may also be caused by impaired plasminogen activator function.
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Patients with diabetes mellitus have an increased risk of cardiovascular disease, especially myocardial infarction (1,2) and cerebrovascular (3) and peripheral vascular diseases (4). These complications account for 80% of the deaths in people with non-insulin-dependent diabetes, with 60% attributable to ischemic heart disease (5). Much attention has been devoted to the pathogenic factors contributing to these complications. Among these factors, altered hemostatic balance, including abnormalities in platelet function, increase in blood coagulability, and an altered fibrinolytic system, have been extensively studied. In this article, I will summarize some of the recent developments in this area.

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uPA, urokinase-type plasminogen activator; tPA, tissue-type plasminogen activator; PAI, plasminogen activator inhibitors; apo(a), apolipoprotein(a); kb, kilobase; bp, base pair.

CHANGES IN BLOOD COAGULATION

There are many observations of increased activation of blood coagulation (6–8), but none of these provides insight into the causes of such changes. Many of the published findings are based on small numbers of patients, and the methodologies used are varied, making comparison of the different series difficult. Thus, it is best to consider only those observed changes that are of some significance as listed in Table 1. The global tests are of limited use in that they merely suggest an increased coagulability of the blood, whereas the increase in the contact activation factors points to a possible cause of the blood coagulability. Also of interest is the increase in fibrinogen level, which is a significant risk factor for ischemic heart disease (9), especially in a diabetic subject (10). In the Framingham study of 1314 subjects over 16 yr, 46 men and 43 women developed diabetes, which predisposed these subjects to all the major cardiovascular disease. These diabetic subjects had a higher fibrinogen level. However, the high fibrinogen level (>312 mg/dl) appears to significantly contribute to the incidence of cardiovascular and coronary heart diseases only in women and not men. There is some basis for the unfavorable effect conferred by the increased plasma fibrinogen level. Fibrinogen is one of the important plasma proteins that contribute to the blood viscosity. Increased blood viscosity is associated with an increased risk of atherosclerosis (11,12). Observations on young male diabetic subjects revealed that blood viscosity is increased, especially among those with retinopathy, and that such an increase is correlated with a higher plasma fibrinogen level (13).

With the increased coagulant activity, the physiologic inhibitors of blood coagulation are also depressed in people with diabetes. These inhibitors include antithrombin III, a physiologic inhibitor of activated factors X and II (14), and protein C (15), which inhibits factors V and VIII.

TABLE 1
Markers of activation of blood coagulation

Global tests	Shortened or normal activated partial thromboplastin time Correlation with platelet volume
Contact activation	Increased kallikrein, factor XII, factor XI
Factor VIII, von Willebrand factor	Increased
Fibrinogen	Increased, Important as risk factor

The cause of these changes in coagulation factors in people with diabetes is not clear. At the cellular level, monocytes in people with diabetes have a greater procoagulant activity (16). On a systemic level, as a result of hyperglycemia, nonenzymatic glycation of plasma proteins may occur. This was postulated to account for many of the changes in blood coagulation and in fibrinolysis. The depressed antithrombin III activity and the acquired protein C deficiency were found to vary with hyperglycemia (14,15). Likewise, glycation of plasminogen may result in its impaired activation by plasminogen activators (17), and glycation of the platelet membrane protein complex IIb-IIIa, which is the platelet receptor for fibrinogen, may account for the increased platelet aggregation in diabetes.

CHANGES IN PLATELET FUNCTION

A detailed description of altered platelet function in diabetes is given in another article in this issue (P.D. Winocour, p. 26) and elsewhere (6,19–23). In Table 2, these changes are listed sequentially in the order of the steps of platelet activation.

TABLE 2
Alterations in markers of activation of platelets

Characteristic	Change
Platelet shape	Discoid; spherical with dentrites
Platelet volume	Increased (controversial)
Platelet survival	Normal or decreased
Platelet adhesion	Increased (decreased after treatment)
Platelet aggregation	Increased spontaneous Increased response to various agonists Increased in vivo release of β-thromboglobulin and PF4 Increased plasma aggregating factor Correlated with micro- and macroangiopathy
Circulation platelet aggregation	Increased
Prostaglandins	Thromboxane B_2 increased (vascular complications) Malondialdehyde increased Decreased platelet sensitivity to prostacyclin
Platelet glycoprotein	Physical changes (increased molecular weight)
Platelet enzymes	Phospholipase A_2 increased Low arachidonic acid

CHANGES IN THE PLASMINOGEN-PLASMIN SYSTEM

The plasminogen-plasmin (fibrinolytic) system is composed of the proteolytic enzyme plasmin, with its precursor plasminogen, and its activators of the urokinase-type and the tissue-type (24,25). The catalytic actions of these proteases are modulated respectively by inhibitors of plasmin and of plasminogen activators. Plasmin inhibitors include α_2-antiplasmin and α_2-macroglobulin, whereas plasminogen activator inhibitors include types 1 and 2. The actions of the plasminogen activators are facilitated by the receptors of plasminogen, uPA and tPA. These receptors are present on many cell surfaces as well as in the circulation.

Measurements of fibrinolytic variables in people with diabetes was done by many investigators (6,8,17,26–32) with mixed results, either normal or impaired overall spontaneous fibrinolytic activity. Tests of the release of fibrinolytic activity after vascular stimulation indicated that such releases are impaired in people with diabetes (30,31). Further analysis of this impaired fibrinolytic activity revealed that tPA is present in normal or increased amounts in the plasma, but is biologically inactive because it is bound in a complex with its inhibitor (26). This binding occurred in both insulin-dependent and non-insulin-dependent diabetic subjects. Obesity and hypertension adversely affected the plasminogen activator inhibitory activity. The decreased fibrinolytic activity was also caused, in part, by glycation of plasminogen, rendering it less susceptible to activation (17). This result also supports the finding that improved glycemic control in a diabetic subject may correct the abnormal tPA level (32) as well as the plasmin level (33).

Under physiological conditions, the fibrinolytic activity of the endothelium can be modulated by several pathways, two of which were recently found to be altered in people with diabetes. One pathway is the thrombomodulin–protein C reaction, a mechanism by which the secretion of thrombomodulin by the endothelial cell activates the circulating protein C. In addition to the inhibition of clotting factors V and VIII, activated protein C serves its antithrombotic role by enhancing fibrinolytic activity through its inhibition of PAI-1 (34). Thus, finding a decreased protein C level in the plasma of a diabetic person (15) points out the dual mechanism by which this abnormality increases the thromboembolic risk. The second pathway involves lipoprotein(a). Recent epidemiologic studies showed that high plasma levels of lipoprotein(a) are associated with an increased risk of atherosclerotic cardiovascular disease (35). A subunit marker of this lipoprotein, apo(a), has a structure with extensive homology to that of plasminogen, and thus, lipoprotein(a) can compete with plasminogen in impairing several fibrinolytic functions, including binding with fibrin, with plasminogen receptors on the endothelial surface, and with heparin-bound tPA (36–39). Though some of this fibrinolytic-inhibiting effect may be offset by concurrent competition of lipoprotein(a) for PAI-1, this lipoprotein is significant because it was recently found to be increased in people with diabetes.

In recent years, attention has been directed to the expression of the various components of the plasmino-

gen-plasmin system at the cellular level (24,40). These components are now believed to be actively involved in many biologic functions in such processes as ovulation, embryogenesis, neuron growth, muscle regeneration, wound healing, angiogenesis, and tumor growth and invasion. The expression of uPA, tPA, and the PAIs in endothelial cells and in smooth muscle cells are also revelant to their role in thrombosis and in atherogenesis, especially during the repair process after vascular injury. Their expression is modulated by the transcriptional regulation of several cytokines, including interleukin 1, interleukin 4, γ-interferon, and tumor necrosis factor; by hormones such as corticosteroids and gonodotrophins; and by growth factors including epidermal growth factor, basic fibroblast growth factor, transforming growth factor–α and –β, platelet-derived growth factors, platelet-derived endothelial cell growth factor, and colony stimulating factors. A major mechanism of action of the components of the plasminogen-plasmin system in these cellular biologic processes is through the focal proteolysis of the extracellular matrix proteins, consisting of collagen, laminin, fibronectin, elastin, vitronectin, and fibrinogen (41–43). uPA and plasmin are the primary catalytic activators of the latent metalloproteinases, such as collagenase, responsible for the proteolysis of the extracellular matrix proteins (44–46). The importance of these interactions became evident in a recent study on the pathogenesis of diabetic microangiopathy (47). This major complication of diabetes arises from the thickening of the capillary basement membrane, which consists of type IV collagen. Through activation of collagenase, uPA can modulate collagen deposition and the resulting basement membrane thickening. Therefore, a high cellular uPA expression would be expected to restrict basement membrane thickening. In an innovative experiment, Miskin et al. generated a transgenic mouse line with a selective organ-specific expression of human uPA (47). Using a fusion gene of a 2.4-kb fragment of the human uPA cDNA and a 409-bp 5′ fragment of the murine ocular lens protein α-crystallin, they produced a transgenic mouse line. The presence of the lens α-crystallin gene resulted in the selected enhanced expression of uPA in the retina, brain, and bone marrow, but not in other organs such as the kidney (47). The phenotypic expression of the transgene was indicated by increased fibrinolytic activity in the eye but not in the kidneys. When experimental diabetes was induced in these transgenic mice with streptozocin, microvascular changes of basement membrane thickening characteristic of diabetic microangiopathy took place in the renal glomerular capillaries but not in the retinal vessels. Such findings support the concept that in people with diabetes, the reduced activity of the fibrinolytic system may be responsible for the basement membrane thickening in the microangiopathy, and that increased uPA activity, locally expressed in the capillaries, may abrogate this process (48).

In summary, changes in the hemostatic components and in the plasminogen-plasmin system may not only cause the increased thromboembolic risk in people with diabetes, but also play an important role in the pathogenesis of vascular complications, including atherosclerosis and microangiopathy. Further studies on the interactions of these components in cellular biologic functions may lead to our better understanding these major complications of diabetes.

REFERENCES

1. Barrett-Connor E, Wingard DL: Sex differential in ischemic heart disease mortality in diabetics: a prospective population-based study. *Am J Epidemiol* 118:489–96, 1983
2. Donahue RP, Abbott RD, Reed DM, Yano K: Postchallenge glucose concentration and coronary heart disease in men of Japanese ancestry: Honolulu Heart Program. *Diabetes* 36:689–92, 1987
3. Abbott RD, Donahue RP, MacMahon SW, Reed DM, Yano K: Diabetes and the risk of stroke: Honolulu Heart Program. *JAMA* 257:949–52, 1987
4. Melton IJ, Makin KM, Palumbo PJ, Elveback LR: Incidence and prevalence of clinical peripheral vascular disease in a population based cohort of diabetic patients. *Diabetes Care* 3:650–54, 1980
5. Harris MI, Entmacher PS: Mortality from diabetes. In *Diabetes in America.* Harris MI, Hamman RF, Eds. Washington, DC, U.S. Govt. Printing Office, 1985, p. 1–48 (NIH publ. no. 85–1468)
6. Fuller JH, Keen H, Jarrett RJ, Omer T, Meade TW, Chakrabarti R, North WRS, Stirling Y: Haemostatic variables associated with diabetes and its complications. *Br Med J* 20:964–66, 1979
7. Jones RL, Peterson CM: Hematologic alterations in diabetes mellitus. *Am J Med* 70:339–52, 1981
8. Ostermann H, van de Loo J: Factors of the hemostatic system in diabetic patients: a survey of controlled studies. *Haemostasis* 16:386–416, 1986
9. Kannel WB, Wolf PA, Costelli WP, D'Agostino RB: Fibrinogen and risk of cardiovascular disease. *JAMA* 258:1183–86, 1987
10. Kannel WB, D'Agostino RB, Wilson PWF, Belanger AJ, Gagnon DR: Diabetes, fibrinogen and risk of cardiovascular disease: the Framingham experience. *Prog Cardiol* 120:672–76, 1978
11. Mayer GA: Blood viscosity in healthy subjects and patients with coronary heart disease. *Can Med Assoc J* 91:951–54, 1964
12. Dormandy JA, Hoare E, Colley J, Arrowsmith DE, Dormandy TL: Clinical, hemodynamic, rheological and biochemical findings in 126 patients with intermittent claudication. *Brit Med J* 4:576–81, 1973
13. Lowe GDO, Lowe JM, Drummond MM, Reith S, Belch JJF, Kesson CM, Wylie WS, Foulds WS, Forbes CD, MacCuish AC, Manderson WG: Blood viscosity in young male diabetics with and without retinopathy. *Diabetologia* 18:359–63, 1980
14. Ceriello A, Giugliano D, Quatraro A, Stante A, Consoli G, Dello Russo P, D'Omoferio R: Daily rapid blood glucose variations may condition antithrombin III biologic activity but not its plasma concentration in insulin-dependent diabetes: a possible role for labile non-enzymatic glycation. *Diabete Metab* 13:16–19, 1987
15. Ceriello A, Quatraro A, Dello Russo P, Marchi E, Barbanti M, Milani MR, Giugliano D: Protein C deficiency in insulin dependent diabetes: a hyperglycemia-related phenomenon. *Thromb Haemost* 65: 104–107, 1990
16. Jude B, Watel A, Fontaine O, Fontaine P, Cosson A: Distinctive features of procoagulant response of monocytes from diabetic patients. *Haemostasis* 19:65–73, 1989
17. Geiger M, Binder BR: Plasminogen activation in diabetes mellitus. Enzyme 40:149–57, 1988
19. Cohen I, Bark D, Fulletton RJ, Veis A, Green D: Nonenzymatic glycation of human blood platelet proteins. *Thromb Res* 55:341–49, 1989
20. Rao AK, Goldberg RE, Walsh PN: Platelet coagulant activities in diabetes mellitus. *J Lab Clin Med* 103:82–92, 1984
21. Kwaan HC, Colwell JA, Cruz S, Suwanwela S, Dobbie JG: Increased platelet aggregation in diabetes mellitus. *J Lab Clin Med* 80:263–46, 1972
22. Winocour PD: The role of platelets in the pathogenesis of diabetic vascular disease. In *Molecular and Cellular Biology of Diabetes Mellitus.* vol. 3. *Complications of Diabetes Mellitus.* Draznin B, Melmed S, LeRoith D, Eds. New York, Liss, 1989, p. 37–47
23. Winocour PD, Kinlough-Rathbone RL, Mustard JF: Platelet survival in rats with spontaneous diabetes mellitus. *J Lab Clin Med* 109:464–68, 1987
24. Kwaan HC: The biologic role of components of the plasminogen-plasmin system. *Prog Cardiovasc Dis* 34:309–16, 1992
25. Bachman F: Fibrinolysis. In *Thrombosis and Hemostasis.* Verstraete M, Vermylen J, Linjen R, Arnout J, Eds. Leuven, Belgium, Leuven University Press, 1987, p. 227–66
26. Auwerx J, Bouillon R, Collen D, Geboers J: Tissue-type plasminogen activator antigen and plasminogen activator inhibitor in diabetes mellitus. *Arteriosclerosis* 8:68–72, 1988

27. Almer LO, Nilsson IM: Fibrinolytic activity and treatment of diabetes. *Lancet* 1:1342, 1974
28. Sharma SC: Platelet adhesiveness, plasma fibrinogen and fibrinolytic activity in juvenile onset and maturity onset diabetes mellitus. *J Clin Pathol* 34:501–503, 1981
29. Almer LO, Sundkvist G, Lilja B: Endothelial factors, toe temperature and leg circulation in diabetics with and without autonomic neuropathy. *Thromb Res* 26:119–28, 1982
30. Almer LO, Milsson IM: On fibrinolysis in diabetes mellitus. *Acta Med Scand* 198:101–106, 1975
31. Fearnly GR, Chakrabartl R, Avis PR: Blood fibrinolytic activity in diabetes mellitus and its bearing on ischaemic heart disease and obesity. *Br Med J* 1:921–23, 1963
32. Juhan-Vague I, Vague P, Poisson C, Aillaud MF, Mendez C, Collen D: Effect of 24 hours of normoglycemia on tissue type plasminogen activator plasma levels in insulin-dependent diabetes. *Thromb Haemost* 51:97–98, 1984
33. Small M, Lowe GDO, MacCuish AC, Forbes CD: Thrombin and plasmin activity in diabetes mellitus and their association with glycaemic control. *Q J Med* 65:1025–31, 1987
34. Esmon CT: Protein C: the regulation of natural anticoagulant pathways. *Science* 235:1348–52, 1987
35. Scanu AM, Fless GM: Lipoprotein(a). *J Clin Invest* 85:1709–15, 1990
36. Gonzalez-Gronow M, Edelberg JM, Pizzo SV: Further characterization of the cellular plasminogen binding site: evidence that plasminogen 2 and lipoprotein(a) compete for the same site. *Biochemistry* 28:2374–77, 1989
37. Plow EF, Felez J, Miles LA: Cellular regulation of fibrinolysis. *Thromb Haemost* 66:32–36, 1991
38. Eldeberg JM, Reilly CF, Pizzo SV: The inhibition of tissue type plasminogen activator by plasminogen activator inhibitor–1: the effects of fibrinogen, heparin, vitronectin and lipoprotein(a). *J Biol Chem* 266:7488–93, 1991
39. Loscalzo J, Weinfeld M, Fless GM, Scanu AM: Lipoprotein(a) fibrin binding and plasminogen activation. *Arteriosclerosis* 10:240–45, 1990
40. Pollanen J, Stephens RE, Vaheri A: Directed plasminogen activation at the surface of normal and malignant cells. *Adv Cancer Res* 57:273–328, 1991
41. Ruoslahti E: Fibronectin and its receptors. *Annu Rev Biochem* 57:375–413, 1988
42. Hynes RO: Integrins: a family of cell surface receptors. *Cell* 48:549–54, 1987
43. Rouslahti E, Pierschbacher MD: New perspectives in cell adhesion, RGD and integrins. *Science* 238:491–97, 1987
44. Testa JE, Quigley JP: The role of urokinase-type plasminogen activator in aggressive tumor cell behavior. *Cancer Metastasis Rev* 9:355–67, 1990
45. Salo T, Liotta LA, Keski-Oja J, Turpeenie MI, Hujanen T, Tryggerasonn K: Secretion of basement membrane collagens degrading enzyme and plasminogen activator by transformed cells—role in metastasis. *Int J Cancer* 30:669–73, 1982
46. Fairbairn S, Gilbert R, Ojkakian G, Schwimmer R, Quigley JP: The extracellular matrix of normal chick embryo fibroblasts: its effect on transformed chick fibroblasts and its proteolytic degradation by the transformation. *J Cell Biol* 101:1790–98, 1985
47. Miskin R, Axelrod JH, Griep AE, Lee E, Belin D, Vassalli JD, Westphal H: Human and murine urokinase cDNAs linked to the murine A-crystallin promoter exhibit lens and non-lens expression in transgenic mice. *Eur J Biochem* 190:31–38, 1990
48. Miskin R, Levinger S: Diabetic mice expressing transgenic urokinase in the retina: limited thickening of retinal capillary basement membrane. In *Proc Int Workshop on Molecular and Cellular Biology of Plasminogen Activation, Elsinore, Denmark 1991*, p. 71