Improved Glycemic Control and Platelet Function Abnormalities in Diabetic Patients With Microvascular Disease

Bijan Roshan, Geoffrey H. Tofler, Larry A. Weinrauch, Ray E. Gleason, Joanne A. Keough, Izabela Lipinska, Annette T. Lee, and John A. D'Elia

Patients with diabetes mellitus have a variety of platelet and coagulation system dysfunctions. At least theoretically, these can contribute to microvascular complications. Intensive glycemic control has been demonstrated to decrease microvascular complications in type 1 diabetics. We studied 16 patients with type 1 diabetes mellitus (11 men and five women; mean age, 39 years) with albuminuria greater than 0.1 g/d and/or proteinuria greater than 0.3 g/d and a creatinine clearance rate higher than 30 mL/min. They received a regimen including three to four injections of insulin per day with or without a weekly infusion of intravenous insulin, and were evaluated for 6 months. We compared the plasma level of von Willebrand factor, platelet aggregation responses to adenosine diphosphate (ADP), epinephrine, and collagen, and platelet adhesion at the beginning of the study and at follow-up intervals. Glycemic control improved significantly. There were no significant differences in the platelet aggregation responses to ADP (1.59 ± 0.34 *v* 1.88 ± 0.23 mmol/L, *P* = .3; normal, 4.6 ± 0.2), epinephrine (0.50 ± 0.20 *v* 1.11 ± 0.31 mmol/L, *P* = .06; normal, 7.6 ± 1.5), or collagen (92.4 ± 6.61 *v* 82.60 ± 3.78 seconds, *P* = .6; normal, 79.1 ± 3.1) or in platelet adhesion (126.31 ± 16.95 *v* 195.08 ± 30.2 platelets, *P* = .34; normal, 68.6 ± 1.4). Baseline von Willebrand factor increased, but not significantly (166.38% ± 10.6% *v* 142.72% ± 14.73%, *P* = .21; normal, 102.0% ± 6.0%). In type 1 diabetic patients with established microvascular complications of nephropathy, a statistically significant improvement in glycemic control did not improve the in vitro platelet function abnormalities. Improved glycemic control delays the progression of microvascular disease through mechanisms not measured by tests of platelet function.

PATIENTS WITH DIABETES mellitus have a variety of platelet and coagulation system dysfunctions. The impaired platelet-mediated vasodilatation,[1,2] increased platelet adhesiveness,[3-10] and increased aggregation[10-20] have been well described. Increases in thromboxane A2 production and release by platelets,[21] platelet-dependent thrombin formation,[22] plasma β-thromboglobulin,[23,24] von Willebrand factor,[25,26] vascular cell adhesion molecule-1,[27] plasma fibrinogen,[24,28] fibrinopeptide A,[29,30] and platelet factor 4[31] all may render the platelet/coagulation system more susceptible to inappropriate in vivo activation in diabetic patients.[23,32-34]

In type 1 diabetic patients, a hyperaggregation of platelets has been demonstrated in association with increased in vitro activity of membrane Ca adenosine triphosphatase (ATPase) and decreased activity of Na-K ATPase.[35] These platelet membrane enzyme activities were independent of the glucose level in the cell culture medium. Platelets of diabetic patients have an increased intracellular level of ionized calcium[36] and show an altered effect of peptide and protein ligands on membrane fluidity.[37] These mechanisms may contribute to microvascular complications in diabetic patients.

Intensive glycemic control has been demonstrated to decrease microvascular complications in type 1 and type 2 diabetic patients.[38-40] We have previously reported a relationship between the progression of renal disease and autonomic dysfunction,[41] as well as a strong correlation between autonomic dysfunction and increased plasma fibrinogen/fibrinolytic activity,[42] in diabetic nephropathy. Aoki et al[43] have documented that intensive glycemic control slows the progression of diabetic renal dysfunction. We hypothesized that intensive insulin treatment would improve some aspects of platelet dysfunction in patients with type 1 diabetes mellitus over time. In the present study, we report the relationship between platelet functional studies and glycemic control in type 1 diabetics with proteinuria.

SUBJECTS AND METHODS

Twenty-two patients were included at the start of the study. Two of them were lost to follow-up study, and one was excluded later due to sepsis that required hospitalization. Three more patients were excluded based on an assay demonstrating inhibition of arachidonic acid by their medications, making platelet assays meaningless. Thus, 16 patients remained in the study (11 men and five women) and were aged 21 to and 65 years (mean age, 39). They all had (1) an onset of insulin-dependent diabetes before age 35, (2) albuminuria greater than 0.1 g/d and/or proteinuria >0.3 g/d on two separate 24-hour urine collections, (3) a creatinine clearance rate higher than 30 mL/min, (4) the ability to be evaluated for a period of 18 months at the Joslin Clinic, (5) a willingness to accept randomization, and (6) an absence of pregnancy or acute or chronic illness that would render the tests uninterpretable (drug dependence, active liver disease, recent myocardial infarction or stroke, etc.). They were assigned to a regimen including four subcutaneous injections of insulin per day or four subcutaneous injections per day plus intravenous insulin once per week. These diabetic patients were prospectively and consecutively enrolled as part of a multicenter study involving seven institutions in the United States, the purpose of which was to determine whether aggressive glycemic control decreases the progression of renal disease in diabetic nephropathy.[44]

In this study, we compared the plasma level of von Willebrand factor, platelet aggregation response to adenosine diphosphate (ADP), epinephrine, and collagen, and platelet adhesiveness at baseline and after 6 months of follow-up study. The von Willebrand antigen concentration

From the Department of Medicine, Beth Israel Deaconess Hospital, Boston; Institute for the Prevention of Cardiovascular Disease, Boston; Clinical Research Section, Mount Auburn Hospital, Cambridge; John Cook Renal Unit, Joslin Diabetes Center, Boston; Harvard Medical School, Boston, MA; and Picower Institute for Medical Research, Manhasett, NY.

Submitted March 8, 1999; accepted June 17, 1999.

Presented in part at the 59th Scientific Session of the American Diabetes Association, San Diego, CA, 1999.

Address reprint requests to John A. D'Elia, MD, John Cook Renal Unit, Joslin Diabetes Center, 1 Joslin Place, Boston, MA 02215.

0026-0495/00/4901-0029$10.00/0

was determined by an enzyme-linked immunosorbent assay (ELISA).[45] Platelet aggregometry was performed[46] with a Biodata Pap-4 aggregometer (Hatboro, PA). ADP and epinephrine (Sigma, St Louis, MO) were added at a concentration of 0.005 to 30 μmol/L. The threshold for each time point was determined as the lowest concentration of agonist sufficient to produce a biphasic response with 50% aggregation. Collagen (Sigma) at a final concentration of 0.19 mg/mL was administered and the time to initial aggregation (lag time) measured. The platelet count in platelet-rich plasma was determined with a ZF Coulter Counter (Coulter Electronics, Hialeah, FL). The method for platelet adhesion uses a modified Hele-Shaw flow chamber system in which platelet-rich plasma is infused at a constant rate over a slide coated with fibronectin. The number of platelets that adhered to the slide was counted after 8 minutes of infusion. Arachidonic acid assay was performed on all samples. Plasma levels of advanced glycosylation end products (AGEs) were measured by ELISA using polyclonal antibodies to AGE-modified proteins.[47]

Quantitative data were tested for significance by an unpaired *t* test between the mean values for different groups. A paired *t* test was used to test for significance between the means of the same groups at different time intervals. All data are expressed as the mean ± SE as a measure of dispersion. An α level of .05 was considered significant. All analyses were performed using SAS software (SAS Institute, Cary, NC).

RESULTS

Baseline measurements of hemoglobin A_{1c}, AGEs, platelet responses to ADP, epinephrine, platelet adhesiveness, and von Willebrand factor were all abnormal for our laboratory. Glycemic control improved significantly (hemoglobin A_{1c}, 9.55% ± 0.35% *v* 8.24% ± 0.37%, $P = .0092$; normal, <6.5%) over 6 months, and AGEs were 11.59 U ± 2.35 at baseline and 8.80 U ± 1.94 at 6 months ($P < .05$). The platelet aggregation response to ADP (1.59 ± 0.34 *v* 1.88 ± 0.23 mmol/L, $P = .3$; normal, 4.6 ± 0.2), to epinephrine (0.50 ± 0.20 *v* 1.11 ± 0.31 mmol/L, $P = .06$; normal, 7.6 ± 1.5), or to collagen (92.4 ± 6.61 *v* 82.60 ± 3.78 seconds, $P = .06$; normal, 79.1 ± 3.1) and platelet adhesion (126.31 ± 16.95 *v* 195.08 ± 30.2 platelets, $P = .34$; normal, 68.6 ± 1.4) did not change significantly. Baseline von Willebrand factor increased but did not change significantly (166.38% ± 10.6% *v* 142.72% ± 14.73%, $P = .21$; normal, 102.0% ± 6.0%) during the study.

DISCUSSION

Platelet function abnormalities that may contribute to microvascular or macrovascular complications are well described in diabetic patients. Better glycemic control in type 1 and type 2 diabetes has been shown to minimize the development or progression of microvascular complications in two large trials (Diabetes Control and Complications Trial and UK Prospective Diabetes Study). In these large trials,[38,40] there has been a trend for fewer complications with larger reductions in hemoglobin A_{1c}.

Published reports of type 1 and type 2 diabetics show significant differences in both methodology and results. Four cross-sectional studies have related glycemic control to platelet function in either type 1 or type 2 diabetic patients. Davi et al[48] studied type 2 diabetics, finding that only with tight glycemic control is an improvement in platelet function noted. In a cross-sectional study of 50 patients with type 1 diabetes, El Khawand et al[49] showed that patients with poor glycemic control had pronounced hemostatic disturbances, but the responses of platelet aggregation to ADP or collagen were not significantly different when well-controlled groups were compared with poorly controlled groups of diabetics. Aoki et al[22] demonstrated a higher rate of platelet-dependent thrombin generation in type 2 diabetics with poor glycemic control compared with a well-controlled diabetic cohort and a cohort of normal patients. Wanger et al[50] found no significant difference between a control group and type 1 diabetic patients with excellent, good, or poor glycemic control with regard to the concentration of platelet β-thromboglobulin or generation of thromboxane B2 and 6-keto-prostaglandin F1α.

Four prospective studies have related glycemia to platelet function in type 1 diabetics. A significant reduction of the platelet synthesis of thromboxane B2 but no reduction in platelet hyperaggregation following continuous insulin infusion in type 1 diabetics has been reported by Mayfield et al.[51] Using continuous insulin infusion, Turk et al[52] noted that the increase in platelet aggregation in response to ADP was significantly decreased toward normal at the 6-month endpoint. McDonald et al[53] postulated that the reduction in platelet thromboxane B2 synthesis in type 1 diabetic patients receiving insulin infusions in their study might be related to a normalization of lipid concentrations. A positive correlation between the low-density lipoprotein cholesterol level and platelet aggregation suggests that insulin infusion has a physiologically important impact on hemostatic and atheroembolic events[52] in type 1 diabetic patients without evidence of microvascular disease. Christiansen et al[54] noted that intensive insulin therapy to near-normoglycemia resulted in no difference in platelet reactivity as compared with conventional insulin therapy in a cohort of type 1 diabetic patients. In our study, despite a statistically significant improvement in glycemic control, there were no physiologically important changes in platelet functional abnormalities. However, our patients had clinical microvascular disease, which was not present in the studies showing a benefit of tight glycemic control. In addition to their microvascular disease, our patients were unable to achieve a glycohemoglobin level within the normal range due to their hypoglycemia, with the resultant catechol excess causing problematic hypertension.

The results of this small study suggest that the moderate improvements in glycemic control attained clinically in a relatively homogeneous group of patients with microvascular disease were not sufficient to reverse the measured platelet hyperaggregation over 6 months. Improved glycemic control in diabetes may delay the progression of microvascular complications through mechanisms of hemostasis not measured by tests of platelet function. This is the first study to concentrate on patients with microvascular disease. A study of continuous insulin infusion in such patients should be undertaken, as supported by a recent editorial suggesting that platelet aggregation in uncontrolled diabetes is reversible in the absence of microvascular disease by improved glycemic control, by reducing the formation of AGEs that may react directly on platelets or indirectly via induction of lipid peroxidation.[55]

REFERENCES

1. Oskarsson HJ, Hofmeyer TG: Platelets from patients with diabetes mellitus have impaired ability to mediate vasodilation. J Am Coll Cardiol 27:1464-1470, 1996

2. Oskarsson HJ, Hofmeyer TG: Diabetic human platelets release a substance that inhibits platelet-mediated vasodilation. Am J Physiol 273:H371-H379, 1997

3. Shaw S, Pegrum GD, Wolff SS, et al: Platelet adhesiveness in diabetes mellitus. J Clin Pathol 20:845-847, 1967

4. Mayne EE, Bridges JM, Weaver JA: Platelet adhesiveness, plasma fibrinogen and factor VIII levels in diabetes mellitus. Diabetologia 6:436-440, 1970

5. Fuller JH, Keen H, Jarrett RJ, et al: Haemostatic variables associated with diabetes and its complications. BMJ 2:964-966, 1979

6. Badawi H, El-Sawy M, Mikhail M, et al: Platelets, coagulation and fibrinolysis in diabetic and non-diabetic patients with quiescent coronary heart disease. Angiology 21:511-519, 1970

7. Hellem AJ: Adenosine diphosphate induced platelet adhesiveness in diabetes mellitus with complications. Acta Med Scand 190:291-295, 1971

8. Mayne EE, Bridges JM, Weaver JA: Platelet adhesiveness, plasma fibrinogen and factor VIII levels in diabetes mellitus. Diabetologia 6:436-440, 1970

9. Odegaard HE, Skalhegg BA, Hellem AJ: Increased activity of anti–Willebrand factor' in diabetic plasma. Thromb Diath Haemorrh 11:27-37, 1964

10. Jones RL, Peterson CM: Hematologic alterations in diabetes mellitus. Am J Med 70:339-352, 1981

11. O'Malley BC, Temperly WR, Ward JD, et al: Platelet abnormalities in diabetic peripheral neuropathy. Lancet 2:1274-1276, 1975

12. Kwaan HC, Colwell JA, Cruz S, et al: Increased platelet aggregation in diabetes mellitus. J Lab Clin Med 80:236-246, 1972

13. Colwell JA, Halushka PV, Sarji KE, et al: Correlation of platelet aggregation, plasma factor and activity, and megathrombocytes in diabetic subjects with and without vascular disease. Metabolism 26:279-285, 1977

14. Heath H, Bridgden WD, Canever JV, et al: Platelet adhesiveness and aggregation in relation to diabetic retinopathy. Diabetologia 7:308-315, 1971

15. Sagel J, Colwell JA, Crook L: Increased platelet aggregation in early diabetes mellitus. Ann Intern Med 82:733-738, 1975

16. Gensini GF, Abbate R, Favilla S, et al: Changes of platelet function and blood clotting in diabetes mellitus. Thromb Haemost 42:983-993, 1979

17. Butkus A, Skriska VA, Schumaker P: Thromboxane production and platelet aggregation in diabetic patients with clinical complications. Thromb Res 19:211-223, 1980

18. Catalano DG, Averna M, Notarbartolo A, et al: Thromboxane biosynthesis and platelet function in type II diabetes mellitus. N Engl J Med 322:1769-1774, 1990

19. Winocour PD: Platelet abnormalities in diabetes mellitus. Diabetes 41:26-31, 1992 (suppl 2)

20. Shukla SD, Paul A, Klachko DM: Hypersensitivity of diabetic human platelets to platelet activating factor. Thromb Res 66:239-246, 1992

21. Halushka PV, Rogers RC, Loadholt CB, et al: Increased platelet thromboxane synthesis in diabetes mellitus. J Lab Clin Med 97:87-96, 1981

22. Aoki I, Shimoyama K, Aoki N, et al: Platelet-dependent thrombin generation in patients with diabetes mellitus: Effect of glycemic control on coagulability in diabetes. J Am Coll Cardiol 27:560-566, 1996

23. Burrows AW, Chavin S, Hockaday TDR: Plasma beta-thromboglobulin concentrations in diabetes mellitus. Lancet 1:235-237, 1978

24. Lowe GDO, Ghafour IM, Belch JJF, et al: Increased blood viscosity in diabetic proliferative retinopathy. Diabetes Res 3:67-70, 1986

25. Lufkin EG, Fass DN, O'Fallon WM, et al: Increased von Willebrand factor in diabetes mellitus. Metabolism 28:63-66, 1979

26. Stehouwer CD, Strose ES, Hackeng WH, et al: Von Willebrand factor and development of diabetic nephropathy in IDDM. Diabetes 40:971-976, 1991

27. Schmidt AM, Crandall J, Hori O, et al: Elevated levels of vascular cell adhesion molecule-1 (VCAM-1) in diabetic patients with microalbuminuria: A marker of vascular dysfunction and progressive vascular disease. Br J Haematol 92:747-750, 1996

28. Poon PYW, Dornan TL, Orde-Peckar C, et al: Blood viscosity, glycemic control and retinopathy in insulin-dependent diabetes. Clin Sci (Colch) 63:211-216, 1982

29. Rosove MH, Frank HJL, Harwig SS: Plasma beta-thromboglobulin, platelet factor 4, fibrinopeptide A, and other hemostatic functions during improved short-term glycemic control in diabetes mellitus. Diabetes Care 7:174-179, 1984

30. Small M, Lowe GD, MacCuish AC, et al: Thrombin and plasmin activity in diabetes mellitus and their association with glycaemic control. Q J Med 65:1025-1031, 1987

31. Muhlhauser I, Schernthaner G, Silberbauer K, et al: Platelet proteins (beta-TG and PF4) in atherosclerosis and related diseases. Artery 8:73-79, 1980

32. Betteridge DJ, Zahavi J, Jones NAG, et al: Platelet function in diabetes in relationship to complications, glycosylated haemoglobin and serum lipoproteins. Eur J Clin Invest 111:272-277, 1981

33. Paulsen EP, McChung NM, Sabio H: Some characteristics of spontaneous platelet aggregation in young insulin dependent diabetic subjects. Horm Metab Res Suppl 11:15-21, 1981

34. Davi G, Gresele P, Violi F, et al: Diabetes mellitus, hypercholesterolemia, and hypertension but not vascular disease per se are associated with persistent platelet activation in vitro. Evidence from the study of peripheral arterial disease. Circulation 96:69-75, 1997

35. Mazzanti L, Rabini RA, Fumelli P, et al: Altered platelet membrane dynamic properties in type 1 diabetes. Diabetes 46:2069-2074, 1997

36. Vicari AM, et al: Deranged platelet calcium homeostasis in diabetic patients with end-stage renal failure. Diabetes Care 19:1062-1066, 1996

37. Watala C, Gwozdzinski K, Pluskota E, et al: Diabetes mellitus alters the effect of peptide and protein ligands on membrane fluidity of blood platelets. Thromb Haemost 75:147-153, 1996

38. Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin dependent diabetes mellitus. N Engl J Med 329:977-986, 1993

39. Eurodiab IDDM Complications Study Group: Microvascular and acute complications in IDDM patients: The Eurodiab Complications Study. Diabetologia 37:278-285, 1994

40. UK Prospective Diabetes Study (UKPDS) Group: Intensive blood-glucose control with sulfonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 352:837-853, 1998

41. Weinrauch LA, Kennedy FP, Gleason RE, et al: Relationship between autonomic function and progression of renal disease in diabetic proteinuria. Am J Hypertens 11:302-308, 1998

42. Weinrauch LA, Gleason RE, Keough J, et al: Relationship between autonomic function and plasma fibrinogen, viscosity, and elements of fibrinolytic activity in diabetic nephropathy. Am J Hypertens 10:454-461, 1997

43. Aoki TT, Benbarka M, Okimura MC, et al: Long-term intermit-

tent intravenous insulin therapy and type 1 diabetes. Lancet 432:515-518, 1993

44. Dailey G, Boden G, Creech R, et al: Weekly pulsatile IV insulin treatments appear to slow progression of diabetic nephropathy. Diabetes 44:24A, 1995 (abstr)

45. Penny W, Weinstein M, Salzman EW, et al: Correlation of circulating von Willebrand factor levels with cardiovascular hemodynamics. Circulation 83:1630-1636, 1991

46. Born GVR: Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 194:927-929, 1962

47. Makita Z, Vlassara H, Cerami A, et al: Immunochemical detection of advanced glycosylation end-products in vivo. J Biol Chem 8:5133-5138, 1992

48. Davi G, Averna M, Catalano I, et al: Platelet function in patients with type 2 diabetes mellitus: The effect of glycemic control. Diabetes Res 10:7-39, 1989

49. El Khawand C, Jamart J, Donckier J, et al: Hemostasis variables in type 1 diabetic patients without demonstrable vascular complications. Diabetes Care 16:1137-1145, 1993

50. Wanger B, Fashing P, Schneider B, et al: Platelet and endothelial function in patients with insulin dependent diabetes mellitus with excellent, good, or poor metabolic control. Microvasc Res 52:183-187, 1996

51. Mayfield RK, Halushka PV, Wohltmann HJ, et al: Platelet function during continuous insulin infusion treatment in insulin-dependent diabetic patients. Diabetes 34:1127-1133, 1985

52. Turk Z, Flego I, Kerum G: Platelet aggregation in type 1 diabetes without microvascular disease during continuous subcutaneous insulin infusion. Horm Metab Res 28:95-100, 1996

53. McDonald JWD, Dupre J, Rodger NW, et al: Comparison of platelet thromboxane synthesis in diabetic patients on conventional insulin therapy and continuous insulin infusions. Thromb Res 28:705-712, 1982

54. Christiansen JS, Ingerslev J, Bernvil SS, et al: Near normoglycemia for 1 year has no effect on platelet reactivity, factor VIII, and von Willebrand factor in insulin-dependent diabetes mellitus: A controlled trial. J Diabetes Complications 1:100-106, 1987

55. Keaney JF, Loscalzo J: Diabetes, oxidative stress, and platelet activation. Circulation 99:189-191, 1999