Platelet Survival in Patients with Diabetes Mellitus

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SUMMARY

Platelet survival by [51]chromium labeling was determined in six normal control subjects and in twelve patients with diabetes mellitus, before and after improved glucose control (mean HbA_{1c} 10.5 ± 2.2% and 7.1 ± 1.8%, respectively). Mean platelet survival was 9.47 ± 0.85 days for the normal subjects and 9.04 ± 1.40 and 9.90 ± 1.05 for the diabetic subjects in hyperglycemic and improved glycemic states, respectively. The differences between these values were not statistically significant. However, platelet survivals performed in three diabetic patients who had severe retinal disease requiring photocoagulation were significantly shortened compared with nonsmoking control subjects ($P < 0.05$) or to patients without severe retinopathy ($P \leq 0.01$). These observations imply that measurable changes in in vivo platelet survival occur after the development of small vessel disease. It remains to be determined whether abnormalities described for diabetic platelets in vitro or changes in in vivo platelet physiology contribute to the initiation or propagation of vascular disease in the hyperglycemic individual. DIABETES *30*:486–489, June 1981.

Since the introduction of insulin therapy, vascular disease has accounted for the majority of the morbidity and mortality in patients with diabetes mellitus. Microvascular disease is manifested most dramatically by retinopathy (the leading cause of new cases of adult blindness in the United States) and nephropathy. Macrovascular disease increases the incidence of coronary artery disease and stroke to twice that of the nondiabetic population.

Recent studies suggest the involvement of the hemostatic system in the initiation or the propagation of vascular lesions. Perturbations of the hemostatic system have been reported in patients with diabetes mellitus.[1,2] Abnormalities of platelet function associated with diabetes include increased platelet adhesion to foreign surfaces,[3,4] increased platelet aggregation in vitro,[5–7] and increased availability of platelet factor 3.[8,9] Since abnormal platelet aggregation in vitro in response to epinephrine reverses with improvement in blood glucose levels,[7] we studied platelet survival in diabetic patients in the hyperglycemic state and after improved glucose control.

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MATERIALS AND METHODS

Twelve nonketotic diabetic patients with hyperglycemia of varying duration were admitted to the Rockefeller University Clinical Research Center. The patients had varying complications, including macrovascular complications, retinopathy, neuropathy, and/or nephropathy as summarized in Table 1. Macrovascular disease was rated by the presence of electrocardiographic abnormalities, absence of peripheral pulses, presence of bruits over vessels, or history of angina or myocardial infarction, with each abnormality rating one (+). Retinopathy was graded by fluorescein angiography: 0, no diabetic changes; +, early change-scattered microaneurysms, rare retinal hemorrhages, or exudates; ++, moderate change-numerous microaneurysms, retinal hemorrhages, hard and soft exudates, slight vitreous hemorrhage, or early neovascularization within one disk diameter of the optic disk; +++, severe change-extensive neovascularization, extensive fibroglial tissue proliferation, extensive vascular beading, extensive microaneurysm formation, marked retinal edema, or lack of profusion of the capillaries over an extended area; and ++++ status post photocoagulation. Neuropathy was graded by: +, presence of abnormalities on surface electrode nerve conduction study; ++, nerve conduction abnormality plus abnormal physical findings on sensory and motor exam; or +++, these findings plus severe debilitation, such as severely impaired ambulation. Renal involvement was judged by creatinine clearance <60 ml/min. No patients had a creatinine clearance <20 ml/min.

Glycemia was quantitated by fasting blood glucose, glu-

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TABLE 1
Clinical manifestations of 12 diabetic patients studied for platelet survival

Patient	Sex/Age	Duration of diabetes mellitus (yr)	Therapy	Complications			
				Vascular	Retinopathy	Neuropathy	Nephropathy
1	F/27	14	Insulin	0	0	0	0
2	F/25	24	Insulin	0	+	0	0
3	M/61	2	Chlorpropamide	++	0	++	0
4	F/62	5	Diet	++	0	+	0
5	F/29	25	Insulin	0	++++	0	0
6	F/26	3	Insulin	0	0	0	0
7	M/55	10	Insulin	+	0	++	0
8	F/36	25	Insulin	+	++++	+	0
9	F/55	10	Chlorpropamide	++	0	++	0
10	F/22	18	Insulin	0	++++	++	+
11	F/56	27	Insulin	+	++	++	+
12	M/20	14	Insulin	0	++++	++	0

cose brackets, and HbA_{1c}. The expression "glucose brackets" is the sum of blood glucose values drawn before and 1 h after each meal. This value represents the patient's ability to metabolize dietary challenges and has been shown to correlate well with 24-h integrated blood glucose concentrations.[10] The blood glucose was brought under control by diet restriction, insulin, or oral hypoglycemic therapy.

Platelet survival was studied with ^{51}Cr-labeled platelets, using sterile techniques, by the method of Harker and Finch with certain modifications.[11] Five hundred milliliters of blood were drawn into a blood pack with ACD and two satellite bags (Fenwal Triple Pack). The blood was centrifuged at 300 × *g* for 15 min and the platelet-rich plasma (PRP) was transferred into one of the satellite bags, while the packed red cells were retrieved and reinfused into the patient. For each 100 ml of PRP, 1 ml of 0.15 M sterile citric acid was added. The PRP was centrifuged into a pellet at 1500 × *g* for 20 min. All but 5 ml of the platelet-poor plasma (PPP) was returned to the second satellite bag. After the platelets were resuspended by gentle inversion of the bag, 300–400 μCi of ^{51}Cr (5–6 μCi/kg body wt) was added to the platelet suspension and incubated for 20 min. All but 40 ml of PPP from bag number 2 was returned to the platelet suspension and the mixture centrifuged at 1500 × *g* for 15 min. The radioactive PPP was decanted. The pellet was washed with an additional 20 ml of PPP, which was also decanted. The remaining radioactive platelets were resuspended in 20 ml of PPP. Red blood cells (RBC) were removed by centrifugation at 200 × *g* for 5 min and the labeled platelets were aspirated into a syringe. One milliliter of platelet suspension was removed for a standard, and 0.1-ml aliquots of platelets were added to 2.0 ml of ammonium oxalate and to 2.0 ml of normal saline to confirm the efficiency of RBC removal. RBC removal was judged adequate if there was less than a 10% difference between the platelet counts from centrifuged pellets of the ammonium oxalate suspension and the normal saline suspension. The radioactive platelets were infused into the patients and 5-ml samples of blood were collected into EDTA at 2 and 4 h after infusion, and daily each morning for 8 days. The samples were counted in vials containing 0.5 ml sodium dodecyl sulfate to ensure constant counting geometry. Platelet survival was analyzed by linear regression, a monoexponential model, and a gamma function model developed by Murphy and Francis.[12]

After the initial platelet survival study, the "hyperglycemic period," blood glucose was brought under optimum control. The period after improvement in blood glucose and repeat platelet survival varied between 2 and 6 wk. For purposes of comparison, six normal volunteers were also studied. Platelet counts were performed by Coulter counter. HbA_{1c} was performed by radioimmunoassay.[13] Where applicable, values are expressed as mean ± SD. The difference between the means was assessed by Student's *t* test for paired data.[14] Differences were termed significant if the *t* value exceeded the 5% level.

RESULTS

Platelet survival studies were performed in 12 diabetic patients during periods of hyperglycemia and after improved "control." The platelet counts and the results of platelet survival studies for the twelve patients and the normal volunteers are shown in Table 2. The status of blood glucose control is also summarized. Carbohydrate control improved significantly in all patients from a mean hyperglycemic (glucose brackets) value of 1364 ± 232 mg/dl to a mean value of 673 ± 136 mg/dl ($P < 0.001$) during the repeat study. HbA_{1c} values also showed a significant improvement from a mean value of 10.53 ± 2.20% to a mean euglycemic value of 7.13 ± 1.76% ($P < 0.001$).

Platelet counts showed a slight but insignificant decrease from a mean value $3.03 \pm 0.80 \times 10^5$ platelets/mm³ during the hyperglycemic phase of the study to a mean value of $2.76 \pm 0.75 \times 10^5$ platelets/mm³ after improved glucose control. Mean platelet survival, using a linear regression model of analysis, showed no significant difference between values obtained during hyperglycemia (9.04 ± 1.40 days) and the values obtained during improved glucose control (9.90 ± 1.05 days). Moreover, these values are not significantly different from the values obtained from the six normal volunteers (mean = 9.47 ± 0.85). Two of the control subjects (4 and 5) were cigarette smokers at the time of the study, and those subjects had the lowest platelet survival of the normal group. If platelet survivals of the smokers are removed from the comparison, there is still no significant difference between the platelet survivals of the diabetic pa-

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TABLE 2
Platelet counts and results of platelet survival studies

Patient	Period	HbA_{1c}	Glucose brackets	Platelet count (× 10^5)	Platelet survival (days)
1	Hyperglycemia	10.0	1525	4.60	9.63
	Euglycemia	6.2	710	3.60	11.37
2	Hyperglycemia	13.3	1810	3.32	9.89
	Euglycemia	8.4	488	2.68	12.04
3	Hyperglycemia	14.3	1359	2.06	8.13
	Eugylcemia	8.7	605	1.90	10.03
4	Hyperglycemia	8.2	1144	2.45	9.04
	Euglycemia	5.4	547	2.29	8.46
5	Hyperglycemia	7.9	1516	3.04	9.06
	Euglycemia	6.0	840	2.80	8.38
6	Hyperglycemia	10.0	1085	2.30	9.94
	Euglycemia	8.0	625	2.34	10.17
7	Hyperglycemia	11.5	1499	2.22	11.37
	Euglycemia	5.0	795	3.10	9.30
8	Hyperglycemia	11.7	1153	3.01	7.31
	Euglycemia	9.2	958	2.38	9.75
9	Hyperglycemia	12.1	1365	2.74	9.60
	Euglycemia	9.5	542	1.48	9.46
10	Hyperglycemia	11.4	1529	2.90	7.34
	Euglycemia	8.1	607	4.02	8.13
11	Hyperglycemia	8.4	1377	4.40	12.43
	Euglycemia	6.8	680	3.68	10.71
12	Hyperglycemia	7.6	1008	3.30	8.67
	Euglycemia	4.2	680	2.86	10.20

Controls	Age/Sex	Platelet count (× 10^5)	Platelet survival (days)
1	36/F	4.90	10.05
2	24/M	2.61	9.39
3	24/F	4.86	10.62
4	24/F	2.96	8.25
5	27/M	4.13	8.87
6	25/M	1.90	9.64
Mean ± SD	26.6 ± 4.7	3.48 ± 1.31	9.47 ± 0.84

tients and the remaining controls. None of the diabetic subjects was a smoker.

Table 3 shows a comparison of mean platelet survival in the subjects when determined by linear regression, a monoexponential model, and a gamma function. Again, no significant differences are seen between diabetics and normals. When patients were categorized according to the presence or absence of vascular disease, no distinct correlations were found between platelet survival and the presence of large vessel disease. However, as seen in Figure 1, the mean platelet survival of patients who had been treated with photocoagulation for diabetic retinopathy was signifi-

TABLE 3
Comparison of mean platelet survival by linear regression, monoexponential function, and gamma function

Diabetes	Linear function	Mono-exponential function	Gamma function
Hyperglycemic	9.04 ± 1.40*	5.92 ± 1.37	8.05 ± 2.18
Normoglycemic	9.90 ± 1.05	6.30 ± 1.14	8.96 ± 1.83
Normal control	9.47 ± 0.85	6.07 ± 0.95	7.27 ± 1.80

* Mean ± SD, days.

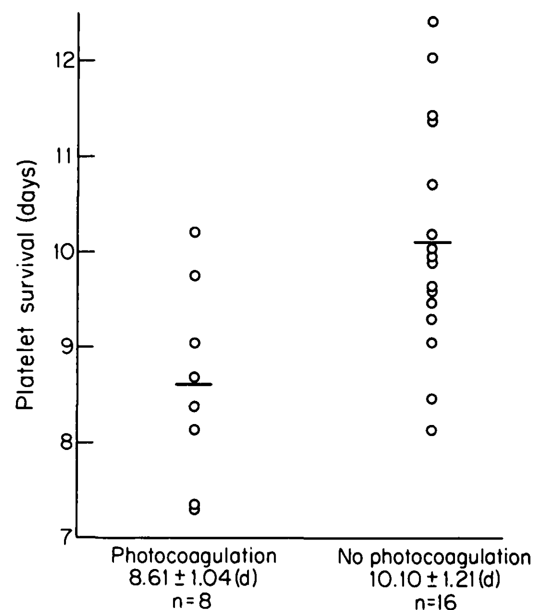

FIGURE 1. Platelet survival studies in diabetic subjects who had been treated with photocoagulation for diabetic retinopathy.

cantly lower than the mean platelet survival of the remainder of the diabetic patients ($P < 0.01$) and the mean platelet survival of the nonsmoking controls ($P < 0.05$).

DISCUSSION

Many reports have described in vitro abnormalities of platelets obtained from diabetic patients. However, the interpretations of these reports as to the etiology of these abnormalities remains inconclusive. Evaluation of these studies is made even more difficult by the various methodologies, patient sources, and conflicting results reported. Hyperaggregation of platelets of diabetic patients has been found to be associated with age,[15] blood glucose control,[7] and the presence or absence of various vascular changes.[16–18] Hyperaggregation has also been reported with a number of circulating substances, including lipids, prothrombin, and von Willebrand factor.[1,2] Each of these factors has been reported to be altered in diabetes and may contribute to the observations related to in vitro platelet changes seen in blood from diabetic patients. Pharmacologic agents such as aspirin[19] and sulfonylureas[18,20] have been found to decrease the aggregation response of the diabetic platelet in vitro, but to date there is no evidence that their use is of benefit in vivo.

The procedure most probably reflective of platelet behavior in vivo is the measurement of platelet survival and turnover. Several reports have appeared in the literature describing decreased platelet survival in patients with diabetes mellitus. Ferguson et al.[21] measured platelet survival in eight diabetic subjects using in vivo labeling with (^{75}Se)-selenomethionine and found a significant reduction. These patients were insulin-dependent and were reported to be free of vascular complications.[21] Another report using ^{51}Cr labelling describes reduced platelet survival only in those patients with vascular complications.[22] In another ^{51}Cr platelet survival study, one-third of diabetics studied were found to have statistically excessive platelet turnover, although there was no statistical difference between the diabetic group and normal controls. No correlation was found

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between platelet consumption and the presence and/or degree of vascular complications in this latter study.[23] In another study using an aspirin labeling technique, platelet survival was reported to be reduced, with no correlation with the presence of vascular disease.[24]

The present study was designed to determine whether improved glucose "control" resulted in a concomitant change in in vivo platelet consumption analogous to the dramatic changes reported in diabetic patients for erythrocyte survival,[7] white cell function,[7] and fibrinogen kinetics.[25] Three methods of data analysis were used to assure that qualitative or quantitative differences in platelet survival would not be missed. We found no statistically significant difference in platelet kinetics between diabetic subjects and nondiabetic controls when taken as a group. We also found no difference in platelet kinetics between hyperglycemic and normoglycemic diabetic patients. There were patients whose platelet survival improved concomitant with improved blood glucose control. These improvements were within the normal range and are of questionable statistical and/or physiologic significance. Even though, overall, we found no statistical difference in platelet kinetics between diabetics and normals, patients with diabetes and small vessel disease of the retina requiring photocoagulation were found to comprise a subgroup with shortened platelet survival. Again, improvement in glucose control in this subgroup led to no consistent change in in vivo platelet survival. These observations would imply that shortened platelet survival results after the development of diabetic microvascular disease, rather than being a primary event in the initiation of vascular problems. Whether platelet changes are influenced by concomitant but clinically silent large vessel disease or whether they contribute to the propagation or acceleration of vascular pathology in the diabetic individual remains to be determined.

The effect of diabetes mellitus on platelet physiology and function remains a confusing picture. The literature indicates that in vitro, several abnormalities related to aggregation, adhesion, and the release reaction are demonstrable. In vivo, the data is more conflicting but it appears that the demonstrable abnormalities may depend on the presence of vascular disease. It is possible that the platelet abnormalities of diabetes mellitus, independent of vascular disease, are too subtle to be demonstrated by the insensitive in vivo techniques available. It is also possible that platelet abnormalities in diabetes are secondary to the vascular complications concomitant with the disease and do not participate in the genesis of vascular lesions. These questions remain to be answered with the advent of more sensitive in vivo markers both for platelet function and vascular disease.

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