Effects of diet and gliclazide on the haemostatic system of non-insulin-dependent diabetics

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Abstract

The effect of the sulphonylurea gliclazide on tests of haemostatic function was studied in 14 newly diagnosed non-insulin-dependent diabetics. After two months' treatment with diet alone 11 of the 14 were given gliclazide; the three others remained on dietary treatment. Compared with pretreatment values, significant reductions in platelet retention, factor VIII-related antigen, factor VIII coagulant activity, and plasma heparin neutralising activity accompanied a fall in the plasma glucose concentration due to either diet alone or diet and gliclazide.

The beneficial effects of gliclazide on platelet abnormalities seem likely to be due to its hypoglycaemic action rather than to any direct effect on haemostatic function.

Introduction

Abnormalities of platelet function and of the coagulation and fibrinolytic systems occur in association with diabetes mellitus.^{1,2} The effect of the degree of metabolic control of diabetes on these tests of haemostasis has received little attention, though adrenaline-induced platelet aggregation³ and factor VIII

function and the possible influence of improved glycaemic control brought about by this sulphonylurea. We therefore assessed for one year the effects of diet and gliclazide on a panel of haemostatic tests in patients with non-insulin-dependent diabetes.

Patients and methods

Fourteen newly diagnosed non-insulin-dependent diabetics completed the study. Table I gives their clinical details. Their mean age was 59.1 (range 42-70) years. Two patients had exudative retinopathy and two had symptoms of peripheral neuropathy at the time of diagnosis, the remainder having no symptoms or signs of vascular disease. Diabetes mellitus was diagnosed on the basis of a 50 g two-hour glucose tolerance test using the criteria proposed by Keen *et al.*⁷

All patients were seen at regular intervals by a dietitian and were educated to follow a conventional carbohydrate-restricted diet. After eight weeks they underwent a second glucose tolerance test. If the two-hour blood glucose concentration exceeded 6 mmol/l (108 mg/100 ml) gliclazide was prescribed in addition to the diet, the dose being adjusted to give optimal control of blood glucose concentrations. No other drugs were prescribed during the study. The patients were asked not to take aspirin-containing compounds and were given paracetamol as a substitute.

After informed consent had been obtained patients were reviewed

TABLE I—Clinical details of diabetics studied

Case No	Age (years)	Sex	Complications	% ideal body weight* at:			Dose of gliclazide (mg/day)
				Onset	2 months	12 months	
<i>Treated by diet and gliclazide</i>							
1	55	F		141	132	126	80
2	62	F		112	103	103	80
3	70	M		107	101	101	160
4	61	M	Peripheral neuropathy	114	111	120	240
5	61	F		156	141	147	160
6	42	F		100	99	103	240
7	55	M	Peripheral neuropathy	123	110	110	80
8	64	M	Exudative retinopathy	106	105	109	240
9	68	F		111	106	107	320
10	66	F		128	122	126	120
11	65	M	Exudative retinopathy	101	103	107	160
<i>Treated by diet alone</i>							
12	56	M		103	102	105	
13	55	M		120	107	105	
14	47	M		110	111	118	

*Calculated using Metropolitan Life Assurance Co tables.

ristocetin cofactor activity⁴ have been reported to be reduced when diabetics were intensively treated with diet and insulin. Recently, the sulphonylurea gliclazide was introduced to clinical practice in the United Kingdom. This drug has been claimed to reduce platelet retention and aggregation when given to patients with maturity-onset diabetes,^{5,6} but studies have not distinguished between a specific drug-induced effect on platelet

at two-monthly intervals throughout the study, when non-fasting venous blood was removed using a 19-gauge butterfly cannula. Blood was obtained at the same time at each visit (one to two hours after breakfast).

Blood glucose concentration was measured by a glucose oxidase method, platelet count with a Coulter Thrombocounter, platelet retention to glass beads by a modification⁶ of Salzman's method, factor VIII coagulant activity by a one-stage clotting assay,⁸ and factor VIII-related antigen by immunoelectrophoresis.⁹ Euglobulin clot lysis time was measured by the method of Blix¹⁰ (results expressed as arbitrary units¹¹), fibrinogen concentration by the method of Ratnoff and Menzie,¹² antithrombin III concentration by immunodiffusion,¹³ heparin neutralising activity of platelet-poor plasma by a heparin-thrombin clotting time assay,¹⁴ and β -thromboglobulin concentration using a radioimmunoassay provided by the Radiochemical Centre (Amersham, England). Results for factor VIII and antithrombin III were expressed as the percentage of the results obtained in a normal plasma pool from 20 healthy male controls. Serum gliclazide con-

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centrations were measured by gas-liquid chromatography.¹⁵ Platelet retention and euglobulin lysis times were measured immediately after blood sampling; the remaining tests were performed on samples frozen and stored at -70°C .

Results were compared with pretreatment values using the Wilcoxon rank sign test for paired samples.

Results

The mean percentage ideal body weight for height at diagnosis was $116.7 \pm \text{SEM } 2.5\%$ and after two months' dietary treatment had fallen significantly to $110 \pm 2.5\%$ ($p < 0.01$). By the end of the study, however, it had increased significantly to $113 \pm 3.4\%$ compared with the mean at two months ($p < 0.01$).

Three patients responded to dietary restriction alone and remained well controlled at the end of the study (mean postprandial plasma glucose concentration 4.3 mmol/l (77 mg/100 ml)). After two months the remaining 11 diabetics were not satisfactorily controlled by diet alone and gliclazide was introduced, daily doses ranging from 80 to 320 mg/day. One patient withdrew from the study at the onset of gliclazide treatment because of dyspepsia (superficial gastritis shown by gastroscopy), which also recurred when another sulphonylurea,

Percentage changes in mean blood glucose concentration, platelet retention, and factor VIII-related antigen (FVIII:Ag) compared with pretreatment values in 11 diabetics treated with diet for 12 months and gliclazide from the second to the twelfth month.

TABLE II—Laboratory results in the 11 diabetics treated with diet for 12 months and with gliclazide from two to 12 months. Values are means ($\pm \text{SEM}$)

	Months of treatment						
	0	2	4	6	8	10	12
Blood glucose (mmol/l)	14.7 (1.5)	9.3† (0.8)	6.6† (0.7)	7.5† (0.6)	6.7† (0.5)	7.0† (0.7)	7.3† (0.7)
Serum gliclazide (mg/l)			5.3 (0.7)	5.0 (1.0)	5.7 (1.1)	4.8 (1.0)	5.3 (1.0)
Platelet retention (%)	35.5 (3.1)	31.3 (2.5)	24.7* (2.1)	26.7* (3.3)	25.0* (2.7)	22.3† (2.0)	29.2 (4.1)
Factor VIII-related antigen (%)	165 (16)	161 (19)	143* (22)	149 (24)	156 (23)	151 (29)	165 (34)
Factor VIII coagulant activity (%)	172 (20)	129* (14)	130* (8)	112† (10)	131* (10)	176 (27)	151 (28)
Heparin neutralising activity (U/ml)	0.21 (0.04)	0.16 (0.03)	0.14* (0.02)	0.11* (0.01)	0.14* (0.03)	0.12* (0.02)	0.13* (0.01)
Euglobulin lysis time (U)	13.2 (0.4)	12.1 (0.6)	12.6 (0.3)	12.7 (0.3)	12.9 (0.3)	12.7 (0.5)	12.4 (0.4)
β -thromboglobulin ($\mu\text{g/l}$)	56.2 (12.4)	68.9 (11.5)	43.4 (6.8)	77.9 (18.1)	64.2 (17.3)	63.2 (10.1)	44.1 (6.0)
Fibrinogen (g/l)	3.00 (0.18)	2.72 (0.17)	2.94 (0.16)	2.81 (0.21)	2.70 (0.24)	2.95 (0.18)	2.77 (0.19)
Antithrombin III (%)	107.8 (4.6)	111.4 (4.5)	112.0 (5.8)	105.0 (4.6)	98.9 (4.7)	99.9 (5.6)	100.5 (3.9)

* $p < 0.05$, † $p < 0.01$ compared with pretreatment values.

‡ $p < 0.01$ compared with values after two months' diet alone.

Conversion: SI to traditional units—Glucose: $1 \text{ mmol/l} \approx 18 \text{ mg/100 ml}$.

TABLE III—Laboratory results in the three patients treated with diet alone

Case No	Months of treatment with diet alone						
	0	2	4	6	8	10	12
Blood glucose (mmol/l)	12 { 14.4 13 { 10.1 14 { 22.0	5.6 5.0 4.0	6.1 4.5 5.2	5.1 4.0 4.8	7.7 3.5 3.6	5.2 3.2 4.5	2.6 4.0 7.7
Platelet retention (%)	12 { 48.0 13 { 17.0 14 { 17.0	16.0 27.9 20.0	17.0 25.0 9.0	20.0 24.0 8.0	8.0 17.0 17.0	26.0 8.0 14.0	24.0 20.0 14.0
Factor VIII-related antigen (%)	12 { 147 13 { 123 14 { 202	105 136 100	88 127 112	116 91 103	104 150 109	98 162 171	126 156 175
Factor VIII coagulant activity (%)	12 { 175 13 { 108 14 { 219	195 98 126	140 121 135	86 121 155	102 93 140	113 120 128	98 123 163
Heparin neutralising activity (U/ml)	12 { 0.172 13 { 0.194 14 { 0.304	0.112 0.120 0.118	0.136 0.218 0.290	0.102 0.204 0.550	0.105 0.092 0.222	0.110 0.128 0.180	0.136 0.124 0.125

Conversion: SI to traditional units—Glucose: $1 \text{ mmol/l} \approx 18 \text{ mg/100 ml}$.

gliclazide, was given. Two patients had symptoms of hypoglycaemia, which were abolished when the dose of gliclazide was reduced.

Table II summarises the laboratory findings in the 11 patients treated with diet and gliclazide. After two months of diet alone significant reductions were observed in plasma glucose concentration and factor VIII coagulant activity. After the introduction of gliclazide there was a further significant fall in plasma glucose concentration.

Platelet retention, factor VIII coagulant activity, factor VIII-related antigen, and plasma heparin neutralising activity were reduced significantly compared with pretreatment values. Mean serum gliclazide concentrations remained constant throughout the treatment period, and the remaining haemostatic tests showed no significant changes. When percentage changes compared with pretreatment values were plotted (figure) the changes in platelet retention and

factor VIII-related antigen followed the same pattern as changes in postprandial plasma glucose concentrations.

Because of the small number of patients who were adequately controlled by diet alone, statistical analysis of this group was not possible. Nevertheless, reductions in mean platelet retention, factor VIII coagulant activity, factor VIII-related antigen, and heparin neutralising activity were observed (table III), while results of the remaining tests did not change.

Discussion

In this study satisfactory glycaemic control was achieved when gliclazide was added to the dietary treatment of newly diagnosed non-insulin dependent diabetics who were not adequately controlled with diet alone. Reduction of blood glucose concentrations was associated with changes in tests of haemostatic function—namely, a reduction in platelet retention, factor VIII coagulant activity, factor VIII-related antigen, and heparin neutralising activity. Smaller changes in these tests occurred after the two months of diet alone, which, with the exception of the change in factor VIII coagulant activity, failed to reach significance. Since the “ideal” weight was not achieved in all these patients, extending the dietary period might possibly have caused further falls in plasma glucose concentration with a corresponding improvement in these haemostatic tests.

Towards the end of the 12 months the results of the tests tended to return towards pretreatment values, whether the patients were treated by diet alone or by diet plus gliclazide. In the gliclazide-treated group mean drug concentrations remained constant, suggesting good compliance with treatment, but there was a significant increase in body weight and a slight rise in blood glucose concentrations at 12 months, which may imply that dietary habits deteriorated towards the end of the study.

Previous workers showed that treatment of maturity-onset diabetes with gliclazide resulted in reduction of platelet retention to glass beads and of adenosine-diphosphate-induced platelet aggregation.^{5,6} In those studies, however, such treatment also produced an improvement in plasma glucose concentrations, and it is therefore uncertain whether the changes in platelet function were due to the drug or to improved metabolic control. The present study appears to favour the latter hypothesis. Treatment by diet alone resulted in improvements in certain haemostatic tests, and falls in plasma glucose concentration were paralleled by a corresponding reduction in factor VIII-related antigen and platelet retention.

The biological implications of such changes are uncertain. Previous studies have shown that haemostatic abnormalities tend to be most pronounced in patients with diabetic vascular complications.^{1,2} If an abnormal haemostatic mechanism plays a part in the pathogenesis of diabetic angiopathies then a return of these tests towards normal might be expected to be beneficial. On the other hand, such abnormalities may be purely secondary to vascular damage. For example, in adequately controlled diabetes the highest concentrations of factor VIII-related antigen are generally found in diabetics with micro-vascular disease, but concentrations rise sharply during diabetic ketoacidosis and return towards normal after correction of the metabolic disturbance.¹⁶ Factor VIII-related antigen is produced by vascular endothelial cells,¹⁷ so it is possible that raised concentrations of this antigen during periods of poor diabetic control indicate vascular endothelial damage. Factor VIII coagulant activity, considered to be an acute-phase reactant,¹⁸ is not synthesised by endothelial cells. Raised factor VIII coagulant activity may therefore represent tissue injury also caused by the disordered carbohydrate and lipid metabolism in uncontrolled diabetes.

The ability of plasma to neutralise heparin is partly due to a factor released from platelets (platelet factor 4). Plasma values of both heparin neutralising activity¹⁹ and platelet factor 4²⁰ have been reported to be increased in diabetes. A proportion of heparin neutralising activity, however, is contributed by

α -glycoproteins,²¹ which are also raised in diabetes.²² As plasma β -thromboglobulin and platelet factor 4 are released simultaneously from platelets²³ the most likely explanation from the present study for a change in heparin neutralising activity during treatment would appear to be a change in α -glycoprotein components.

In conclusion, the results of this study do not support the view that gliclazide per se exerts a beneficial effect on the haemostatic function of non-insulin-dependent diabetes. Nevertheless, control of plasma glucose concentrations by diet and gliclazide led to an improvement in some tests of coagulation and platelet function. Whether such changes lead to a reduction in diabetic vascular complications must await the results of controlled prospective clinical trials.

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