Free Radical Scavenging Activity of Sulfonylureas: A Clinical Assessment of the Effect of Gliclazide

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In long-term clinical studies the beneficial effects of gliclazide on platelets have been related to a reduction in oxidative stress. This property is because of gliclazide's free radical scavenging ability that relates to the unique amino azabicyclo-octare ring, which is grafted on to the sulfonylurea. During a blinded clinical trial, the possible effects of gliclazide were assessed in 30 non-insulin-dependent diabetic patients. All patients had been treated for diabetes for more than 2 years (mean 8 years) and had been established on glibenclamide for over 2 years with or without adjunctive metformin therapy. Patients were studied for 6 months and randomized to continue either their present dose of glibenclamide or to be converted to an equipotent dose of gliclazide. Measurements were taken of hemostatic variables, the oxidative status of the plasma, and the redox status, both extracellularly as plasma albumin-thiols (PSH) and lipid peroxides, and intracellularly as red blood cell superoxide dismutase activity (SOD). At 3 months, diabetic control was unaltered, but there were significant improvements in the oxidative status of the gliclazide-treated patients. Lipid peroxides decreased (8.3 ± 1.1 to 7.0 ± .06 μmol/L, $P < .01$) and red blood cell SOD increased (135 ± 21 to 152 ± 36 μg/mL, $P < .05$). PSH levels were unaltered at 458 ± 38 μmol/L, whereas they had decreased significantly in the glibenclamide patients (414 ± 34 μmol/L, $P < .05$), resulting in a significant difference between the 2 treatment groups ($P < .004$). Platelet reactivity to collagen also improved in the gliclazide-treated patients, decreasing from 65.1% ± 14% to 50.8% ± 24% ($P < .01$). The reactivity of the platelets remained unaltered in the glibenclamide patients. At 6 months, the significant differences between the 2 treatment groups remained. Hence, gliclazide was shown in a clinical study to have free radical scavenging activity independent of glycemic control.

BOTH THE END PRODUCTS of glycosylation in the form of advanced glycation end products (AGE) and the free radicals generated in this process can be implicated in the accelerated atherosclerosis and the vascular and prothrombotic microangiopathic changes typified by diabetes. The rate of formation of free radicals is dependent on the rate of protein glycosylation and, therefore, the level and duration of hyperglycemia.[1] AGE are cross linked and heavily oxidized. Glycation and oxidation are inextricably linked.[2]

The importance of the demonstration of the mechanisms whereby hyperglycemia contributes to vascular damage opens exciting therapeutic options. It is now conceivable to use specific agents to block protein glycosylation or scavenge free radicals, which will have effects independent of improving diabetic control. There is reduced free radical scavenging in diabetes, and a compound which could directly scavenge free radicals as well as reduce blood sugar levels may be more beneficial than a pure hypoglycemic agent alone.

Gliclazide is a second-generation sulfonylurea that has been shown in vitro to be a general free radical scavenger.[3] This effect could explain the previously documented effects on prostanoid release and platelet function both in animal[4] and human platelets.[5] These appear to be independent of any effects on glycemic control and are not necessarily mimicked by other oral hypoglycemic agents. A clinical study documented a decline in the vasoconstrictor prostanoid TXA_2 together with a decline in lipid peroxides that was independent of glycemic control.[6] It is suggested that the effect may be because of direct free radical scavenging. This was further investigated in a glibenclamide-controlled study over a 6-month period.

MATERIALS AND METHODS

Thirty non-insulin-dependent diabetic patients were studied. Twenty men aged 39 to 65 years (mean age, 58.1 years) were recruited into the study. All patients had been treated for diabetes for more than 2 years (mean 8 years) and had been established on glibenclamide for more than 2 years with or without adjunctive metformin therapy. The dosage of glibenclamide had not been altered at 2 consecutive visits before enrollment in the trial, and all subjects had postprandial blood glucose values below 15 mmol/L. Patients taking additional drugs known to influence platelet aggregation (eg, nonsteroidal anti-inflammatory agents) or to have free radical scavenging properties (eg, vitamins C or E, probucol, and captopril) were not studied.

Patients were only studied if they had retinopathy and/or incipient nephropathy. However, they were excluded if they required laser therapy or had undergone laser treatment in the previous 6 months, or if the serum creatinine was greater than 200 mmol/L. Patients were also excluded if they had clinically significant large-vessel disease, a history of intermittent claudication, myocardial infarction, angina or cerebrovascular ischemia, or signs of vascular disease on examination, such as absent foot pulses or electrocardiogram evidence of ischemia.

To assess the degree of oxidative stress found in the patients studied, 2 nondiabetic control groups were compared. The degree of plasma oxidation as lipid peroxides and plasma thiols was assessed. These were age- and gender-matched nondiabetic subjects; one group was healthy controls taking no medication with no history of vascular disease; the second group was patients with proven ischemic heart disease awaiting coronary artery bypass surgery.

Patients were studied at −1, 0, 1, 3, and 6 months and randomized at time point 0 to continue either their present dose of glibenclamide, or to be converted to an equivalent dose of gliclazide. Five milligrams of glibenclamide was considered to be equivalent to 80 mg of gliclazide. Randomization details were not available to the scientific staff until all biochemical and platelet investigations were completed.

Venous samples were taken through a 21-gauge butterfly cannula, without venous stasis, after 30 minutes in the recumbent position, 2 hours after tablets, and a standard breakfast. Diabetic control was assessed by HbA_1 and blood glucose measurement at the same time.

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Measurements were taken of hemostatic variables, the oxidative status of the plasma, and the redox status, both extracellularly as plasma albumin-thiols (PSH) and intracellularly as red blood cell superoxide dismutase activity (SOD). The following standard assays were performed within 2 hours of the venepuncture, and samples were coded so that the clinical status of the patient or control was unknown to the investigator performing the assay.

ASSAYS

For the PSH concentration, the plasma was separated by centrifugation at 1,500*g* for 15 minutes at 4°C. PSH levels were measured using a spectrophotometric technique. Lipid peroxides were determined as malondialdehyde-like material by the spectrophotometric method of Aust.[7] SOD was detected in erythrocytes that had been separated, washed, and then lysed. The red blood cell SOD was measured by the method of Misra and Fridovich.[8] Platelet aggregation to collagen in whole blood was quantified by measuring the decrease in single platelet count in response to 1 μg/mL collagen using a whole blood platelet counter (Clay Adams Ultra Flo 100, Becton-Dickinson, Cowley, Oxford, UK). Glycosylated hemoglobin was measured by agar gel electrophoresis (Corning Medical, Halstead, UK) (normal range 6.0% to 8.0%). Plasma glucose was measured on an autoanalyzer using the glucose oxidase method (Cobas Fara, Roche Diagnostics, Basel, Switzerland).

STATISTICAL ANALYSIS

Analysis of variance was performed together with the Mann-Whitney *U* test comparing the results obtained between patients continuing on glibenclamide and those changing to gliclazide.

RESULTS

One subject randomized to continue on glibenclamide was withdrawn after 6 weeks after sustaining a nonfatal myocardial infarction. His results are not included in the analysis. When subjects are under oxidative stress, lipid peroxides increase, while PSH and red blood cell SOD decreases. Table 1 shows that the diabetic patients, on entry to the trial, were under oxidative stress, as their lipid peroxides were greater than that seen in the age-matched control groups and their PSH levels were reduced.

There were no differences between the values obtained for any of the measurements at −1 month, baseline, and 1 month after randomization. For subsequent analyses, comparisons were made between baseline values and 3- and 6-month values. Baseline characteristics were similar between patients continuing on glibenclamide and those randomized to change to gliclazide (Table 2).

Table 1. Comparison of Oxidative Status in Diabetic Patients, Healthy Subjects, and Nondiabetic Controls With Ischemic Heart Disease

	Normal Controls (n = 29)	Controls With IHD (n = 30)	Diabetic Patients (n = 29)
Age (yr)	59.5 (40-68)	60 (45-68)	58.1 (38-65)
PSH (μmol/L)	506 (433-462)	435 (361-462)*	463 (420-490)*
Lipid peroxides (μmol/L)	7.0 (5.4-9.6)	8.4 (6.5-10.1)*	8.7 (6.6-11.3)*

NOTE. Values are the mean, with ranges in parentheses. Significant differences between patient groups and normal controls.

Abbreviation: IHD, ischemic heart disease.

*$P < .05$ for all values. No differences between diabetic patients and IHD patients.

Table 2. Baseline Characteristics of the Two Patient Groups

	Gliclazide-Treated (n = 15)	P	Glibenclamide-Treated (n = 14)
Blood glucose (mmol/L)	8.6 (3.1)	.16	10.6 (4.7)
HbA_1 (%)	8.2 (2.3)	.23	9.1 (2.0)
Lipid peroxides (μmol/L)	8.3 (1.1)	.11	9.0 (1.2)
PSH (μmol/L)	458 (42)	.70	451 (63)
SOD (μg/mL)	135 (21)	.75	132 (19)
Platelet aggregation (%)	65.1 (14)	.97	70.2 (14)

NOTE. Results are presented as the mean, with the SD in parentheses. Significance tested by the Mann-Whitney *U* test comparing gliclazide-treated with glibenclamide-treated patients.

At 3 months, diabetic control was unaltered, but there were significant improvements in the oxidative status of the gliclazide-treated patients. Lipid peroxides decreased (8.3 ± 1.1 to 7.0 ± 0.6 μmol/L, $P < .01$) and red blood cell SOD increased (135 ± 21 to 152 ± 36 μg/mL, $P < .05$). PSH levels were unaltered at 458 ± 38 μmol/L, whereas they had decreased significantly in the glibenclamide-treated patients (414 ± 34 μmol/L, $P < .05$), resulting in a significant difference between the 2 treatment groups ($P < .004$; Table 3, Fig 1).

Platelet reactivity to collagen also improved in the gliclazide-treated patients, decreasing from 65.1% ± 14% to 50.8% ± 24% ($P < .01$). The reactivity of the platelets remained unaltered in the glibenclamide-treated patients. At 6 months, the significant differences between the 2 treatment groups remained, although there were no further improvements in the gliclazide-treated patients (Table 4, Fig 1).

CONCLUSION

Previous studies on gliclazide have shown that the molecule possesses free radical scavenging effects in vitro in concentrations within the therapeutic range found in serum after a dose of 80 mg.[3] Furthermore, this concentration of gliclazide inhibits the oxidation of LDL cholesterol[9] and the adhesion of monocytes to endothelial cells induced by oxidatively modified LDL.[10] This effect has not been seen with other sulfonylureas in

Table 3. Three-Month Characteristics of the Two Patient Groups

	Gliclazide-Treated (n = 15)	P	Glibenclamide-Treated (n = 14)
Blood glucose (mmol/L)	9.7 (2.8)	.72	10.1 (3.8)
HbA_1 (%)	8.1 (1.9)	.21	9.2 (2.1)
Lipid peroxides (μmol/L)	7.0 (0.6)	.0002	8.3 (0.8)
PSH (μmol/L)	458 (38)	.004	414 (34)
SOD (μg/mL)	152 (36)	.016	123 (16)
Platelet aggregation (%)	50.8 (24)	.006	72.3 (15)

NOTE. Results are presented as the mean (SD) significance tested by the Mann-Whitney *U* test comparing gliclazide-treated with glibenclamide-treated patients.

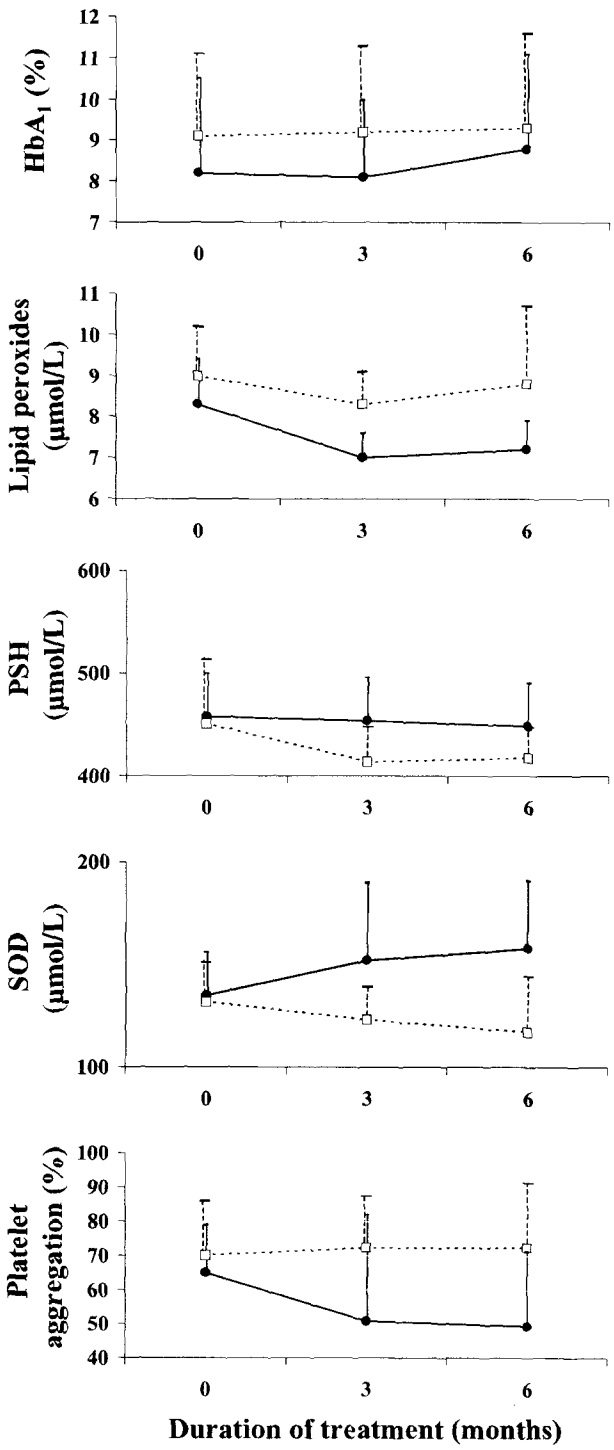

Fig 1. Effect of 3 and 6 months' duration of treatment with gliclazide (●; n = 15) or glibenclamide (□; n = 14) on measures of oxidative stress in patients with non-insulin-dependent diabetes. Results expressed as means and SD bars. Significant differences ($P < .05$) were seen in lipid peroxides, PSH, SOD, and platelet aggregation at 3 and 6 months.

Table 4. Six-Month Characteristics of the Two Patient Groups

	Gliclazide-Treated (n = 15)	*P*	Glibenclamide-Treated (n = 14)
Blood glucose (mmol/L)	8.8 (2.9)	.63	9.6 (4.5)
HbA_1 (%)	8.8 (2.3)	.51	9.3 (2.3)
Lipid peroxides (µmol/L)	7.2 (0.7)	.009	8.8 (1.9)
PSH (µmol/L)	449 (42)	.007	418 (30)
SOD (µg/mL)	158 (33)	.003	117 (27)
Platelet aggregation (%)	49.3 (22)	.004	72.4 (19)

NOTE. Results are presented as the mean (SD). Significance tested by the Mann-Whitney *U* test comparing gliclazide-treated with glibenclamide-treated patients.

vitro. All hypoglycemic therapies reduce the amount of oxidation as this corrects the metabolic derangements that are fundamental to the development of diabetic vascular disease. These clinical studies have demonstrated that patients showed significant improvements in their oxidative status after 3 months' treatment. This improvement in oxidative status, which was associated with a reduction in platelet reactivity, remained constant for the rest of the study period. The effect was independent of glycemic control and confirms preliminary observations shown by a decrease in lipid peroxides in an uncontrolled open study.[6]

Many clinical studies have shown increased oxidative stress in association with the vascular complications of diabetes and improvements in these parameters with the use of a free radical scavenger as used in this study. The demonstration of a benefit to clinical vascular disease has proved difficult to achieve in all studies of non-insulin-dependent diabetes. This is because of the multifactorial nature of complications and the long duration of disease required before microvascular complications such as retinopathy became apparent.

The Japanese Diabetic Retinopathy Program[11] studied the progression of retinopathy over a 5-year period comparing gliclazide with other sulfonylureas and with placebo. The study suggested that with equivalent metabolic control there was a trend towards a lower rate of deterioration of retinopathy and a significantly lower incidence of preproliferative retinopathy in the group receiving gliclazide compared with patients receiving other sulfonylureas or placebo.

There is little comparative evidence on the effect of specific sulfonylureas on large-vessel disease, although improvement in parameters of hyperglycemia is associated with an improvement in morbidity from large-vessel disease. In type 2 diabetes, atherosclerosis coexists in the majority of patients and often predates the clinical diagnosis of diabetes. The presence of atherosclerosis, which often determines the ultimate fate of the patient, further increases the level of lipid peroxidation of oxidative stress, amplifying the effects of hyperglycemia and potentiating vascular damage. In diabetes, therefore, where increased glycation and oxidation are fundamental in the pathogenesis of diabetic vascular disease, agents such as gliclazide with antioxidant activities may have an enhanced therapeutic role.

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