The Effects of Gliclazide and Other Sulfonylureas on Low-Density Lipoprotein Oxidation In Vitro

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Diabetes is associated with increased oxidant stress. This may contribute to the development of diabetic macrovascular complications through increased oxidation of low-density lipoprotein (LDL), which is thought to be a crucial step in the development of atherosclerosis. The sulfonylurea gliclazide has been shown to have free radical–scavenging activity in vitro, but its effects on LDL oxidation, and these effects of other sulfonylureas, are unknown. To investigate this, we studied the effects of in vitro supplementation with gliclazide 1 μmol/L on copper-induced oxidation of LDL isolated from 20 control subjects and 22 type II diabetic patients. The effects of 1 μmol/L vitamin C, a known water-soluble antioxidant, were studied simultaneously. The resistance to oxidation, expressed as the lag time between the addition of copper and commencement of oxidation, was significantly increased by both gliclazide and vitamin C, and the effect was similar for LDL from diabetic and control subjects. The baseline oxidation lag time was 63.4 ± 2.1 minutes, and increased to 108 ± 4.4 minutes with gliclazide and 88.7 ± 5.6 minutes with vitamin C ($P = .0001$, baseline *v* either treatment). The increase in lag time with gliclazide of 70% ± 3% was greater than the 30% ± 5% increase with vitamin C ($P < .0005$). In a separate experiment, LDL isolated from eight control and 10 diabetic subjects was supplemented with 1 μmol/L gliclazide, glibenclamide, glipizide, and tolbutamide. For each LDL sample, all drugs were studied simultaneously and the oxidation lag time was compared against that of untreated LDL. Gliclazide increased the lag time from 53.7 ± 2.4 minutes to 108.4 ± 4.5 minutes ($P = .0001$). None of the other sulfonylureas had any effect on lag time. These findings demonstrate that gliclazide is an effective inhibitor of in vitro LDL oxidation, and in this respect, it is more potent on a molar basis than vitamin C. This antioxidant property of gliclazide was not shared by the other sulfonylureas studied.

ATHEROSCLEROSIS is the cause of death in approximately 80% of diabetic patients, and diabetes confers a twofold to fourfold relative risk of developing coronary artery disease.[1] Even when adjusted for factors such as hypertension and hyperlipidemia, diabetes remains an important cardiovascular risk factor.[2] The hypothesis that increased oxidative stress may be partially responsible for this excess atherosclerotic risk is becoming increasingly popular. Diabetes can increase oxidative stress by several mechanisms.[3] Advanced glycation end products, which progressively accumulate in diabetes, can generate free radicals capable of oxidizing lipid in the arterial wall.[4] Glucose can catalyze lipid peroxidation in vitro,[5] and antioxidant defenses including glutathione,[6] vitamin E,[7] and vitamin C[8] have been reported to be reduced in diabetic patients.

Gliclazide, a sulfonylurea in routine clinical use in many countries, has been reported to have free radical–scavenging properties at pharmacological concentrations, a characteristic not shared by glibenclamide.[9] Two clinical studies have found reduced circulating lipid peroxides in diabetic patients treated with gliclazide, suggesting an effect on lipid peroxidation in vivo.[10,11] The oxidative modification of low-density lipoprotein (LDL) is considered an important step in the development of atherosclerosis. After initial oxidation of the polyunsaturated fatty acid component of LDL, peroxide products attach to the lysine groups of apoprotein B, altering its receptor affinity and allowing uptake by the scavenger receptor on macrophages.[12] Therefore, an agent that inhibits LDL oxidation has the potential to prevent or retard the development of atherosclerosis. This report examines the effects of gliclazide on LDL oxidation in vitro and compares them against the effects of vitamin C, a known water-soluble antioxidant, and the sulfonylureas glibenclamide, glipizide, and tolbutamide. We used a copper-based oxidation method first developed by Esterbauer et al[13] and recently refined to improve its practical application.[14] The technique is clinically relevant, with the susceptibility of LDL to oxidation previously shown to correlate with the extent of coronary atherosclerosis.[15]

SUBJECTS AND METHODS

Subjects and Sample Collection

Two separate experiments were performed. The first compared the effects of gliclazide and vitamin C on LDL oxidation, and the second compared the effects of gliclazide against those of other sulfonylureas. In the first experiment, there were 22 diabetic patients (18 men and four women; mean age, 59 ± 10 years) and 20 control subjects (seven women and 13 men; age, 50 ± 8 years). In the second, the were 10 diabetic patients (five women and five men; age, 54 ± 5 years) and eight controls (three women and five men; age, 50 ± 4 years). Recruitment and blood sampling procedures were the same for both experiments. All diabetic patients had non–insulin-dependent diabetes mellitus (NIDDM) as defined by the criteria of the US National Diabetes Data Group,[16] and were recruited from the Diabetes Outpatient Clinic at Monash Medical Centre. They were receiving treatment with diet, oral hypoglycemic agents, or insulin. Control subjects were recruited from apparently healthy hospital staff and had no history of significant illness or family history of diabetes and were on no medication. All control subjects had a random blood glucose less than 5.5 mmol/L and hemoglobin A_{1C} less than 6.0%. No subject was taking antioxidant vitamin supplements.

On the morning of study, 10 mL fasting blood was drawn into an EDTA tube, transferred to the laboratory on ice, and centrifuged at 3,500 rpm for 10 minutes, and the plasma was separated. LDL was separated by gradient-density ultracentrifugation using a minor modification of the method of McDowell et al.[14] Briefly, 4 mL fresh EDTA

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plasma was adjusted to density 1.21 g/mL by addition of potassium bromide (KBr) and overlayed by a KBr solution of density 1.019 g/mL. The sample was centrifuged at 70,000 rpm at 4°C for 3 hours in a Beckman (Palo Alto, CA) L-8 70M ultracentrifuge with a 70.1 Ti rotor. The LDL fraction was collected by aspiration, and 0.5 mL was analyzed on a Sephadex G 25 PD-10 column (Pharmacia, Uppsala, Sweden) loaded with phosphate-buffered saline (PBS). The first 2-mL fraction was discarded, and the second 2 mL of the collection, which contained the LDL fraction, was retained. The cholesterol content of the fraction was measured on a Cobas Bio centrifugal analyzer (Hoffman-La Roche, Basel, Switzerland), and the LDL was further diluted with PBS to yield a final cholesterol concentration of 0.15 mmol/L.

Supplementation With Test Compound

Gliclazide was supplied by Servier Laboratories (Courbevoie, France); glibenclamide and tolbutamide by Hoechst Pharmaceuticals (Melbourne, Australia); glipizide by Alphapharm Pharmaceuticals (Brisbane, Australia); and vitamin C by BDH (Poole, England; catalog no. 10303). In the first experiment, because gliclazide and vitamin C are water-soluble, they were dissolved directly in PBS, and these solutions were used to dilute the LDL for final concentrations of 1 μmol/L test compound. In experiment 2, because only gliclazide and tolbutamide are water-soluble, all test solutions were prepared by initially dissolving the drug in 2 mL pure ethanol that was diluted to 100 mL using PBS. LDL was then supplemented in a manner similar to experiment 1. The concentration of 1 μmol/L is within the therapeutic range of gliclazide and above the therapeutic range of the other sulfonylureas.[17-19]

LDL Oxidation

The LDL isolated from each subject was studied individually, and the oxidation reaction with each test compound was studied simultaneously in a seven-sample, thermostatted diode-array spectrophotometer (Hewlett Packard 8452A, Palo Alto, CA). Native LDL and LDL aliquots supplemented with each of the test compounds were placed in quartz cuvettes, and copper sulfate was added to yield a final copper concentration of 5 μmol/L. The oxidation reaction proceeded at 37°C, and absorbance at 234 nm, which corresponds to the production of conjugated dienes,[13] was measured every 2 minutes. LDL susceptibility to oxidation was expressed as the lag time between the addition of copper and the appearance of conjugated dienes.

Statistical Analysis

All paired data were analyzed with a Wilcoxon test, and unpaired data with a Mann-Whitney test, using the statistical package Statview (Abacus Concepts, Berkeley, CA). Results are expressed as the mean ± SE.

RESULTS

Experiment 1

There was no difference between the diabetic and control groups with respect to the oxidation lag time at baseline (diabetic *v* control, 61.8 ± 3.1 *v* 65.3 ± 3 minutes, $P = .39$). Supplementation with both gliclazide and vitamin C at a final concentration of 1 μmol/L produced a significant increase in the oxidation lag time of both diabetic and control LDL, indicating inhibition of LDL oxidation (Fig 1). The lag time increased from 63.4 ± 2.1 minutes at baseline to 108 ± 4.4 minutes with gliclazide and 88.7 ± 5.6 minutes with vitamin C ($P = .001$ *v* baseline for both agents). The percentage increase in lag time was significantly greater with gliclazide (69% ± 3%) than with vitamin C (30% ± 5%, $P < .005$).

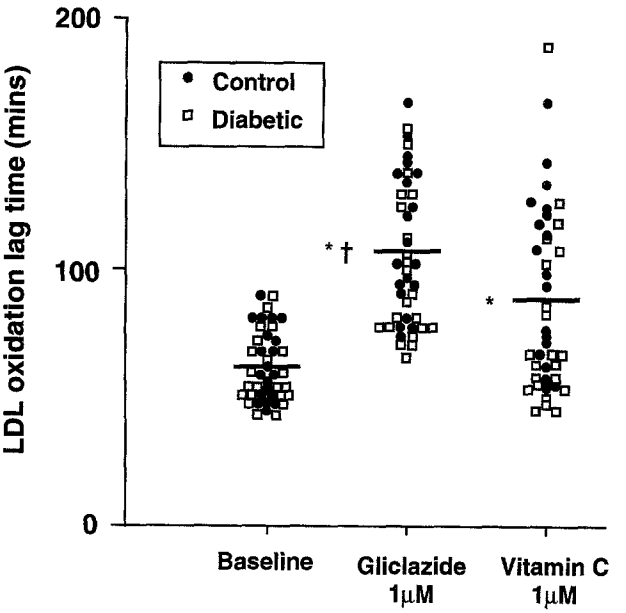

Fig 1. Effect of supplementation with 1 μmol/L gliclazide or vitamin C on oxidation of LDL isolated from 20 control and 22 diabetic subjects. *$P = .001$ *v* baseline; †$P < .005$ *v* vitamin C.

Experiment 2

Of the sulfonylureas tested, only gliclazide produced an increase in oxidation lag time (Fig 2). The baseline lag time was 53.7 ± 2.4 minutes, and increased to 108 ± 4.5 minutes with gliclazide ($P < .0002$). The postsupplementation lag time was 54.0 ± 2.5 minutes with glibenclamide, 53.2 ± 2.7 minutes with tolbutamide, and 53.5 ± 2.5 minutes with glipizide (all $P < .0002$ *v* gliclazide). LDL from diabetic and control subjects responded in a similar manner, and individual values are listed in Table 1.

DISCUSSION

These studies demonstrate that gliclazide is an effective inhibitor of in vitro LDL oxidation. On a molar basis, gliclazide

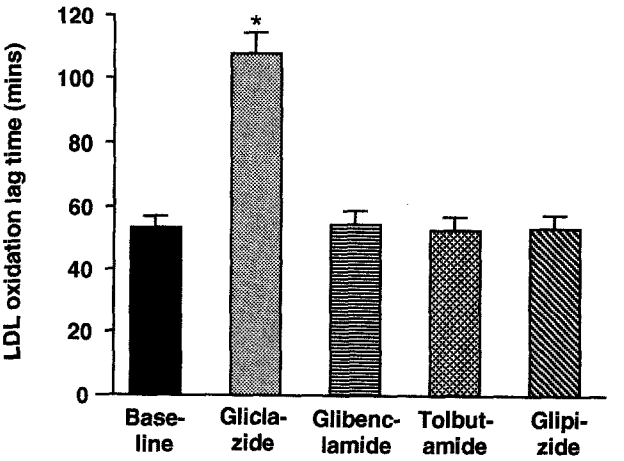

Fig 2. Effect of supplementation with 1 μmol/L sulfonylurea on oxidation of LDL isolated from 8 control and 10 diabetic subjects. *$P < .002$ *v* baseline and other drugs.

Table 1. Effects of Supplementation With 1 μmol/L Sulfonylurea on LDL Oxidation Lag Time (min) in Eight Control and Ten Diabetic Subjects

Group	Baseline	Gliclazide	Glibenclamide	Tolbutamide	Glipizide
Control	54.2 ± 3.2	109 ± 5.8*	53.2 ± 3.3	54.1 ± 3.6	53.6 ± 3.6
Diabetic	53.2 ± 3.6	108 ± 6.9*	54.6 ± 3.9	52.5 ± 4.1	53.4 ± 3.7

*$P < .0005$ *v* baseline and other drugs.

was more potent than vitamin C in this respect; however, the normal range for vitamin C in our laboratory is 40 to 120 μmol/L, so the concentration used in this study was well below physiological levels. Peak plasma gliclazide concentrations after an oral dose range from 6 to 25 μmol/L, and the concentration used in this study was at the lower end of the therapeutic range.[17] Since vitamin C levels have been reported to be reduced in diabetes,[8,20] it is possible that the in vivo antioxidant effect of gliclazide could approach that of vitamin C. This hypothesis is supported by two previous studies demonstrating reduced levels of circulating lipid hydroperoxides in diabetic patients treated with gliclazide.[10,11] One of these, a randomized study comparing gliclazide and glibenclamide in NIDDM patients, found a decline in lipid peroxides after 3 months of gliclazide therapy, suggesting that lipid peroxidation had been inhibited.[10] In that study, the oxidative status was not completely normalized by gliclazide, an outcome that is not surprising given that there are many other factors that protect LDL from oxidation. Principal among these are the lipid-soluble antioxidants, especially α-tocopherol (vitamin E) and the carotenoids, which are carried on LDL.[21] Water-soluble chain-breaking antioxidants such as vitamin C act by regenerating α-tocopherol from its oxidized radical form. Therefore, abnormalities of lipid-soluble antioxidants, such as reduced levels of vitamin E (reported in diabetes[7]), will also contribute to the increased lipid oxidation seen in diabetes.

The baseline oxidation lag time in the diabetic group was not different from that of the control group in this study. This is in contrast to the findings of Tsai et al,[22] who found that the oxidation lag time for LDL from patients with poorly controlled insulin-dependent diabetes was shorter than for LDL from matched nondiabetic controls. There could be several explanations for this difference, such as the degree of glycemic control or the type of diabetes studied. However, our study was not designed to test for possible differences between diabetic and nondiabetic groups: diabetic patients were included simply to confirm that the in vitro effects of antioxidant supplementation could be demonstrated with diabetic and control LDL. We did not attempt to match the groups with respect to age or lipid levels, and therefore, no significant inferences should be drawn from the baseline data.

Of the sulfonylureas studied, only gliclazide inhibited LDL oxidation. This is consistent with the study by Scott et al,[9] who found that gliclazide but not glibenclamide possessed free radical–scavenging activity. The in vivo correlate, circulating lipid peroxides, was also unaffected by glibenclamide in the study by Jennings et al,[10] and we are not aware of studies involving other sulfonylureas. The site of gliclazide's antioxidant activity is unknown, but it is likely to involve the azabicyclooctyl ring, which is not present in other sulfonylureas.[23]

We have demonstrated that gliclazide can inhibit LDL oxidation at therapeutic concentrations, and this property is not shared by glibenclamide, glipizide, or tolbutamide. Previous studies suggest that this antioxidant property may be active in vivo, leading to potential beneficial effects on the development of atherosclerosis. Further studies are required in patients with NIDDM to determine the possible influence of gliclazide on diabetic macrovascular disease.

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