CURRENT THERAPEUTIC RESEARCH
VOLUME 63, NO. 7, JULY 2002

Antioxidant Effects of Gliclazide, Glibenclamide, and Metformin in Patients with Type 2 Diabetes Mellitus

Anna Maria Signorini, MD,[1] Cecilia Fondelli, MD,[2] Elisabetta Renzoni, MD,[2] Cheti Puccetti, BD,[2] Giorgio Gragnoli, MD,[2] and Giorgio Giorgi, BD[1]

[1]Department of Pharmacology "G. Segre," Siena University, and [2]Siena University Hospital, Siena, Italy

ABSTRACT

Background: Hyperglycemia increases oxygen-reactive species generation and reduces the protective capabilities of antioxidant defense systems. In patients with type 1 or 2 diabetes mellitus (DM), the increased production of oxygen free radicals may be linked to the development of chronic complications of diabetes. In vitro studies have demonstrated that oral antidiabetic drugs have antioxidant effects that might be secondary to an inhibiting role in lipid peroxidation.

Objective: The purpose of this study was to determine the effects of 3 common oral antidiabetic agents on oxidized anti–low-density lipoprotein antibody (o-LAb) plasma levels and on total antioxidant status (TAS).

Methods: We studied in vivo patients with type 2 DM treated with long-term gliclazide, glibenclamide, or metformin therapy along with healthy control subjects. The diabetic patients were randomized to the following treatment groups: group 1, gliclazide 80 to 240 mg/d; group 2, glibenclamide 10 to 15 mg/d; and group 3, metformin 1500 mg/d.

Results: Ninety-two patients with type 2 DM (group 1, 18 females, 15 males [mean age, 62.8 ± 9.5 years]; group 2, 19 females, 11 males [mean age, 63.2 ± 8.8 years]; group 3, 16 females, 13 males [mean age, 62.0 ± 5.3 years]) and 28 age- and sex-matched healthy control subjects (18 females, 10 males; mean age, 59.1 ± 5.3 years) were enrolled. In group 1, both the o-LAb level and TAS were similar to those of the control group. The o-LAb level was highest and the total antioxidant plasma level was lowest in group 3, whereas intermediate levels were found in group 2. Multivariate analysis using stepwise regression showed that the type of oral antidiabetic agent was independently associated with o-LAb and total antioxidant levels, as well as with sex, age, and type of antidiabetic agent.

Conclusion: In the patients with type 2 DM in this study, gliclazide had a positive effect on the oxidation-reduction system.

Accepted for publication May 13, 2002.

0011-393X/02/$19.00

Key words: gliclazide, glibenclamide, metformin, oxidation-reduction system. (*Curr Ther Res Clin Exp.* 2002;63:411–420)

INTRODUCTION

Diabetes mellitus (DM) is a condition of increased oxidative stress due to an altered balance between radical production and protective antioxidant defenses.[1] Hyperglycemia causes release of tissue-damaging reactive oxygen species[2] and diminishes antioxidant agents.[3–5] Several hypotheses have been put forth to explain how hyperglycemia is linked to complications of diabetes. Known mechanisms of action include: (1) metal-catalyzed oxidation of glucose, described as auto-oxidative glycosylation,[6] generates superoxide anion and idroxylic radicals; (2) nonenzymatic glycation and oxidation of proteins and lipids generate advanced glycation end products; and (3) glycated proteins and advanced glycation end products react by glyco-oxidation,[7,8] resulting in the production of oxygen-derived free radicals. Chronic hyperglycemia also induces the transformation of glucose in sorbitol by the activation of the nicotinamide adenine dinucleotide phosphate–dependent polyol pathway, leading to the depletion of protective endothelial substances, such as nitric oxide. Fructose is more reactive than is glucose in auto-oxidative processes.[9]

Irreversible oxidative tissue damage may play a role in the development of diabetic micro- and macroangiopathy.[10–12] However, whether oxidative stress is primarily a cause of vascular diabetic complications or a secondary consequence of tissue damage is unclear.[13,14] In either case, oxygen free radicals increase endothelial damage.

Antioxidant therapy represents a possible intervention to prevent or to slow the progression of diabetic micro- and macroangiopathic complications. In vitro studies have demonstrated that gliclazide, an antidiabetic oral agent, has a significant effect on intracellular processes stimulated by oxidative stress.[13,14] O'Brien and Luo[15] suggested that gliclazide in vitro inhibits copper-induced oxidation of low-density lipoprotein (LDL). Noda et al[16] concluded that (1) gliclazide has an in vitro scavenger effect on superoxide anion that is only slightly less than that of ascorbic acid and (2) that gliclazide has the ability to remove the idroxylic radicals to detoxify the cells. In patients with type 2 DM, gliclazide treatment has been reported to reduce thiobarbituric acid–reactive plasma substances, indicating a decrease in lipoperoxidation and an increase in antioxidant enzyme activity.[17] Renier et al[18] reported a decrease in LDL oxidation and monocyte adhesion to the endothelium, whereas Jennings and Belch[19] found a free-radical scavenging effect of gliclazide that led to improvement in oxidative status.

We measured oxidized anti-LDL antibody (o-LAb) level and assessed total antioxidant status (TAS) in patients with type 2 DM treated with 3 oral antidiabetic drugs to investigate the effects of oral antidiabetic agents commonly used in our clinical practice on oxidation-reduction (redox) status.

PATIENTS AND METHODS

Patients and Control Subjects

Patients with type 2 DM receiving long-term gliclazide, glibenclamide, or metformin therapy and age- and sex-matched healthy control subjects were eligible. The diabetic patients were randomized to the following treatment groups: group 1, gliclazide 80 to 240 mg/d; group 2, glibenclamide 10 to 15 mg/d; and group 3, metformin 1500 mg/d.

Inclusion criteria were age, normotensive state,[20] absence of clinical micro- or macrovascular complications, and therapy with oral gliclazide, glibenclamide, or metformin ≥6 months before enrollment. Patients with newly diagnosed diabetes and patients having ongoing oral antidiabetic secondary failure were excluded.

Arterial hypertension was defined according to the World Health Organization guidelines.[20] Clinical micro- and macrovascular complications were determined using electrocardiography, echocardiography, epiaortic Doppler ultrasonography, and ankle–arm blood pressure index determined using Doppler ultrasonography to detect macrovascular disease[21]; fundus oculi examination (with or without fluoroangiography) to detect diabetic retinopathy[22]; albumin excretion rate in 3 different specimens collected over 1 year to detect diabetic nephropathy[23]; and Semmes-Weinstein 10-g monofilament for esthesiometry,[24] biothesiometry for sensory perception threshold,[25] and cardiovascular tests for autonomic neuropathy[26,27] to detect neuropathy.

Study Protocol

For both diabetic patients and control subjects, a complete history, physical examination, and calculation of body mass index (BMI)[28] were performed by the authors during an outpatient visit. After patients and subjects fasted overnight (12 hours), all blood specimens for laboratory investigations were drawn by a laboratory technician. Fasting plasma glucose level and serum levels of total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and LDL cholesterol were assayed by the laboratory technician using an autoanalyzer. Glycosylated hemoglobin (HbA_{1c}) level was determined using high-performance liquid chromatography.[29] Total urinary albumin excretion (UAE) was measured by a laboratory technician from the morning spot urine specimen[30] using immunonephelometry.[31] UAE was used to calculate the albumin-to-creatinine ratio (urinary albumin [mg]/urinary creatinine [mg]) by a laboratory technician. All of these tests are routinely performed at least twice a year during outpatient visits. We drew an additional 6 mL of blood to assess redox status.

All participants gave written informed consent for the study. Institutional review board approval of the study protocol was obtained.

Assessment of Oxidation-Reduction Status

TAS is an indicator of the ability to defend against free-radical damage (the major antioxidant defenses in plasma include radical scavengers [eg, as-

corbate, protein thiols, bilirubin, urate, α-tocopherol] and preventive antioxidants [eg, ceruloplasmin, transferrin, iron-scavenging proteins]). TAS was assessed using colorimetry (Total Antioxidant Status test, Randox Laboratories, Ltd, Crumlin, United Kingdom).[32] The chromogen 2,2′-azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS) is incubated with a peroxidase (metmyoglobin) and hydrogen peroxide in a stabilized formulation to produce the radical cation $ABTS^{+}$, which is characterized by a blue-green color and the absorbance of which is measured at 600 nm using spectrophotometry. TAS due to the presence of antioxidants in the tested plasma samples inhibits the reaction and the color production. Plasma total antioxidant levels are expressed as millimoles per liter (mmol/L).[32]

Oxidative stress was assessed using the quantification of autoantibodies against peroxidized LDL molecules. If LDL is altered by oxidation, it becomes immunogenic, and specific autoantibodies against epitopes (eg, malonyldialdehyde lysine) of the oxidized LDL can be detected in serum. Oxidized LDL autoantibodies have been proposed as an index of in vivo oxidation and can be measured using an enzyme-linked immunosorbent assay with high sensitivity (0–2000 mIU/mL) and specificity (100% anti-LDL antibodies and 5% native LDL).[33] For both methods, immediately processed plasma was used.

Statistical Analysis

Data are expressed as mean ± SD. Comparisons between groups were performed using the Scheffé test for variables displaying a normal distribution and the Kruskal-Wallis test for variables showing a skewed distribution (albumin-to-creatinine ratio, TAS, and o-LAb). Statistical analysis was completed with multivariate analysis using stepwise regression, which is performed to better disclose the relationship of serum o-LAb level and TAS with clinical variables (ie, age, sex, BMI, diabetes duration, fasting plasma glucose level, lipidic fractions, UAE, and antidiabetic agents). Significance was set at $P < 0.05$. The statistical software SPSS (SPSS Science, Chicago, Ill) was used for all analyses.

RESULTS

Ninety-two patients with type 2 DM (group 1, 18 females, 15 males [mean age, 62.8 ± 9.5 years]; group 2, 19 females, 11 males [mean age, 63.2 ± 8.8 years]; group 3, 16 females, 13 males [mean age, 62.0 ± 5.3 years]) and 28 age- and sex-matched healthy control subjects (18 females, 10 males; mean age, 59.1 ± 5.3 years) were enrolled (Table I).

Diabetes duration, BMI, laboratory data, and metabolic control parameters of the control group and of the patients, subdivided into groups 1, 2, and 3, are shown in Table II.

Fasting plasma glucose, HbA_{1c}, and UAE levels were significantly higher in the diabetic groups than in controls ($P < 0.05$). Diabetes duration, BMI, and levels of fasting plasma glucose, HbA_{1c}, total cholesterol, HDL cholesterol, and

Table I. Mean age and sex of the study population (N = 120).

Group	Mean Age ± SD, y	Sex
Group 1 (n = 33)	62.8 ± 9.5	18 F, 15 M
Group 2 (n = 30)	63.2 ± 8.8	19 F, 11 M
Group 3 (n = 29)	62.0 ± 5.3	16 F, 13 M
Control group (n = 28)	59.1 ± 5.3	18 F, 10 M

UAE were not significantly different between the 3 diabetic groups. Triglyceride levels were significantly higher in group 3 ($P < 0.05$) and LDL cholesterol in group 2 ($P < 0.05$) compared with the other diabetic groups. Although metabolic control parameters were not within normal limits,[34] they were similar in all diabetic patients.

Plasma levels of TAS were 1.42 ± 0.092 mmol/L in the control group, 1.39 ± 0.111 mmol/L in group 1, 0.99 ± 0.042 mmol/L in group 2 ($P < 0.05$), and 0.85 ± 0.083 in group 3 ($P < 0.05$; Figure 1). Plasma concentrations of o-LAb were 263.3 ± 24.25 mIU/mL in the control group, 236.6 ± 31.56 mIU/mL in group 1, 391.5 ± 17.85 mIU/mL in group 2 ($P < 0.05$), and 435.7 ± 11.25 mIU/mL in group 3 ($P < 0.05$; Figure 2). These statistically significant differences were observed

Table II. Clinical data and laboratory findings (mean ± SD) by treatment group (N = 120).

Parameter	Controls (n = 28)	Group 1 (n = 33)	Group 2 (n = 30)	Group 3 (n = 29)
Diabetes duration, mo	N/A	120.7 ± 78.6	151.1 ± 92.5	130.0 ± 89.7
Body mass index, kg/m^2	26.2 ± 3.9	27.3 ± 5.8	27.7 ± 4.3	27.8 ± 6.7
Fasting plasma glucose, mg/dL	86.3 ± 5.4*	142.8 ± 66.2	150.6 ± 55.4	143.7 ± 61.3
Glycosylated hemoglobin, %	4.1 ± 0.6*	8.8 ± 1.8	8.5 ± 2.1	8.4 ± 2.1
Serum total cholesterol, mg/dL	196.2 ± 39.0	218.4 ± 33.3	200.0 ± 49.9	206.6 ± 40.6
Serum HDL cholesterol, mg/dL	51.3 ± 8.9	46.8 ± 10.2	44.8 ± 11.8	42.9 ± 9.6
Serum LDL cholesterol, mg/dL	121.2 ± 12.7*	145.6 ± 22.8	170.9 ± 37.8†	152.0 ± 22.9
Serum triglycerides, mg/dL	120.0 ± 30.6	141.0 ± 78.5	152.7 ± 98.9	184.1 ± 91.0†
UAE, mg/mg	0.012 ± 0.004*	0.068 ± 0.031	0.065 ± 0.054	0.079 ± 0.077

HDL = high-density lipoprotein; LDL = low-density lipoprotein; UAE = urinary albumin excretion.
*$P < 0.05$ versus diabetic groups.
†$P < 0.05$ within group.

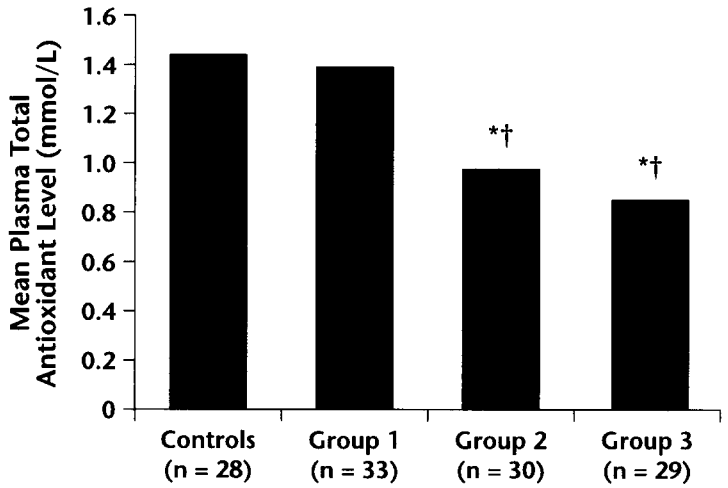

Figure 1. Plasma total antioxidant levels (mean ± SD, mmol/L) in the study population (N = 120). *$P < 0.05$ versus controls. †$P < 0.05$ within group.

for the comparisons between groups 2 and 3 versus the control group as well as for the comparisons between groups 1, 2, and 3.

Statistical analysis has shown that group 1 had circulating TAS higher than groups 2 and 3 and similar to the control group, whereas o-LAb levels in group 1 were lower than in groups 2 and 3 and without statistical difference versus the control group.

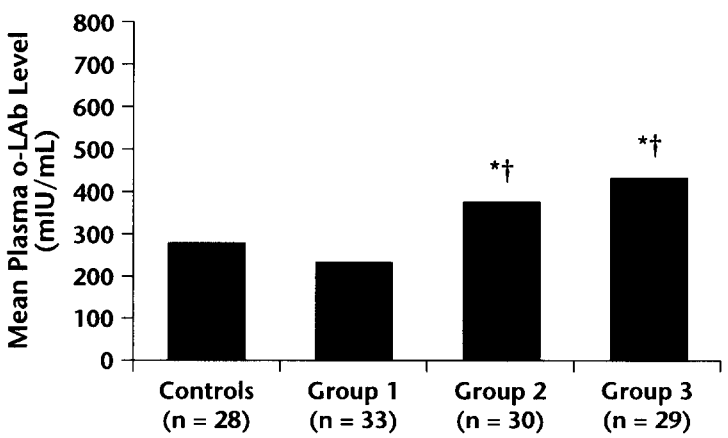

Figure 2. Plasma oxidized anti–low-density lipoprotein antibody (o-LAb) levels (mean ± SD, mIU/mL) in the study population (N = 120). *$P < 0.05$ versus controls. †$P < 0.05$ within group.

Multivariate analysis by stepwise regression showed a significant association with sex (with females having lower circulating o-LAb plasma levels; $P < 0.004$), age ($P < 0.019$), type of antidiabetic agent (with the gliclazide group having the lowest values; $P < 0.001$), and an inverse correlation with TAS ($P < 0.012$). When TAS was considered as the dependent variable, an inverse correlation with o-LAb level ($P < 0.002$) and with the type of therapy ($P < 0.007$) was observed.

DISCUSSION

The long-term effects of oxidative stress on the vascular complications of diabetes and on the worsening of insulin deficit are still being debated. The role of hyperglycemia in inducing oxidative damage has been investigated in several diabetic micro- and macroangiopathic complications, including Schwann fiber demyelinization,[35] glomerular lesions,[36] and endothelial cell damage leading to atherosclerotic plaque formation.[37]

The observation that oxygen free radicals mediate beta cell destruction in the animal model of streptozocin-induced diabetes suggests that oxidative stress also may play a role in the initial stages of type 1 DM.[35] In humans with type 1 DM, this mechanism could be involved in the development of autoimmune insulitis by inducing apoptosis. Oxygen free radicals also may contribute to insulin resistance and thus to the development and progression of type 2 DM because hydrogen peroxide has been observed to potently inhibit insulin signaling in target cells.[38]

The importance of the redox state in patients with type 1 or 2 DM is such that the search for pharmacologic agents capable of significantly inhibiting oxidative phenomena or of enhancing antioxidant systems is of particular relevance. Among oral antidiabetic agents, gliclazide has received special attention for its putative antioxidant role.

The results of this study appear particularly relevant, as we have confirmed the possibility of quantifying in vivo the beneficial effects of gliclazide on redox status and of comparing these effects with those of other antidiabetic oral agents in a carefully selected group of metabolically stable patients free of vascular manifestations.

The results of this study demonstrate that gliclazide therapy for patients with type 2 DM induces a redox status similar to that of healthy subjects. This effect most likely is mediated by the chemical structure of the molecule, containing an azabicyclo-octil ring and a sulfhydryl group known to neutralize oxidant-mediated effects secondary to a variety of mechanisms, including hyperglycemia.[39]

CONCLUSIONS

In this study population, the ability to reduce oxidative stress, which may be unique to gliclazide and not shared by the other antidiabetic agents, is of

considerable interest but needs further study. The effects of gliclazide on additional cohorts of diabetic patients with different degrees of chronic complications, as well as other conditions in which oxygen free radicals are involved, should be assessed.

REFERENCES

1. Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. 2nd ed. New York: Oxford University Press; 1989.
2. Oberley LW. Free radicals and diabetes. *Free Radic Biol Med*. 1988;5:113–124.
3. Santini SA, Marra G, Giardina B, et al. Defective plasma antioxidant defenses and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM. *Diabetes*. 1997;46:1853–1858.
4. Will JC, Byers T. Does diabetes mellitus increase the requirement for vitamin C? *Nutr Rev*. 1996;54:193–202.
5. Jain SK, McVie R. Effect of glycemic control, race (white versus black), and duration of diabetes on reduced glutathione content in erythrocytes of diabetic patients. *Metabolism*. 1994;43:306–309.
6. Wolff SP, Dean RT. Glucose autoxidation and protein modification: The potential role of 'autoxidative glycosylation' in diabetes. *Biochem J*. 1987;245:243–250.
7. Mossine VV, Linetsky M, Glinsky GV, et al. Superoxide free radical generation by Amadori compounds: The role of acyclic forms and metal ions. *Chem Res Toxicol*. 1999;12:230–236.
8. Bonnefont-Rousselot D, Bastard JP, Jaudon MC, Delttre J. Consequences of the diabetic status on the oxidant/antioxidant balance. *Diabetes Metab*. 2000;26:163–176.
9. Lee AY, Chung SS. Contributions of polyol pathway to oxidative stress in diabetic cataract. *FASEB J*. 1999;13:23–30.
10. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes*. 1991;40:405–412.
11. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care*. 1996;19:257–267.
12. Semenkovich CF, Heinecke JW. The mystery of diabetes and atherosclerosis: Time for a new plot. *Diabetes*. 1997;46:327–334.
13. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. *Diabetes*. 1999;48:1–9.
14. Matteucci E, Giampietro O. Oxidative stress in families of type 1 diabetic patients. *Diabetes Care*. 2000;23:1182–1186.
15. O'Brien RC, Luo M. The effects of gliclazide and other sulfonylureas on low-density lipoprotein oxidation in vitro. *Metabolism*. 1997;46(Suppl 1):22–25.
16. Noda Y, Mori A, Packer L. Gliclazide scavenges hydroxyl, superoxide and nitric oxide radicals: An ESR study. *Res Commun Mol Pathol Pharmacol*. 1997;96:115–124.
17. Desfaits AC, Serri O, Renier G. Normalization of plasma lipid peroxides, monocyte adhesion, and tumor necrosis factor-alpha production in NIDDM patients after gliclazide treatment. *Diabetes Care*. 1998;21:487–493.
18. Renier G, Desfaits AC, Serri O. Gliclazide decreases low-density lipoprotein oxidation and monocyte adhesion to the endothelium. *Metabolism*. 2000;49(Suppl 1):17–22.
19. Jennings PE, Belch JJ. Free radical scavenging activity of sulfonylureas: A clinical assessment of the effect of gliclazide. *Metabolism*. 2000;49(Suppl 1):23–26.

20. 1999 World Health Organization–International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Subcommittee. *J Hypertens.* 1999; 17:151–183.
21. Ceriello A, Boemi M, Cucinotta D, et al, for the Società Italiana di Diabetologia, the Diabetes and Atherosclerosis Study Group, Associazione Medici Diabetologi. Recommendations for cardiovascular assessment and risk reduction in diabetes—1999. *Cardiologia.* 1999;44:751–758.
22. Zingirian M, Cardillo Piccolino F, Ghiglione D. Protocollo di screening, diagnosi e follow-up della retinopatia diabetica. *Il Diabete.* 1993;5:44–49.
23. Diabetic nephropathy. *Diabetes Care.* 2000;23(Suppl 1):S69–S72.
24. Sosenko JM, Sparling YH, Hu D, et al. Use of the Semmes-Weinstein monofilament in the strong heart study: Risk factors for clinical neuropathy. *Diabetes Care.* 1999;22: 1715–1721.
25. Sosenko JM, Boulton AJ, Kubrusly DB, et al. The vibratory perception threshold in young diabetic patients: Associations with glycemia and puberty. *Diabetes Care.* 1985;8:605–607.
26. Ewing DJ, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care.* 1985;8:491–497.
27. Spallone V, Menzinger G. Diagnosis of cardiovascular autonomic neuropathy in diabetes. *Diabetes.* 1997;46(Suppl 2):S67–S76.
28. Keys A, Fidanza F, Karvonen MJ, et al. Indices of relative weight and obesity. *J Chronic Dis.* 1972;25:329–343.
29. Dunn PJ, Cole RA, Soeldner JS. Further development and automation of a high pressure liquid chromatography method for the determination of glycosylated hemoglobins. *Metabolism.* 1979;28:777–779.
30. Signorini AM, Fondelli C, Manetti E, et al. Follow-up a cinque anni della nefropatia in una popolazione di pazienti con diabete non insulino-dipendente (NIDDM) afferente a un servizio di Diabetologia. *G Ital Diabetol.* 1997;17:163–170.
31. Hilborne LH, Lin PC, Higgins SA, Rodgerson DO. Evaluation of the Behring Nephelometer for detection of low level urinary albumin. *Am J Clin Pathol.* 1990;93,405–410.
32. Miller NJ, Rice-Evans C, Davies MJ, et al. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci (Lond).* 1993;84:407–412.
33. Festa A, Kopp HP, Schernthaner G, Menzel EJ. Autoantibodies to oxidised low density lipoproteins in IDDM are inversely related to metabolic control and microvascular complications. *Diabetologia.* 1998;41:350–356.
34. The 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.* 1993;329:977–986.
35. West IC. Radicals and oxidative stress in diabetes. *Diabet Med.* 2000;17:171–180.
36. Bendayan M. Immunocytochemical detection of advanced glycated end products in rat renal tissue as a function of age and diabetes. *Kidney Int.* 1998;54:438–447.
37. Lopes-Virella MF, Virella G, Orchard TJ, et al. Antibodies to oxidized LDL and LDL-containing immune complexes as risk factors for coronary artery disease in diabetes mellitus. *Clin Immunol.* 1999;90:165–172.
38. Hansen LL, Ikeda Y, Olsen GS, et al. Insulin signaling is inhibited by micromolar concentrations of H_2O_2. Evidence for a role of H_2O_2 in tumor necrosis factor alpha–mediated insulin resistance. *J Biol Chem.* 1999;274:25078–25084.

39. Scott NA, Jennings PE, Brown J, Belch JJ. Gliclazide: A general free radical scavenger. *Eur J Pharmacol.* 1991;208:175–177.

Address correspondence to:
Anna Maria Signorini, MD
Dipartimento di Farmacologia "G. Segre"
Università degli Studi di Siena
Strada Delle Scotte 6
53100 Siena
Italy
E-mail: signorini@unisi.it