Effects of Irbesartan on Intracellular Antioxidant Enzyme Expression and Activity in Adolescents and Young Adults With Early Diabetic Angiopathy

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OBJECTIVE — Defective intracellular antioxidant enzyme production (IAP) has been demonstrated in adults with diabetic nephropathy. The objective of this study was to evaluate the effects of irbesartan, an angiotensin II receptor antagonist, on IAP in adolescents and young adults with type 1 diabetes and early signs of retinopathy and nephropathy.

RESEARCH DESIGN AND METHODS — This prospective, matched case-control study was conducted between November 2001 and December 2002 among 14 type 1 diabetic patients with early signs of angiopathy (ages 14–21 years), 11 type 1 diabetic patients without angiopathy (ages 12–22 years), and 10 healthy volunteers (ages 16–22 years). Skin fibroblasts were obtained by skin biopsies from the anterior part of the forearm and cultured in Dulbecco's modified Eagle's medium. The activity and mRNA expression of CuZn superoxide dismutase (CuZnSOD), Mn superoxide dismutase (MnSOD), catalase (CAT), and glutathione peroxidase (GPX) were measured before and after 6 months of treatment with irbesartan (150 mg/day); on both occasions, antioxidant enzyme activity was evaluated at different glucose concentrations (5 and 22 mmol/l).

RESULTS — At a normal glucose concentration (5 mmol/l), the activity and mRNA expression of CuZnSOD (0.50 ± 0.21 units/mg protein, 4.4 ± 1.5 mRNA/glyceraldehyde-3-phosphate dehydrogenase), MnSOD (0.26 ± 0.04 units/mg protein, 0.08 ± 0.07 mRNA), CAT (0.32 ± 0.08 units/mg protein, 4.8 ± 1.3 mRNA), and GPX (0.53 ± 0.09 units/mg protein, 2.2 ± 0.9 mRNA) were not different among the three groups (only values of diabetic subjects with angiopathy are shown). At high glucose concentrations, the activity and mRNA expression of CuZnSOD increased similarly in all groups (diabetic subjects with angiopathy: 0.93 ± 0.26 units/mg protein, 9.4 ± 2.1 mRNA); that of CAT and GPX increased in only control subjects and diabetic subjects without angiopathy (diabetic subjects with angiopathy: 0.33 ± 0.09 units/mg protein and 5.0 ± 1.4 mRNA; 0.54 ± 0.10 units/mg protein and 2.3 ± 1.0 mRNA, respectively). MnSOD did not change in any group. Treatment with irbesartan in adolescents with diabetic angiopathy was able to restore CAT and GPX activity and mRNA expression after exposure to high glucose concentrations. Markers of oxidative stress (serum malondialdehyde, fluorescent products of lipid peroxidation, monocyte chemoattractant protein-1, and 8-isoprostanes prostaglandin $F_{2\alpha}$) were significantly reduced after treatment with irbesartan.

CONCLUSIONS — Adolescents and young adults with early signs of diabetic angiopathy have defective intracellular antioxidant enzyme production and activity. Treatment with irbesartan can substantially improve the activity and production of these enzymes in skin fibroblasts.

Diabetes Care 28:1690–1697, 2005

It has recently been shown that oxidative stress may play a role in the pathogenesis of diabetic vascular complications (1). Hyperglycemia can induce increased production of reactive oxygen metabolites and species. Elevated glucose concentrations may also increase the levels of oxygen radical scavenging enzymes in cultured endothelial cells (1) and the kidney of rats with streptozotocin-induced diabetes (1). The formation of oxygen-derived free radicals and the activation of oxidative stress through nonenzymatic glycation of proteins, autooxidative glycation, activation of protein kinase C, and the increased polyol pathway may be induced by hyperglycemia.

In normal subjects, exposure to high glucose concentrations induces an antioxidant defensive mechanism in skin fibroblasts; in adult type 1 diabetic subjects with macroalbuminuria and overt nephropathy, this defensive mechanism is markedly impaired (2). A defective antioxidant response to hyperglycemia has been recently demonstrated in peripheral blood mononuclear cells of patients with type 1 diabetes and diabetic nephropathy (3).

Recently, we demonstrated that vitamin E supplementation (600 mg, b.i.d. for 3 months) is unable to significantly modify antioxidant mechanisms in skin fibroblasts of adolescents and young adults with type 1 diabetes and angiopathy (retinopathy or nephropathy) (4).

Irbesartan is a potent and selective angiotensin II (ANG-II) subtype 1 receptor antagonist widely used in adult patients

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Received for publication 26 January 2005 and accepted in revised form 28 March 2005.

Abbreviations: AER, albumin excretion rate; ANG-II, angiotensin II; CAT, catalase; CuZnSOD, CuZn superoxide dismutase; DMEM, Dulbecco's modified Eagle's medium; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPX, glutathione peroxidase; MCP-1, monocyte chemoattractant protein 1; MnSOD, Mn superoxide dismutase; PGF_{2a}, prostaglandin F_{2a}; RAS, renin-angiotensin system; SOD, superoxide dismutase; SSC, standard sodium citrate.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Characteristics of subjects*

	Diabetic subjects with angiopathy	Diabetic subjects without angiopathy	Nondiabetic control subjects
n (female/male)	14 (6/8)	11 (6/8)	10 (5/5)
Age (years)	17.4 (14–21)	16.9 (12–22)	18.1 (16–22)
Diabetic duration (years)	14.1 (11–19)	14.5 (11–20)	—
AIC (%)	10.2 (8.3–11.5)*	8.1 (6.9–9.3)	4.5 (4.0–4.9)
Insulin requirement (units · kg^{-1} · day^{-1})	1.2 (0.9–1.5)	1.1 (0.8–1.2)	—
Mean blood pressure (mmHg)	97.5 (90–103)*	94.9 (88–99)	92 (89–94)
Albumin excretion rate (μg/min)	72 (28–135)*	11 (6–20)	5 (3–9)
Glomerular filtration rate (ml · min^{-1} · 1.73 m^{-2})	153 (142–160)*	130 (124–137)	—

Data are median (range). *$P < 0.01$.

with hypertension and diabetic nephropathy. Data from two large randomized, double-blind, placebo-controlled studies, the Irbesartan Microalbuminuria Type 2 Diabetes Mellitus in Hypertensive Patients study (5), and the Irbesartan Type 2 Diabetic Nephropathy Trial (6), show that irbesartan can slow the development of overt nephropathy and the progression of renal disease in hypertensive patients with type 2 diabetes and suggest that the renoprotective effect of irbesartan is at least in part independent of its blood pressure−lowering effect.

In the present study, we evaluated intracellular antioxidant enzyme production in skin fibroblasts of young diabetic patients with persistent microalbuminuria and early diabetic angiopathy. We also investigated whether the administration of irbesartan (150 mg/day) is able to modify this cellular antioxidant mechanism.

RESEARCH DESIGN AND METHODS

— All patients gave their informed consent to the study, which was approved by the Ethics Committee of the School of Medicine, University of Chieti, Italy. Subjects ≥18 years of age signed their own consent; for subjects ≤17 years of age, their parents signed consent and the adolescents signed their own assent.

The study group was comprised of 14 adolescents or young adults with type 1 diabetes (ages 14–21 years, duration of diabetes 11–19 years). Of the 14 subjects, 9 had retinopathy (6 with background retinopathy and 3 with preproliferative retinopathy), 5 had persistent microalbuminuria (defined as an albumin excretion rate [AER] >20 μg/min in two of three overnight urinary collections), and 5 had both conditions. The control groups were comprised of 11 type 1 diabetic subjects without angiopathy (ages 12–22 years) and 10 healthy volunteers (ages 16–22 years).

Blood samples were obtained from all participants after a 12-h fast. Each participant performed an overnight urine collection before blood samples were taken. The antioxidant 4-hydroxy-TEMPO (1 mmol/l; Sigma, St. Louis, MO) was added to the urine samples, which were then stored at −20°C until extraction. Blood pressure and AER were measured before and after 3 and 6 months of irbesartan treatment.

Skin fibroblasts were obtained by 5-mm punch biopsies taken under local anesthetic from the anterior surface of the forearm. The fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM; ICN Biochemicals, Thame, U.K.). CuZn superoxide dismutase (CuZnSOD), Mn superoxide dismutase (MnSOD), catalase (CAT), and glutathione peroxidase (GPX) activity and mRNA expression were measured before and after 6 months of treatment with irbesartan (150 mg/day). On both occasions, antioxidant enzyme activity was evaluated ex vivo at different glucose concentrations (5 and 22 mmol/l). Clinical characteristics of the subjects participating in the study are summarized in Table 1.

Arterial blood pressure was measured in all patients and control subjects following the recommendations of the American Heart Association and the American Academy of Pediatrics. The glomerular

Table 2—*Antioxidant enzyme activity in skin fibroblasts in normal and high glucose conditions*

	Diabetic subjects with angiopathy		Diabetic subjects without angiopathy		Nondiabetic control subjects	
	Normal glucose	High glucose	Normal glucose	High glucose	Normal glucose	High glucose
CuZnSOD (units/mg protein)	0.50 ± 0.21 (0.28–0.73)	0.93 ± 0.26* (0.70–1.18)	0.56 ± 0.24 (0.33–0.81)	0.97 ± 0.30* (0.72–1.27)	0.63 ± 0.22 (0.34–0.88)	0.89 ± 0.24* (0.71–1.26)
MnSOD (units/mg protein)	0.26 ± 0.04 (0.20–0.31)	0.27 ± 0.05 (0.20–0.33)	0.25 ± 0.05 (0.19–0.32)	0.27 ± 0.06 (0.20–0.34)	0.24 ± 0.04 (0.19–0.30)	0.27 ± 0.07 (0.21–0.35)
Catalase (units/mg protein)	0.32 ± 0.08 (0.23–0.41)	0.33 ± 0.09 (0.22–0.41)	0.33 ± 0.06 (0.24–0.40)	0.71 ± 0.09* (0.60–0.82)	0.33 ± 0.07 (0.23–0.41)	0.72 ± 0.10* (0.61–0.84)
GPX (units/mg protein)	0.53 ± 0.09 (0.42–0.61)	0.54 ± 0.10 (0.43–0.64)	0.56 ± 0.10 (0.43–0.67)	0.94 ± 0.13* (0.80–1.08)	0.60 ± 0.11 (0.48–0.72)	0.94 ± 0.11* (0.81–1.07)

Data are means ± SD (range). Normal glucose, 5 mmol/l; high glucose, 22 mmol/l. *$P < 0.01$ for 5 vs. 22 mmol/l glucose.

Table 3—*mRNA expression of antioxidant enzymes in skin fibroblasts in normal and high glucose conditions*

	Diabetic subjects with angiopathy		Diabetic subjects without angiopathy		Nondiabetic control subjects	
	Normal glucose	High glucose	Normal glucose	High glucose	Normal glucose	High glucose
CuZnSOD	4.4 ± 1.5	9.4 ± 2.1	4.7 ± 2.0	9.5 ± 2.2	4.9 ± 2.0	9.1 ± 2.2
(units/mg protein)	(2.9–5.7)	(7.2–11.4)	(2.8–6.2)	(7.3–11.6)	(2.9–6.5)	(7.1–11.6)
MnSOD	0.8 ± 0.07	0.9 ± 0.08	0.8 ± 0.09	0.9 ± 0.10	0.74 ± 0.09	0.81 ± 0.11
(units/mg protein)	(0.7–0.9)	(0.7–1.0)	(0.7–1.0)	(0.8–1.1)	(0.6–0.9)	(0.7–1.0)
Catalase	4.8 ± 1.3	5.0 ± 1.4	4.6 ± 1.3	9.0 ± 2.2	4.9 ± 1.4	9.2 ± 2.1
(units/mg protein)	(3.6–6.0)	(3.7–6.5)	(3.5–6.0)	(6.9–11.1)	(3.4–6.2)	(7.0–11.4)
GPX	2.2 ± 0.9	2.3 ± 1.0	2.3 ± 1.0	4.2 ± 1.4	2.4 ± 0.9	4.3 ± 1.2
(units/mg protein)	(1.2–3.2)	(1.3–3.4)	(1.2–3.4)	(2.9–5.7)	(1.3–3.4)	(3.0–5.7)

Data are means ± SD (range). Normal glucose, 5 mmol/l; high glucose, 22 mmol/l.

filtration rate was measured as previously described (7). No patient was taking any other medications at the time of the study.

Cell culture

Fibroblasts were cultured in DMEM (ICN) supplemented with 20% FCS (Life Technologies, Paisley, U.K.), 2 mmol/l glutamine (Sigma, Dorset, U.K.), 50 units/ml penicillin (Life Technologies), and 50 μg/ml streptomycin (Life Technologies). At the fourth passage, cells were cooled gradually and then frozen at −180°C in 10% DMSO in DMEM until used for the experiments. It is well recognized that even long-term cryopreservation does not affect fibroblasts' functional activities.

All of the experiments were conducted between the sixth and eighth passages with the same batches of medium and FCS. Using these passages is considered to be a suitable method for studying fibroblasts from donor patients (1,2). The purchased medium contained 5 mmol/l glucose, to which mannitol or glucose was added to ensure that the high and normal glucose culture media had the same osmolarity. Cells were cultured in isosmotic normal (5 mmol/l) and high ex vivo glucose (22 mmol/l) concentrations.

Each sample of cells was grown for 12 weeks, with the medium being renewed every 2nd day. For each culture condition (normal or high glucose), 12 80-cm^2 plastic tissue culture flasks were used: 3 for RNA extraction, 3 for enzyme activity measurement, 3 for the evaluation of cell membrane lipid peroxidation, and 3 to determine cell number.

Cell counting

The medium was aspirated, and the monolayers were washed twice with PBS and detached by treatment with 2.5 ml trypsin-EDTA (Life Technologies) for 4–6 min at 37°C. Trypsin activity was stopped by adding 7 ml of medium containing serum after complete detachment of the cells was verified under the microscope. The cell suspension was passed several times through a fine Pasteur pipette to disaggregate cell clumps and 1 ml was counted in an electronic Coulter counter (ZBI model; Coulter Electronics, Luton, U.K.) equipped with a 100-μm aperture.

Antioxidant enzyme activity

Catalase and glutathione peroxidase activity. The monolayers were rinsed twice with ice-cold PBS, and the cells were harvested with a sterile rubber cell scraper. The cells were sedimented for 4 min at 1,600*g* and processed for enzyme/protein or mRNA analyses. For enzyme/protein lysates, cells were resuspended in 50 mmol/l potassium phosphate buffer containing 0.5% Triton X-100 and sonicated in an ice-water bath for two 30-s bursts on a Branson sonicator B15 (position 2, continuous setting; Branson Ultrasonic, Danbury, CT) with a 30-s cooling interval. Total protein concentration was determined as previously described (4). For CAT and GPX activities, sonicates were first spun for 5 min at 800*g* (4°C). The supernatants were assayed according to the procedure of Clairborne (8) for CAT activity and Gunzler and Flohè for GPX activity, as previously described (4).

Superoxide dismutase measurements. Cells were suspended in 100 mmol/l triethanolamine-diethanolamine buffer and homogenized with a Teflon glass Dounce homogenizer. The homogenate was centrifuged at 105,000*g* for 1 h (4°C), and the supernatant was passed through a small Sephadex G25 (coarse) column to remove low−molecular-weight substances that might interfere with the enzyme assay. An aliquot of the eluate was applied onto a 5.5% polyacrylamide gel to localize superoxide dismutase (SOD) activity, with the exception that no tetramethylethylenediamine was used for staining.

Mn superoxide dismutase activity. MnSOD activity was determined in mitochondrial fractions prepared by differential centrifugation, as previously described (4). Mitochondria were disrupted by a freeze-thaw procedure in a high ionic-strength buffer (0.25 mmol/l sucrose, 0.12 mol/l KCl, and 10 mmol/l Tris-HCl; pH 7.4). Mitochondrial membranes were removed by sedimentation at 105,000*g* for 1 h (4°C) and enzyme activity was measured in the supernatant.

Northern blot analysis

Total RNA was prepared according to the procedure of Chirgwin et al. (8a), as previously described (4). Briefly, 10 μg total RNA were electrophoresed on a 1.4% agarose-formaldehyde gel and then transferred to gene screen membranes. The filters were prehybridized in 50 mmol/l Tris-HCl (pH 7.5), 0.1% sodium pyrophosphate, 0.2% Ficoll, 5 mmol/l EDTA, 1% SDS, 2.2% poly(vinylpyrrolidone), 50% formamide, 0.2% BSA, 1 × standard sodium citrate (SSC), and 150 μg/ml denatured salmon sperm DNA at 65°C for 6 h. Blots were hybridized with ^{32}P-labeled probes for human CuZnSOD, CAT, and MnSOD and bovine GPX to a specific activity of 1×10^6 cpm/ml in hybridization fluid at 65°C overnight (4). The filters were washed at 65°C twice for 15 min with 2 × SSC (0.1%(and twice for 15 min with 0.1 × SSC (0.1%) SDS and then subjected to autoradiography using

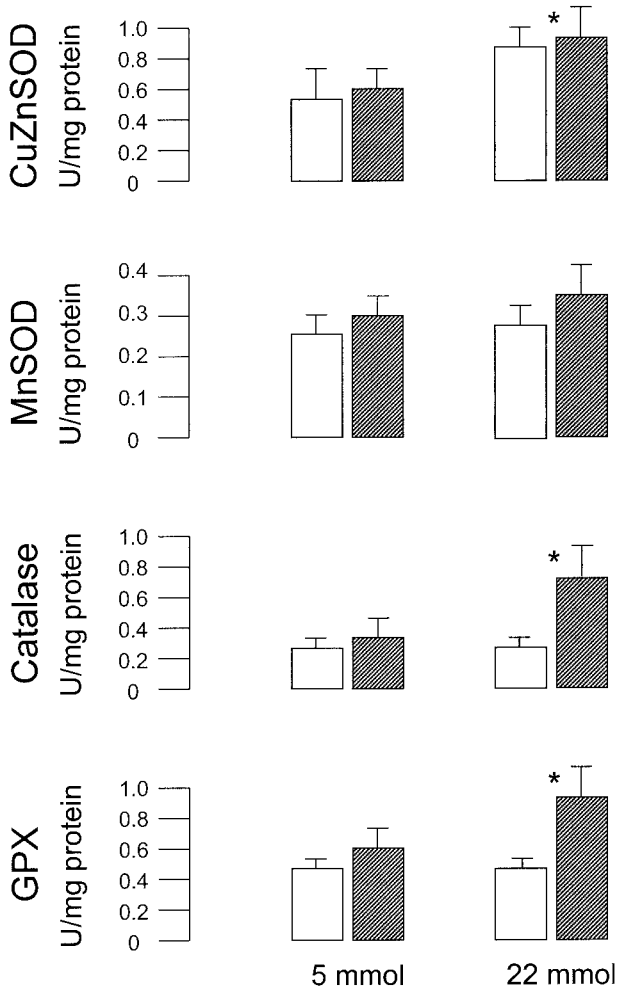

Figure 1—*Antioxidant enzyme activity in skin fibroblasts from adolescents with diabetic angiopathy before (□) and after (▨) treatment with irbesartan (150 mg/day). Enzyme activity was measured in normal (5 mmol/l) and high (22 mmol/l) glucose conditions.* *P $<$ *0.001.*

an intensifying screen at −85°C. Densitometry was performed on an LKB laser scanning densitometer. Hybridization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was used as an internal control to correct for loading inequalities.

The filters were probed for the four antioxidant enzymes separately and GAPDH was also used separately. The results were normalized against an ideal reference value obtained from healthy individuals at 5 mmol glucose/l ex vivo.

Lipid peroxidation

Cells were trypsinized and centrifuged at 250*g* for 10 min at 4°C. Cell pellets were resuspended in 1 ml cold PBS for assay of thiobarbituric acid−reactive substances and conjugated dienes, as previously described (4).

Assays

Urinary 8-isoprostanes prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) excretion rates were measured by previously described radioimmunoassay methods (9). Lipid peroxidation in native LDLs and the plasma lipid peroxide content were evaluated as previously reported (10). Concentrations of monocyte chemoattractant protein 1 (MCP-1) were determined in triplicate by enzyme-linked immunosorbent assay (11). Endothelial dysfunction was evaluated by measuring von Willebrand factor; its propeptide, prothrombin fragment 1 and 2 (F_{1+2}); and tissue-plasminogen activator, as previously described (12,13).

Statistical analyses

ANOVA was used to test differences among the three groups. For each group of fibroblasts, a paired *t* test was used to compare the results under conditions of normal versus high ex vivo glucose concentration, and Fisher's least significant differences test was used to evaluate the differences among the three different groups in the normal or high glucose condition. $P < 0.05$ was considered significant. Data are expressed as means ± SD or as medians (ranges).

RESULTS

Antioxidant enzyme activity

CuZn superoxide dismutase. In normal ex vivo glucose concentrations, CuZnSOD activity and mRNA expression were not different among the three groups. In high ex vivo glucose conditions, CuZnSOD mRNA and activity increased similarly in all groups (NS by ANOVA).

Mn superoxide dismutase. In normal ex vivo glucose concentrations, MnSOD activity and mRNA expression was not different among the three groups. In high ex vivo glucose conditions, MnSOD did not change in any group.

Catalase and glutathione peroxidase. In normal ex vivo glucose concentrations, CAT and GPX activity and mRNA expression were not different among the three groups. In high ex vivo glucose conditions, CAT and GPX mRNA ($P < 0.001$) and activity ($P < 0.001$) were significantly different among the groups by ANOVA (Tables 2 and 3). In high-glucose conditions before irbesartan treatment, CAT and GPX mRNA expression and protein activity were significantly higher in control subjects and diabetic subjects without angiopathy versus angiopathic diabetic subjects (Table 2 and 3); there was no difference among the control subjects and those without diabetic angiopathy.

Lipid peroxidation

High ex vivo glucose concentrations significantly increased lipid peroxidation in every group of cells. Higher levels were found in cells of adolescents and young adults with diabetic angiopathy ($P <$ 0.001).

Treatment with irbesartan

Treatment with irbesartan (150 mg/day) lasted for 6 months. No adverse events were evident in any patient. Irbesartan ameliorated the antioxidant enzymatic activity in both normal and hyperglycemic conditions (Fig. 1). mRNA expres-

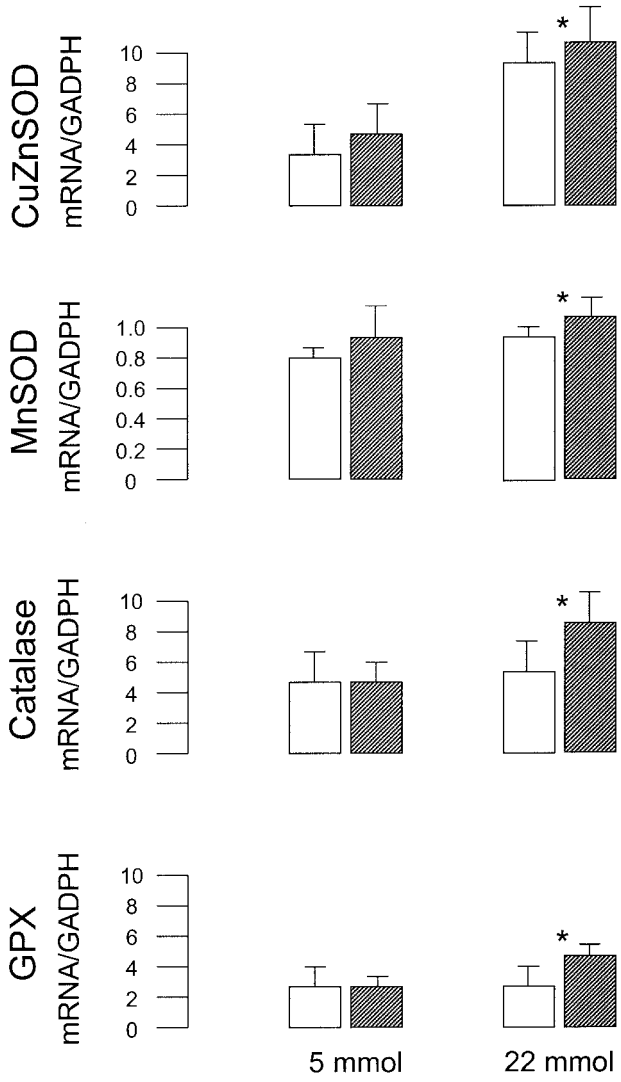

Figure 2—*mRNA expression of antioxidant enzymes in skin fibroblasts from adolescents with diabetic angiopathy before (□) and after (▨) treatment with irbesartan (150 mg/day). mRNA expression was measured in normal (5 mmol/l) and high (22 mmol/l) glucose conditions.* *$P < 0.001$.

sion was also increased after treatment with irbesartan (Fig. 2).

Irbesartan reduced lipid peroxidation in cells of adolescents and young adults with diabetic angiopathy. Serum markers of oxidant status and MCP-1 levels were decreased after the 6-month treatment (Fig. 3); urinary excretion of 8-isoprostanes $PGF_{2\alpha}$ was also reduced by inhibition of the ANG-II receptor (Fig. 3). Endothelial dysfunction was significantly improved after treatment with irbesartan (Fig. 4).

The urinary AER was reduced after treatment with irbesartan (median 72 [range 28–135] vs. 32 [10–54] μg/min; $P < 0.01$). Mean blood pressure was also reduced by irbesartan (97.5 [90–103] vs. 93.1 [88–96] mmHg; $P < 0.05$).

No relation between HbA_{1c} (A1C) values and markers of oxidative stress was found. No significant difference in A1C was observed after 6 months of treatment with irbesartan in patients with diabetic microangiopathy.

CONCLUSIONS — Our findings indicate that exposure to high ex vivo glucose concentrations induced an increase in mRNA levels and the biological activity of CuZnSOD, CAT, and GPX in fibroblasts from control subjects and adolescents without diabetic angiopathy; in contrast, the levels and activity of only CuZnSOD were increased in fibroblasts from diabetic adolescents with angiopathy. This finding may have important consequences with regard to glucose-induced oxidative stress injury to cells; in fact, glucose-induced oxidative stress has been demonstrated to damage several cells, including endothelial and mesangial cells (1).

Both CuZnSOD, which is located primarily in the cytoplasm, and MnSOD, a structurally distinct protein located in the mitochondria, catalyze the reaction $O_2^- + O_2^- + 2H^+ = O_2 + H_2O_2$ (1). H_2O_2 is converted to H_2O in peroxisomes by the antioxidant enzyme CAT and in the cytoplasm by GPX (14). These antioxidant enzymes protect the cell from oxidative stress, but the threshold of protection can vary dramatically as a function of the activity and balance of these enzymes (15). CAT and GPX are far more efficient than CuZnSOD in protecting fibroblasts against oxidative stress (15). However, in several instances, cells with increased levels of CuZnSOD are hypersensitive to oxidative stress rather than protected from it (15). This occurs because CuZnSOD increases the formation of H_2O_2, which, if not efficiently converted to H_2O by an adequate level of CAT and GPX, may be detrimental to the cell (15). It is therefore not surprising that an increase in CuZnSOD is generally accompanied by a concomitant increase in CAT and GPX (15). We confirmed this phenomenon in fibroblasts derived from control subjects and young diabetic patients without microvascular complications in the presence of high ex vivo glucose concentrations. In the fibroblasts of our subjects with childhood-onset diabetes and angiopathy, a high glucose concentration induced a significant increase only in CuZnSOD, with no change in the activity of CAT and GPX. These results confirm previous data obtained in adult type 1 diabetic patients with macroproteinuria and overt nephropathy (2) and suggest that cells of type 1 diabetic adolescents and young adults with incipient angiopathy are not able to adjust their antioxidant mechanisms when high ex vivo glucose–induced oxidative stress is produced; consequently, they are more susceptible to oxidative stress. Alternatively, it could be argued that in the absence of the ability to increase CAT and GPX, the cells may "decide" not to enhance CuZnSOD and MnSOD; in that situation, the mechanism

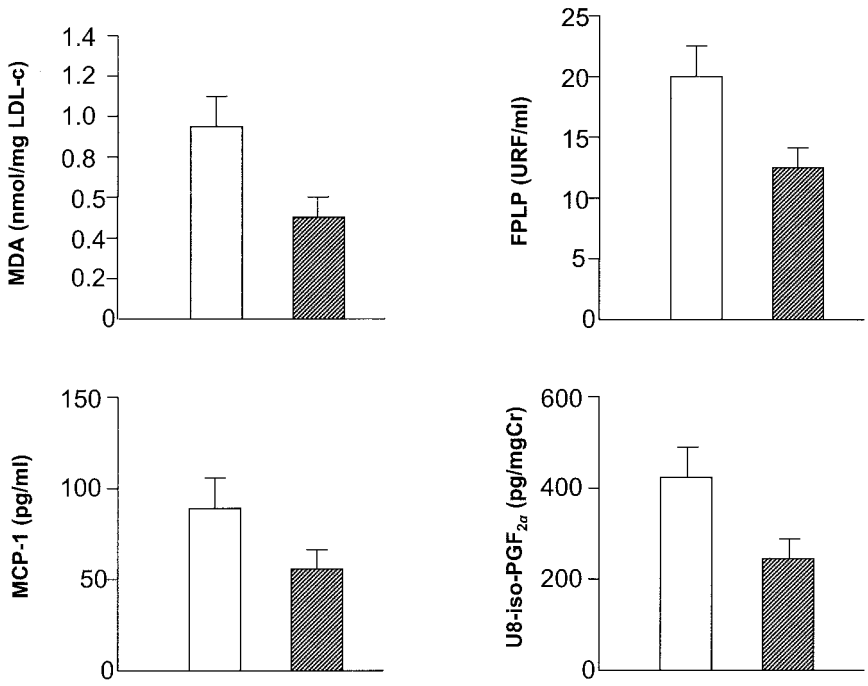

Figure 3—*Serum malondialdehyde (MDA), fluorescent products of lipid peroxidation (FPLP), MCP-1, and urinary excretion of 8-isoprostanes $PGF_{2\alpha}$ (U8-iso-$PGF_{2\alpha}$) before (□) and after (▨) treatment with irbesartan. Cr, creatinine; LDL-c, LDL cholesterol; URF, units of fluorescence.*

could simply be switched off. However, this event should also be operative in diabetic patients without angiopathy and control subjects.

High glucose concentrations in vitro and hyperglycemia in vivo are well-known stimuli for the production of free radicals and the generation of oxidative stress, with a consequent increase in the expression and activity of antioxidant enzymes (1), which act as a defense system against cell damage (14). The observation that despite hyperglycemia, only a portion of type 1 diabetic patients will progress to diabetic microangiopathy might indicate that there is individual diversity in cell response to high ex vivo glucose concentrations. It is therefore of great relevance that a disturbance in the mechanisms of protection from oxidative stress was found only in the cells of adolescents with angiopathy. By contrast, in adolescents and young adults with long-term type 1 diabetes but no angiopathy, a group that appears to be protected from vascular complications, the defense mechanisms against high glucose-induced oxidative stress were intact, as they were in our nondiabetic control subjects.

The novel finding of this study is that treatment with the selective ANG-II receptor antagonist irbesartan (150 mg/day) was able to substantially improve antioxidant enzyme production and activity in young patients showing early signs of diabetic angiopathy. Irbesartan was effective in decreasing markers of oxidant status and MCP-1 levels. Urinary excretion of 8-isoprostanes $PGF_{2\alpha}$ was also reduced and endothelial dysfunction was significantly improved after 6-month treatment with irbesartan.

Considerable evidence suggests that the intrarenal renin-angiotensin system (RAS) plays an important role in the development of diabetic nephropathy (16). Blockade of the RAS with either ACE inhibitors or ANG-II receptor antagonists delays the progression of renal injury associated with diabetes (17). In addition, inhibition of ACE by lisinopril may decrease retinopathy progression in normotensive patients with type 1 diabetes (18) and losartan (an angiotensin II receptor antagonist) may also be effective in preventing diabetic retinopathy (19).

Irbesartan is a newly approved ANG-II receptor antagonist with higher bioavailability, lower plasma protein binding, and a longer half-life than other similar drugs (e.g., losartan, valsartan). It selectively binds to the ANG-II receptor subtype 1, thereby inhibiting the activity of ANG-II. Recent studies in rats with streptozocin-induced diabetes have clearly demonstrated that irbesartan ex-

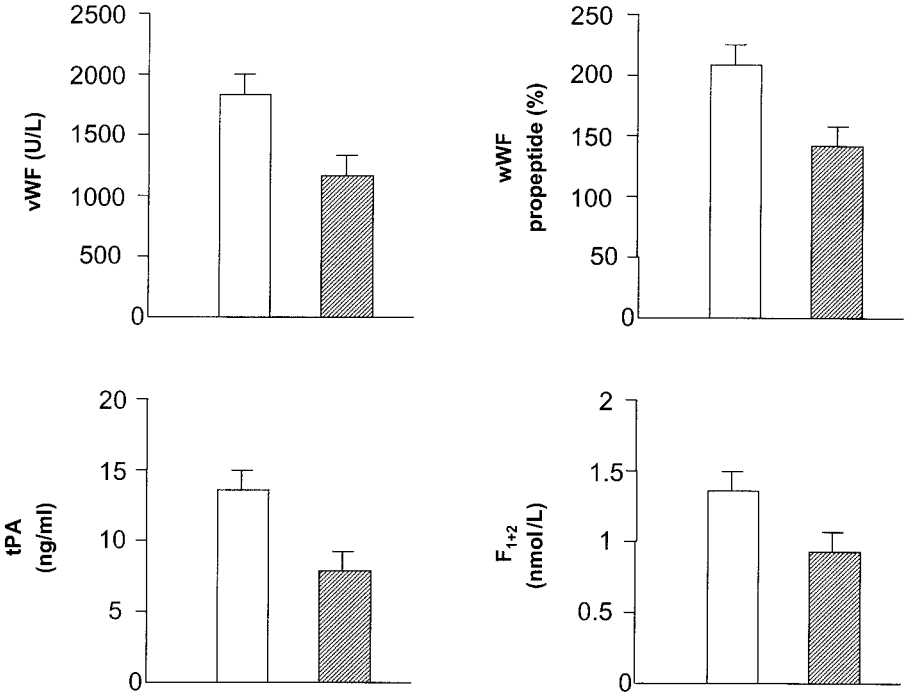

Figure 4—*von Willebrand factor (vWF), vWF propeptide, tissue-plasminogen activator (tPA), and prothrombin fragment 1 and 2 (F_{1+2}) in young patients with diabetic angiopathy before (□) and after (▨) treatment with irbesartan.*

erts a renal protective effect, reducing proteinuria and albuminuria and preventing renal hyperfiltration (20). Specifically, these recent results suggest that the renoprotective effect of irbesartan may be related to its inhibition of renal hypertrophy and expression of growth factors such as transforming growth factor-β_1 and connective tissue growth factor (20). More recently, a new ANG-II receptor blocker (L-158,809) has demonstrated a role in attenuating overexpression of vascular endothelial growth factor in diabetic podocytes (21). Taken together, these data provide robust evidence that ANG-II may be as dangerous as hyperglycemia in inducing vascular and renal damage in diabetic individuals (22). In a recent study, irbesartan was shown to be capable of reducing the incidence of congestive heart failure in patients with type 2 diabetes and overt nephropathy (23).

This is the first study to give solid evidence that irbesartan (150 mg/day) is able to significantly improve intracellular antioxidant enzyme production and activity in skin fibroblasts of adolescents and young adults with childhood-onset type 1 diabetes. This finding is not surprising because it is now well recognized that ANG-II and activated angiotensin I receptors produce intracellular oxidative stress; furthermore, recent data have shown that hyperglycemia is able to directly modulate cellular angiotensin production (24). Indeed, ACE inhibitors and ANG-II receptor antagonists have been demonstrated to act as causal antioxidants; it has been suggested that this property may account for their beneficial effect on diabetic complications (8).

The finding that irbesartan was able to significantly reduce serum markers of oxidative stress and improve endothelial function in young patients with diabetic angiopathy gives further support to the role of ANG-II in the progression of vascular damage in diabetes. Irbesartan has beneficial effects not only on blood pressure and microalbuminuria, but also on antioxidant capacity.

In conclusion, this study confirms that exposure to high ex vivo glucose concentrations induces an antioxidant defense mechanism in skin fibroblasts of normal young subjects and that a failure of this defensive mechanism is present in fibroblasts of young patients with childhood-onset diabetes and early signs of diabetic retinopathy and nephropathy. Treatment with irbesartan, at a dosage of 150 mg/day, significantly improves these cellular antioxidant mechanisms, reduces serum markers of oxidative stress, and ameliorates endothelial dysfunction.

Acknowledgments— This study was supported by a grant from the Italian Ministry of Research and a generous donation by Fondazione Carlo Erba, Italy.

We acknowledge the editorial assistance provided by Antonella Bascelli. We also thank the Regional Juvenile Diabetes Association and in particular Doriana D'Alimonte D'Attilio for support in childhood diabetes care and research.

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