Effect of Insulin Versus Sulfonylurea Therapy on Cardiovascular Risk Factors and Fibrinolysis in Type II Diabetes

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In non–insulin-dependent diabetes mellitus (NIDDM), cardiovascular risk factors improve during treatment, but whether insulin (I) differs from sulfonylurea (SU) therapy is unclear. To separate the contributions of improved diabetic control versus treatment regimen to risk factors, we examined the effects of SU and I on insulin sensitivity, basal and post–glucose load levels of insulin-like molecules, fibrinolysis, and lipid concentrations. Twenty poorly controlled, diet-treated NIDDM subjects were given I or SU each for a period of 16 weeks in a randomized crossover study, with a 4-week washout period between each treatment. Subjects were studied at the baselines (B1 and B2) and after each treatment. Treatment with I or SU produced similar improvements in glycemia (hemoglobin A_1 [HbA_1] B1, 11.7% ± 2.1%; SU, 8.5% ± 0.9%; I, 8.6% ± 1.2%) and the metabolic clearance rate of glucose ([MCR-G] B1, 1.86 ×/÷ 1.4; SU, 2.36 ×/÷ 1.4 ($P = .005$ *v* B1); I, 2.27 ×/÷ 1.4 ($P = .07$ *v* B1) ml · kg^{-1} · min^{-1}). On SU therapy, subjects had higher fasting and post–glucose load levels of intact proinsulin compared with B1 and I (fasting, 13.9 ×/÷ 2.6 *v* 9.5 ×/÷ 2.2 ($P = .004$) and 9.1 ×/÷ 2.4 pmol · L^{-1} ($P = .01$), respectively). Plasminogen activator inhibitor-1 (PAI-1) activity and antigen were higher than at B1 on SU therapy (23.7 *v* 19.9 AU · mL^{-1} ($P = .02$) and 47.6 *v* 32.2 ng · mL^{-1} ($P = .006$), respectively), but not on I. There were no changes compared with B1 and no differences between the two therapies in total, very–low-density lipoprotein (VLDL), and intermediate-density lipoprotein (IDL) cholesterol and triglyceride, low-density lipoprotein (LDL), high-density lipoprotein 2 (HDL_2) and HDL_3 cholesterol, apolipoprotein (apo) A1, A2, and B1, or lipoprotein (a) [Lp(a)] levels. In conclusion, (1) treatment with SU or I resulted in equal improvement in glycemia and insulin sensitivity, (2) intact proinsulin and PAI-1 antigen and activity were higher on SU, and (3) there were no differences in lipid concentrations with improved glycemia or between therapies.

CONVENTIONAL RISK FACTORS such as smoking, lipids, and blood pressure (BP) do not fully explain the excess cardiovascular risk in subjects with non–insulin-dependent diabetes mellitus (NIDDM), and it does not appear to relate to glycemic control.[1] It is important that hypoglycemic therapy does not worsen this risk, but there is little information available on the effects of different modes of therapy for NIDDM on cardiovascular risk factors. Insulin treatment in NIDDM has been shown to reduce very–low-density lipoprotein (VLDI) triglyceride and elevate high-density lipoprotein (HDI) cholesterol concentrations,[2] but it is difficult to separate the direct effects of insulin from its indirect effects via insulin-induced improvements in glycemic control.

Recently, several novel cardiovascular risk factors have been proposed in NIDDM. One such factor is an elevated concentration of circulating proinsulin-like molecules, which in NIDDM comprise 20% to 60% of fasting immunoreactive insulin concentrations,[3] and which in cross-sectional studies correlate more closely with conventional risk factors than insulin levels measured with specific assays.[4] A second novel risk factor is plasminogen activator inhibitor-1 (PAI-1), an inhibitor of fibrinolysis. PAI-1 levels are elevated in numerous disease states such as NIDDM[5] and coronary heart disease (CHD),[6] resulting in diminished fibrinolytic potential. Insulin,[7] proinsulin-like molecules,[8] lipids,[9] and glucose[10] all increase PAI-1 production in vitro, and infusions of insulin and proinsulin in rabbits augment PAI-1 production.[11]

In a cross-sectional study of 146 NIDDM subjects, we have previously demonstrated that insulin therapy (I) is associated with lower concentrations of proinsulin-like molecules compared with treatment with sulfonylurea (SU) or metformin.[12] We have also demonstrated a reduction in PAI-1 activity on metformin.[13] In a crossover design, we compared the effect of therapy with SU or I, without changes in glycemia or insulin sensitivity, on PAI-1 activity in 11 poorly controlled NIDDM subjects,[14] and I treatment was associated with reduced PAI-1 activity. Our current study was designed to improve on the prior crossover study by studying a larger number of patients for a longer treatment period, with the aim of improving glycemic control rather than keeping it static. The aims of our study were (1) to separate the contributions of improved control versus treatment regimen to cardiovascular risk factors, including PAI-1 and proinsulin-like molecules; (2) to investigate whether more vigorous treatments with SU and I produce greater stimulation and suppression of proinsulin-like molecules and consequently larger changes in PAI-1 than found in our previous study in which poor glycemic control was unaltered; and (3) to study a larger number of patients for a longer duration, with a washout period between study periods.

SUBJECTS AND METHODS

Subjects

Twenty NIDDM subjects attending the Diabetic Clinic at Whittington Hospital were studied. NIDDM was diagnosed according to World Health Organization criteria.[15] All subjects were in poor diabetic control on diet alone and were studied before commencement of additional hypoglycemic therapy. Subjects with a previous history of ischemic heart disease, thromboembolic disease, and clinical or biochemical evidence of renal or hepatic disease were excluded. Subjects were on no other medication, including aspirin, at the time of study, and all provided informed written consent. The study was approved by the Ethical Committee of the Islington Health Authority.

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Study Design

Subjects were treated with I (premixed human soluble and isophane 30:70, Humulin M3; Lilly, Indianapolis, IN) or SU (glibenclamide) once or twice daily each for a period of 16 weeks in a randomized crossover study, with a 4-week washout period between each treatment. Subjects were asked to perform fasting and pre-supper home blood glucose monitoring, and dosage adjustments were made by telephone every 3 to 4 days. In addition, each subject was evaluated weekly for the first 4 weeks and then monthly to adjust the therapy and to measure fasting plasma glucose concentrations to achieve fasting normoglycemia (<6 mmol $\cdot$ l^{-1}). I treatment was commenced at 10 U/d for subjects of ideal body weight, with a dosage increase of 25% for each 10% over the ideal body weight, and the dose was increased by 2 U/d according to home blood glucose monitoring (mean, 27.3 $\pm$ 11.7 U/d). Glibenclamide was started at 2.5 mg/d, and increased by 2.5-mg increments according to home blood glucose monitoring to a maximum of 20 mg/d (mean dose, 8.8 $\pm$ 5.6 mg/d). Subjects were studied on four occasions: visit 1, baseline (B1); visit 2, after 16 weeks of treatment with SU or I; visit 3, after a 4-week washout period (second baseline [B2]); and visit 4, after 16 weeks of treatment with I or SU.

During the 4-week washout period, despite stopping treatment, it was not believed justifiable to discontinue dietary recommendations or home blood glucose monitoring. At each visit, subjects attended the Clinical Investigation Unit of the Department of Medicine, Whittington Hospital, between 8 and 9 AM after an overnight fast. When on treatment, subjects were advised to omit the evening and morning dose of SU or I prior to the visit to avoid hypoglycemia during the insulin sensitivity test. Weight and height were recorded with the patients in light clothing and without shoes. The waist measurement was taken as the minimum circumference in centimeters at or just above the umbilicus, and hip girth was recorded at the site of the greater trochanter. Skinfold measurements were performed with Holtain calipers (Holtain, Crymych, UK), including three measurements of subscapular and triceps skinfold. The body mass index (BMI), waist to hip ratio (WHR), and subscapular to triceps ratio (STR) were calculated. After 10 minutes of rest, BP was recorded twice in the right arm using a random-zero sphygmomanometer (Hawksley, Lancing, UK), with diastolic BP recorded at phase V of the Korotkoff sounds. The mean of two BP readings was used.

Blood Sampling

Fasting venous blood samples were taken without venous stasis for measurement of plasma glucose, hemoglobin A_1 (HbA_1), fructosamine, insulin, proinsulin-like molecules, serum lipids, and apolipoproteins. Samples for fibrinogen, PAI-1 activity, and PAI-1 and tissue plasminogen activator (t-PA) antigens were collected in tubes containing 3.8% sodium citrate, centrifuged at 3,000 *g* for 10 minutes at 4°C, and stored immediately at −70°C.

Insulin Sensitivity

An insulin sensitivity test was performed with the technique of Harano et al,[16] with modifications previously described.[17] Values for insulin sensitivity measured by this method correlate well with those obtained by the euglycemic clamp.[17] After an overnight fast, the right antecubital vein was cannulated for infusion of insulin and glucose, and the left distal forearm vein was cannulated retrogradely and warmed in an insulating blanket at 50°C for venous sampling of arterialized blood. Each subject was administered a 150-minutes continuous infusion of glucose 6 mg $\cdot$ kg^1 $\cdot$ min^{-1} and human soluble insulin 50 mU $\cdot$ kg^{-1} $\cdot$ h^{-1} (Actrapid; Novo Nordisk, Basingstoke, UK) in 50 mL Haemaccel (Hoechst, Hounslow, UK) as separate infusions using Travenol Flowgard pumps (Baxter Health Care, Compton, UK). Arterialized blood samples were drawn from the insulated arm every 5 minutes for the last 30 minutes for assay of plasma glucose. The steady-state plasma glucose concentration was calculated from the mean of seven samples taken from 120 to 150 minutes. Insulin sensitivity was calculated as the metabolic clearance rate of glucose (MCR-G) by dividing the glucose infusion rate by the steady-state plasma glucose concentration, and was expressed as milliliters per kilogram per minute.

Oral Glucose Tolerence Test

This was performed on a separate visit within 7 days of the insulin sensitivity test. Subjects were again advised to omit the evening and morning dose of SU or I prior to the visit to avoid hypoglycemia during the test. After an overnight fast, the patients underwent a 75-g oral glucose tolerence test with measurement of glucose, insulin, and proinsulin-like molecule levels at 0, 30, 60, and 120 minutes.

Biochemical Methods

The plasma glucose level was measured by the glucose oxidase method (Glucose II Analyzer; Beckman, Brea, CA), with an interassay coefficient of variation (CV) of 1.7%. Plasma cholesterol levels were measured by an enzymatic colorimetric method using the Kit C system high-performance CHOD-PAP method (Boehringer Mannheim, Lewes, UK; interassay CV, 1.6%), plasma triglyceride levels were measured using the GPO-PAP kit (Wako Chemicals, Neuss, Germany; interassay CV, 2.6%), and HDL cholesterol levels were measured in solution after precipitation of low-density lipoprotein (LDL) and VLDL using sodium heparin and manganese chloride (interassay CV, 5.4%). Plasma lipoproteins were separated by ultracentrifugation. Apolipoprotein (apo) A1, A2, and B were analyzed by immunoturbidimetric assays using a Cobas Bio Centrifugal analyzer (Hoffmann-la Roche, Basel, Switzerland; interassay CV: apo A1, 2.8%; apo A2, 3.1%; apo B, 2.7%). Lipoprotein(a) content was measured by a one-step sandwich enzyme-linked immunosorbent assay (Immunozym Lp(a); Immuno, Heidelberg, Germany).

PAI-1 activity was measured by a chromogenic substrate method using a commercial kit (Spectrolyse/pL; Biopool, Umeå, Sweden). The intraassay CV was 6.5%, and interassay CV 8.5%. The kit does not accurately detect values less than 5 AU $\cdot$ mL^{-1}. PAI-1 antigen was measured using an enzyme-linked immunoadsorbent assay detecting PAI-1 complexed to t-PA, as well as free active and latent PAI-1 (Tint-Elize PAI-1; Biopool), with intraassay and interassay CVs of 2.0% and 6.8%, respectively. The level of t-PA antigen was measured using a commercial kit that detects both active and complexed forms of single-chain and two-chain t-PA (Tint-Elize t-PA; Biopool; intraassay and interassay CV, 4.0% and 6.0%, respectively). Fibrinogen was analyzed by the von Claus method (BCL, Lewes, UK). The intraassay and interassay CVs were 1.4% and 3.0%.

Insulin, intact proinsulin, and des 31,32 proinsulin were assayed using specific in-house two-site immunometric assays[18] with previously characterized monoclonal antibodies 14B, 3B1, and A6 (Serono-Diagnostics, Woking, UK) and an anti–C-peptide antibody, PEP001 (Novo Nordisk, Copenhagen, Denmark). There was no cross-reaction with insulin in either assay, but there was 78% cross-reaction with des 64,65 proinsulin in the intact proinsulin assay and 59% and 54% cross-reaction with intact and des 64,65 proinsulin, respectively, in the des 31,32 proinsulin assay. Des 31,32 proinsulin concentrations were calculated by subtracting the cross-reactivity of measured intact proinsulin. The intraassay and interassay CVs were 7.9% and 14.3% (insulin), 6.3% and 9.8% (intact proinsulin), and 8.6% and 12.6% (des 31,32 proinsulin), respectively.[18] The detection limits (mean plus 3 SD of zero signal) for the same three assays were 3.0, 0.25, and 0.125 pmol $\cdot$ L^{-1}, respectively. The C-peptide level was measured using a radioimmunoassay kit (Biodata, Montecelio, Italy) with intraassay and interassay CVs of 3.5% and 8.0%, respectively. The HbA_1 level was

measured using electroendosmosis (Corning, Palo Alto, CA; normal range, 6.5% to 8.5%) with intraassay and interassay CVs of 3.0% and 5.5%, respectively. Each subject collected three separate, timed overnight urine samples in the week preceding the study. Urinary albumin was determined by a rapid competitive enzyme immunoassay[19] with an interassay CV of 9.5%. The albumin excretion rate (AER) was calculated and expressed as micrograms per minute, and the mean of the three AERs was taken.

Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS) and are expressed as the mean ± SD for normally distributed data and the geometric mean ×/÷ SD for skewed data (insulin-like molecules, C-peptide, MCR-G, AER, fibrinolytic parameters, triglyceride, VLDL, and LDL). The variables after SU treatment were compared with those after I treatment, and both sets of variables were compared with B1, with the differences analyzed by paired Student's *t* test for normally distributed data and Wilcoxon's rank-sum test for skewed data. We additionally tested for (1) treatment effect by calculating the difference and average of the observations after B1 to I (d_I and a_I) and B1 to SU (d_{SU} and a_{SU}) therapy for each subject, and averaging these for each group (d_I, a_I, d_{SU}, and a_{SU}, respectively); (2) period effect using a two-sample *t* test comparing d_I with d_{SU}; and (3) treatment-period interaction by a two-sample *t* test comparing a_I and a_{SU}.

ANOVA was used to study the independent relationship between two variables while allowing for confounding variables using logarithmic transformation of skewed variables. Statistical significance was indicated by a *P* value less than .05.

RESULTS

Clinical Characteristics

Twenty NIDDM subjects (12 men and eight women) with a mean age of 55.9 ± 8.3 years and mean diabetes duration of 4 ± 6.2 years completed both phases of the study (Table 1). Two subjects withdrew from the study, one due to renal calculi and the other to deciding against insulin therapy. Because it was believed unethical to withdraw dietary and monitoring recommendations in the 4-week washout period, values for fasting glucose, fructosamine, and HbA_1 following the washout period (second baseline, B2) did not return to the initial pretreatment range. Hence, all comparisons on the effects of treatment with I or SU were made with the first baseline, B1.[20] Analyses showed no period effect and no treatment-period interaction, implying that the difference between B1 and B2 did not preclude analysis as a crossover design.[21] The BMI was higher on both treatments than at B1, but did not differ between treatments. There were no changes compared with B1 or differences between the two therapies in BP or AER.

Glycemic Control and Insulin Sensitivity

Treatment with I or SU produced a similar improvement in glycemia as judged by fasting glucose, HbA_1, and fructosamine and in the MCR-G as compared with B1 (Table 2). Levels of glucose after the glucose load were similar on both treatments (Fig 1a).

Insulin-Like Molecules

Insulin concentrations, both fasting (Table 2) and stimulated (Fig 1b to c), were higher on treatment than at B1, but did not differ significantly between treatments. Fasting and post–glucose load intact proinsulin concentrations were higher on SU than on I or B1. Fasting des 31,32 proinsulin concentrations did not differ significantly between treatments or compared with B1; however, values at 30, 60, and 120 minutes on both I and SU were higher than at B1. Fasting C-peptide levels were higher on SU therapy compared with B1, but did not differ significantly between the two treatments. All differences in the concentrations of insulin-like molecules persisted after correcting for BMI and for treatment sequence.

PAI-1 activity and antigen were higher on SU therapy than at B1. PAI-1 antigen was significantly higher on SU therapy than on I. There were no significant differences in t-PA antigen or fibrinogen at the end of the two treatment phases. There were no significant correlations between the change in PAI-1 activity or antigen compared with baseline and changes in any of the insulin-like molecules (Table 3).

Lipids and Lipoproteins

There were no significant differences in lipoprotein or lipoapoprotein concentrations between treatments or baseline (Table 4).

DISCUSSION

The increased risk of cardiovascular disease in NIDDM appears to be independent of the duration of diabetes and glycemic control,[1] and it is important that hypoglycemic therapy in the long term should improve, not worsen, this risk. Our study differs from previous studies on the effect of hypoglycemic therapy on cardiovascular risk factors, in that subjects were studied at baseline, having never been subjected to any form of therapy apart from diet alone, and then in a

Table 1. Clinical Characteristics of Subjects at Both Baselines and 16 Weeks After SU and I Therapy

Characteristic	B1	B2	Post-SU	Post-I	*P*		
					SU *v* I	SU *v* B1	I *v* B1
BMI (kg · m^{-2})	29.2 ± 3.8	30.4 ± 4.3*	30.8 ± 4.3	30.4 ± 4.3	.30	<.0001	<.0001
WHR	0.99 ± 0.11	0.96 ± 0.12	0.93 ± 0.08	0.93 ± 0.09	.92	.02	.04
STR	1.66 ± 0.67	1.82 ± 0.80	1.90 ± 0.79	2.22 ± 1.00	.03	.03	.005
SBP (mm Hg)	135.5 ± 19.4	128.6 ± 19.3*	135.9 ± 19.5	136.8 ± 23.4	.79	.91	.94
DBP (mm Hg)	80.1 ± 15.3	79.3 ± 12.7	80.7 ± 14.1	84.6 ± 14.0	.14	.84	.16
AER (μg · min^{-1})	8.8 ×/÷ 2.0	9.3 ×/÷ 2.2	8.6 ×/÷ 2.0	7.0 ×/÷ 1.9	.32	.79	.21

NOTE. Data are the arithmetic mean ×/÷ SD, or geometric means ± SD for skewed data (AER). Significance of differences between SU and I therapy and versus B1 and B2 was assessed using Student's *t* test for normally distributed data and the Wilcoxon rank test for skewed data.

*Significant difference *v* B1.

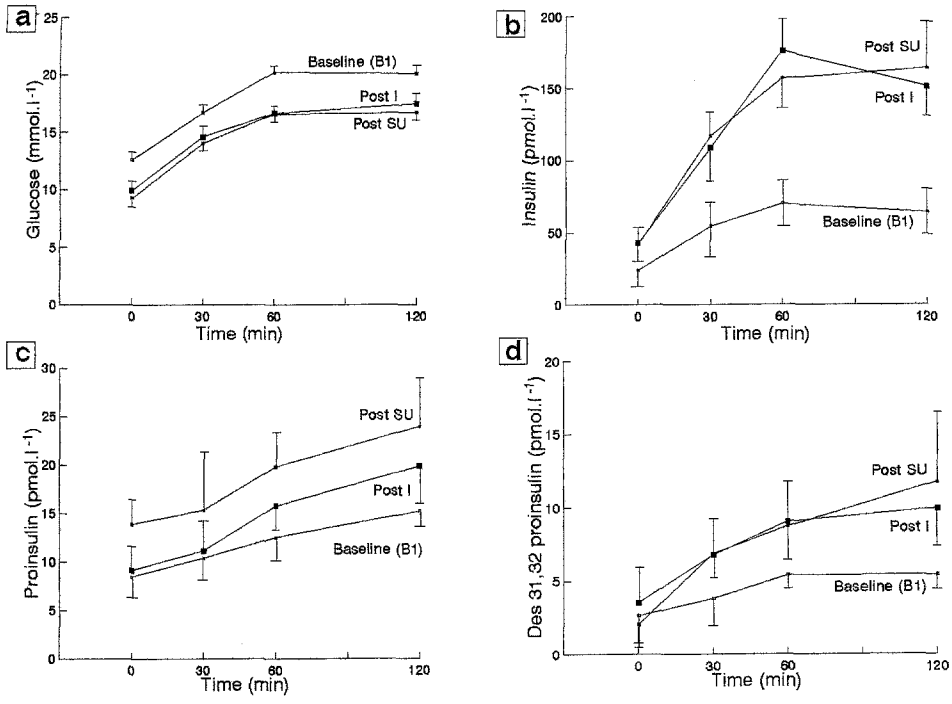

Fig 1. (a) Glucose, (b) insulin, and (c) intact and (d) des 31,32 proinsulin concentrations during the oral glucose tolerance test, at (B1) and on SU and I therapy. Data are the gemoetric mean ×/÷ SEM. Differences between the area under the curve on SU and I therapy: I, *P* = .55; intact proinsulin, *P* = .03; des 31,32 proinsulin, *P* = .32; glucose, *P* = .55.

crossover fashion with a 4-week washout period. By achieving similar improvements in glycemia and insulin sensitivity on both treatments, we were able to dissect the contributions of improved control versus treatment regimen.

Our study demonstrated that SU therapy results in higher circulating levels of intact proinsulin and PAI-1 activity and antigen compared with baseline. In addition, levels of PAI-1 antigen and intact proinsulin were significantly higher on SU therapy compared with I therapy. There were no differences in other insulin resistance syndrome variables between SU and I treatment. These data support our previous cross-sectional study of 146 NIDDM subjects, wherein subjects on SU therapy had higher levels of proinsulin compared with subjects treated with insulin,[12] and our previous crossover study in which insulin therapy in NIDDM subjects decreased both proinsulin and PAI-1 activity.[14] The consequences of increased levels of proinsulin and PAI-1 in NIDDM patients on SU therapy are unclear.

In NIDDM, proinsulin-like molecules may contribute up to 60% of immunoreactive insulin,[3] and in previous studies, these concentrations correlate more closely with cardiovascular risk factors, including PAI-1, than specific levels of insulin.[4] Proinsulin induces PAI-1 synthesis in vitro, acting via the insulin receptor,[8] and intravenous infusions of proinsulin increase plasma PAI-1 activity and PAI-1 mRNA in aorta and liver.[11] A study examining the effects of therapeutic human proinsulin was terminated due to an increased incidence of cardiovascular events,[22] and it could be postulated that this was due to diminished fibrinolytic activity. Although not widely accepted, the University Group Diabetes Program Study suggested an increased cardiovascular mortality on SU therapy,[23] which may have resulted from increased proinsulin production and hence PAI-1.

Gliclazide, a second-generation SU, has been shown to increase t-PA activity in subjects with NIDDM; there was no change observed in PAI-1 activity, and the effect was indepen-

Table 2. Glycemic Control, Insulin Sensitivity, and Insulin-Like Molecules at B1 and 16 Weeks After SU and I Therapy

Parameter	B1	Post-SU	Post-I	*P* SU *v* I	*P* SU *v* B1	*P* I *v* B1
Fasting glucose (mmol · L^{-1})	12.8 ± 3.5	9.1 ± 2.5	8.9 ± 1.9	.77	.001	.001
HbA_1 (%)	11.7 ± 2.1	8.5 ± 0.9	8.6 ± 1.2	.63	<.0001	<.0001
Fructosamine (mmol · L^{-1})	2.5 ± 0.7	1.9 ± 0.5	2.0 ± 0.3	.77	<.0001	<.0001
MCR-G (mL · kg^{-1} · min^{-1})*	1.86 ×/÷ 1.4	2.36 ×/÷ 1.4	2.27 ×/÷ 1.4	.37	.0005	.07
Insulin (pmol · L^{-1})*	26.4 ×/÷ 2.2	38.6 ×/÷ 1.9	42.7 ×/÷ 2.0	.20	.015	.002
Proinsulin (pmol · L^{-1})*	9.8 ×/÷ 2.3	12.8 ×/÷ 2.7	8.0 ×/÷ 2.1	.001	.001	.19
Des 31,32 proinsulin (pmol · L^{-1})*	3.6 ×/÷ 2.6	3.4 ×/÷ 3.5	2.9 ×/÷ 3.8	.88	.88	.99
Proinsulin ratio (%)*	33.0 ×/÷ 1.6	28.2 ×/÷ 1.7	20.8 ×/÷ 1.5	.05	.22	.002
C-peptide (ng · mL^{-1})*	0.7 ×/÷ 0.3	0.9 ×/÷ 0.4	0.8 ×/÷ 0.4	.10	.0007	.39

NOTE. Data are the arithmetic mean ± SD or geometric mean ×/÷ SD for skewed data (asterisk). Significance of differences between SU and I therapy and versus B1 was assessed using Student's *t* test for normally distributed data and the Wilcoxon rank test for skewed data.

Table 3. Fibrinolytic Parameters at B1 and 16 Weeks After SU and I Therapy

Parameter	B1	Post-SU	Post-I	P		
				SU v I	SU v B1	I v B1
PAI-1 activity (AU · L^{-1})	19.9 ×/÷ 1.8	23.7 ×/÷ 2.4	22.2 ×/÷ 2.1	.54	.02	.22
PAI-1 antigen (ng · mL^{-1})	32.2 ×/÷ 1.8	47.6 ×/÷ 2.0	36.5 ×/÷ 2.2	.05	.0006	.35
t-PA antigen (ng · mL^{-1})	11.3 ×/÷ 2.9	12.5 ×/÷ 2.4	12.4 ×/÷ 3.3	.21	.09	.15
Fibrinogen (mg/dL)	256.4 ×/÷ 43.9	239.2 ×/÷ 31.0	243.3 ×/÷ 46.8	.79	.13	.34

NOTE. Data are the geometric mean ×/÷ SD for skewed data. Significance of differences between SU and I therapy and versus B1 was assessed using the Wilcoxon rank test.

dent of metabolic control.[24] Our study did not measure t-PA activity, and differs from the study by Gram et al[24] in that we aimed to improve glycemic control. The mechanism for the independent effect of gliclazide on t-PA activity is unknown. Although none of the studies have measured proinsulin-like molecules, it may be a direct effect on endothelial t-PA production.

Reduced fibrinolysis will predispose the individual to an increased risk of atherogenesis and thrombosis. PAI-1 is elevated in NIDDM[5] and in subjects with CHD, where it is a predictor of reinfarction.[25] Recently, the DIGAMI (Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction) Study demonstrated that insulin-glucose infusions followed by intensive multidose I treatment reduced mortality in NIDDM patients with acute myocardial infarction by one third, and the effect persisted for at least 3.5 years.[26,27] The DIGAMI Study postulated that the improvement in mortality on I therapy may be due to lower PAI-1 activity, although this was not tested.

Our study showed that levels of PAI-1 antigen were higher on SU compared with I therapy, but PAI-1 activity and t-PA antigen did not differ significantly between treatments. This may be explained in part by an increase in BMI in subjects on both therapies. This was an unfortunate consequence of strict diabetic control, and occurred despite dietetic advice. Although this increase was similar on both treatments and was corrected for in our statistical analyses, this may have accounted for the increase in PAI-1 activity at the end of the study on both I and SU therapy. PAI-1 activity is closely associated with the BMI, and the possible decrease in PAI-1 activity on I therapy might have been counteracted by an increase in the BMI; PAI-1 antigen, on the other hand, remained significantly increased in subjects on SU therapy.

The concept of portal versus peripheral insulin levels in determining PAI-1 activity must also be considered. The increase in PAI-1 activity on SU could be due to an increased level of portal insulin. Previous studies have attempted to dissect this difference by administering peripheral insulin infusions[28,29] and attempting to stimulate portal insulin with an oral glucose tolerance test.[29-31] None of the studies have been able to show a convincing elevation of PAI-1; in contrast, some studies demonstrate that oral glucose reduces PAI-1 activity. These studies have been criticized for their short duration, but no study has directly implicated portal insulin. Although portal insulin levels and hepatic PAI-1 production may have contributed to our findings, we have no evidence to support this theory.

In subjects with insulin-dependent diabetes mellitus (IDDM), the data on PAI-1 activity are conflicting, although most studies show a decrease in PAI-1 activity. This may be due to low portal and hepatic insulin concentrations in IDDM subjects.

PAI-1 in blood exists in three different molecular forms: latent (or inactive), active, and PAI-1 complexed to t-PA.[32] The PAI-1 antigen assay measures total antigen and is unable to differentiate between the latent, active, and complexed PAI-1 forms, while the t-PA assay measures t-PA complexed to PAI-1 and PAI-2. t-PA antigen is largely bound to PAI-1 and relates inversely to fibrinolysis.[33] The relative proportions of latent, active, and complexed PAI-1 are influenced by several factors, including age, obesity, time of sample, and disease process,[34,35]

Table 4. Lipid and Lipoprotein Concentrations at B1 and 16 Weeks After SU and I Therapy

Parameter	B1	Post-SU	Post-I	P		
				SU v I	B1 v S	B1 v I
Total cholesterol (mmol · L^{-1})	5.9 ×/÷ 1.4	5.9 ×/÷ 1.3	5.8 ×/÷ 1.1	.76	.82	.59
Total triglyceride (mmol · L^{-1})*	1.61 ×/÷ 1.94	1.66 ×/÷ 1.98	1.67 ×/÷ 1.88	.65	.74	.68
VLDL cholesterol (mmol · L^{-1})*	0.35 ×/÷ 2.52	0.39 ×/÷ 2.12	0.36 ×/÷ 2.68	.56	.95	.56
VLDL triglyceride (mmol · L^{-1})*	0.83 ×/÷ 2.55	0.93 ×/÷ 2.26	0.90 ×/÷2.59	.91	.51	.33
IDL cholesterol (mmol · L^{-1})*	0.13 ×/÷ 2.00	0.12 ×/÷ 2.00	0.12 ×/÷ 1.70	.53	.95	.28
IDL triglyceride (mmol · L^{-1})*	0.09 ×/÷ 2.08	0.08 ×/÷ 1.81	0.08 ×/÷ 1.74	.54	.49	.32
LDL cholesterol (mmol · L^{-1})*	3.03 ×/÷ 1.34	2.98 ×/÷1.37	2.75 ×/÷ 1.25	.08	.76	.10
HDL_2 (mmol · L^{-1})	0.47 ± 0.27	0.46 ± 0.23	0.50 ± 0.18	.29	.60	.58
HDL_3 (mmol · L^{-1})	0.77 ± 0.19	0.83 ± 0.21	0.85 ± 0.20	.66	.11	.11
Apo A1 (g · L^{-1})	1.34 ± 0.25	1.30 ± 0.24	1.35 ± 0.26	.06	.75	.76
Apo A2 (g · L^{-1})	0.53 ± 0.14	0.53 ± 0.11	0.52 ± 0.12	.68	.81	.46
Apo B (g · L^{-1})	1.21 ± 0.36	1.20 ± 0.31	1.20 ± 0.32	.93	.64	.93
Lp(a) (mg · dL^{-1})	20.00 ± 4.61	16.77 ± 6.42	18.58 ± 5.55	.93	.64	.93

NOTE. Data are the mean ± SD or the geometric mean ×/÷ SD for skewed data (asterisk). Significance of differences between SU and I therapy and B1 was assessed using the Wilcoxon rank test.

and it is possible that hypoglycemic therapy alters the amount of PAI-1 present in different molecular forms. There has been no study to date examining the effect of diabetic therapy on the relative concentrations of the various molecular forms. Furthermore, the clinical relevance of the latent antigen to the overall fibrinolytic potential needs to be evaluated.

In conclusion, our study showed that 16 weeks of treatment with SU or I resulted in equal improvement in glycemia and insulin sensitivity. Proinsulin and PAI-1 antigen levels were higher on SU therapy, but there were no differences in other cardiovascular risk factors between the two treatments. Our results may help explain the improved mortality observed post–myocardial infarction in insulin-treated NIDDM subjects in the DIGAMI Study. We anxiously await the results of the UK Prospective Diabetes Study,[36] which will ultimately answer the question of the effects of long-term therapy with SU and I on the incidence of cardiovascular events.

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