Progressive decrease of proinsulin secretion in sulphonylurea-treated type 2 diabetes

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ABSTRACT

Progressive deterioration of β-cell function is proposed as a disease-related factor of sulphonylurea (SU) failure in type 2 diabetes. If it gradually worsens over time then disease duration may mirror the progressive β-cell deterioration. The aim of the present study is to assess whether or not disease duration is influential in remodelling the secretion pattern of insulin-like molecules and in glucose control of SU-treated type 2 diabetes. A research model is used to investigate proinsulin secreting capacity over time, using two groups of patients: i) disease duration <5 years (n= 62), comprising SU responders (SUr; n=48) and SU failures (SUf; n=14); and ii) disease duration ≥ 5 years (n= 37), comprising an SUr group (n=17) and an SUf group (n=20). Blood samples are taken at 0 h, 0.5 h 1 h, 2 h and 3 h during a standard oral glucose tolerance test and measured for glucose, total proinsulin (TPI), intact proinsulin (IPI) and specific insulin (SI) concentrations. Pairwise comparison of estimated marginal means of blood glucose, SI, IPI and TPI levels at each time point are carried out between groups and subgroups. (SUr *vs*. SUf). Homa insulin resistance index (IR index) is applied to analyse IR between the groups. It was found that patients with shorter disease duration had higher proinsulin (TPI and IPI) levels at all time points (P<0.05), together with a lower glucose level at 2 h and 3 h (P<0.05). Homa insulin index analysis showed no difference between the two groups (P=0.26). Results also showed that the SUr group had a significantly lower glucose level at 0h and 3h (P<0.05), although no significant difference in insulin and proinsulin levels was found between the SUr and SUf groups. In conclusion, proinsulin may play an important role in glucose control in SU-treated type 2 diabetes, but the effect is reduced in SUf patients.

KEY WORDS: Diabetes mellitus. Insulin. Proinsulin. Sulfonylurea compounds.

Introduction

In the human pancreatic islet, processing of proinsulin to insulin occurs predominantly in secretory vesicles, which provide a large intracellular store of insulin for rapid release when the β-cells receive the appropriate stimulation.[1] *In vitro* experiments in the 1980s showed that decreased secretory response or reversible impairment of insulin secretion occurs when pancreatic islets experience prolonged exposure to a stimulatory concentration of glucose or sulphonylurea (SU) compounds.[2–5] This phenomenon was described as 'desensitisation of insulin secretion' and is thought to be an important step in the manifestation of type 2 diabetes in those patients who fall into the SU failure (SUf) group.[5–8]

In SU-treated diabetes it is believed that the pancreatic islets are gradually over-stimulated as a result of long-term exposure to a full dose of sulphonylurea, and this leads to the depletion of releasable insulin in β-cells.[9–11] In previous work, the authors found that type 2 diabetics showed severe insulin deficiency and that those with SUf were less able to control blood glucose when responding to a glucose challenge.[12] The mechanisms underlying such pathological changes remain unclear, however, but it was proposed that desensitisation to stimuli is an evolving process that worsens over time, and that disease duration might mirror progressive β-cells deterioration.[5]

This study aims to discover whether or not disease duration influences glucose control and effects a remodelling of the secretory pattern of insulin-like molecules in SU-treated type 2 diabetes.

Materials and methods

A total of 124 patients with type 2 diabetes (diagnosed according to WHO 1999 guidelines) volunteered for the study. All were subjected to clinical or biochemical screen for evidence of cardiac, hepatic, kidney or thyroid abnormalities. Patients with no complications and not on insulin treatment (only full-dose SU treatment) were recruited for a glibenclamide challenge test[13] to assess response to SU treatment.

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Finally, 99 patients completed the study, as 13 were excluded on evidences of other disease, seven failed to attend for oral glucose tolerance test (OGTT) and five were excluded because they missed certain timed laboratory tests.

Glibenclamide challenge test

Glibenclamide (7.5 mg) was taken orally after a fasting glucose test, followed by a series of blood glucose tests at 60 min, 120 min and 180 min. Glucose decrease rate (%) was calculated using the formula $(Glu_{0min}–Glu_{(x)min})/Glu_{0min} \times 100\%$, where $Glu_{(x)min}$ denotes glucose level at a certain time. A patient with a glucose decrease rate greater than 25% at any time point was classified as SUr, while the remainder were classified as SUf.

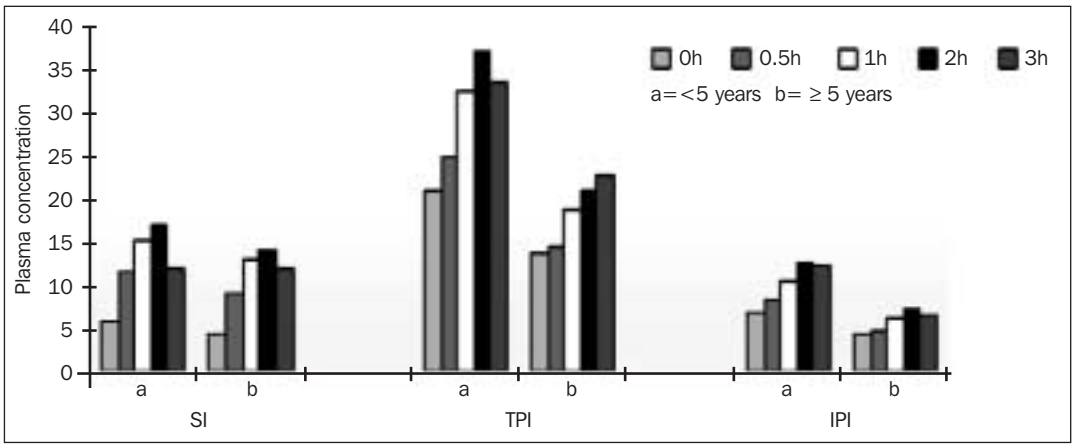

Fig. 1.
Comparison of group mean insulin levels between groups with disease duration <5 years and ≥ 5 years (SI: $P>0.05$, TPI: $P<0.03$, IPI: $P<0.03$).

Study group details

Details of the group that comprised patients with disease duration <5 years were as follows: $n=62$ (male: 42, female: 20); mean age: 58 (range: 28–65); Sur: 48, SUf: 14.

Details of the group that comprised patients with disease duration ≥ 5 years were as follows: $n=37$ (male: 22; female: 15); mean age: 60.2 (range: 39–83); SUr: 17, SUf: 20

Blood sample collection

All patients took a last dose of oral hypoglycaemic agent the day before the study. After overnight fast, all subjects had a standard 75-g OGTT. Five blood samples were collected at 0 min, 30 min, 60 min, 120 min and 180 min for glucose, TPI, IPI and SI analysis. Sera were separated within 30 min of collection. Glucose and SI were measured immediately after separation, while samples for TPI and IPI were stored at –20°C for later analysis.

Sample analysis

Glucose samples were analysed on Beckman CX5 autoanalyser using a glucose oxidase method. SI samples were analysed on an Access chemiluminescent immunoassay system (Beckman Instruments, Cheska, USA). Analytical sensitivity (95% confidence) was 0.03 miu/L, with a reportable range of 0.03–300 miu/L. Total imprecision was <10% across the assay range. No cross reactivity was detected when up to 4000 pmol/L proinsulin or 20,000 pmol/L C-peptide were added. Reference fasting range was 1.9–8.2 miu/L.

TPI and IPI samples were analysed on a Bio-Rad autoimmunoassay system using enzyme-linked immunosorbent assay (ELISA) kits (Dako, Cambridgeshire, UK). Assay parameters were provided by the manufacture (detection limit – TPI: 0.07 pmol/L, IPI: 0.13 pmol/L). Inter- and intra-assay coefficients of variation were <10% for both TPI and IPI. No cross-reaction was detected when up to 3600 pmol/L and 8400 pmol/L of SPI added for IPI and TPI, respectively. Reference fasting concentrations were 3.4–27.3 pmol/L for TPI and 0.95–10.6 pmol /L for IPI.

Data analysis

A general linear model multivariate procedure (SPSS software) was used for data calculation and statistical analysis. Serum glucose, SI, TPI and IPI levels at each time point were analysed as dependent variables, while disease duration and SU status were fixed factors. Patient age, sex and body-mass index (BMI) were selected as covariates in the analytical model.

Table 1. Between-subject effects (covariates).

Dependent variable	R^2	Source Sex *P*	Age *P*	BMI *P*
BG 0h	0.149	0.05	0.124	0.548
BG 0.5h	0.123	0.025	0.508	0.809
BG 1h	0.103	0.138	0.124	0.633
BG 2h	0.114	0.234	0.450	0.531
BG 3h	0.231	0.128	0.255	0.923
SI 0h	0.270	0.976	0.065	0.000
SI 0.5h	0.159	0.024	0.095	0.028
SI 1h	0.233	0.001	0.062	0.016
SI 2h	0.182	0.021	0.159	0.007
SI 3h	0.195	0.002	0.021	0.075
TPI 0h	0.082	0.818	0.357	0.060
TPI 0.5h	0.146	0.953	0.026	0.034
TPI 1h	0.188	0.121	0.013	0.063
TPI 2h	0.251	0.762	0.000	0.008
TPI 3h	0.130	0.216	0.005	0.600
IPI 0h	0.113	0.550	0.355	0.005
IPI 0.5h	0.167	0.823	0.025	0.005
IPI 1h	0.134	0.736	0.036	0.029
IPI 2h	0.164	0.780	0.004	0.062
IPI 3h	0.165	0.323	0.007	0.153

BG=Blood glucose SI=Specific insulin.
TPI=Total proinsulin IPI=Intact proinsulin.

As this study had an unequal *n* design, estimated marginal means were reported and used for pairwise comparison. All dependent variables at each time point were compared respectively between two main groups (<5 years *vs.* ≥ 5 years) and two subgroups (SUr *vs.* SUf). $P<0.05$ was considered significant. Homa IR index[14] was applied to analyse insulin resistance between the groups.

Results

Results of tests of between-subject effects (related to age, sex and BMI) are shown in Table 1. Estimated marginal mean and mean difference for all variables and for pairwise comparison outcomes (*P*) are listed in Table 2 (SUr *vs.* SUf)

Table 2. Estimated marginal mean and pairwise comparison between groups (SUr *vs*. SUf).

Dependent variable	Estimated marginal mean*				Mean difference (a–b)*	Std error	Pairwise comparison *P*
BG 0h (mmol/L)	a	11.28	b	13.64	–2.35	0.84	0.006
BG 0.5h (mmol/L)	a	16.56	b	18.53	–1.97	1.01	0.055
BG 1h (mmol/L)	a	21.11	b	22.37	–1.25	1.11	0.261
BG 2h (mmol/L)	a	22.79	b	25.27	–2.48	1.28	0.055
BG 3h (mmol/L)	a	18.74	b	22.93	–4.19	1.14	0.000
SI 0h (miu/L)	a	5.20	b	5.09	0.11	0.82	0.890
SI 0.5h (miu/L)	a	11.31	b	9.03	2.28	2.37	0.338
SI 1h (miu/L)	a	16.14	b	11.81	4.33	2.68	0.109
SI 2h (miu/L)	a	17.93	b	13.24	4.69	3.36	0.166
SI 3h (miu/L)	a	13.69	b	9.97	3.73	2.17	0.089
TPI 0h (pmol/L)	a	16.31	b	17.96	–1.65	3.21	0.608
TPI 0.5h (pmol/L)	a	19.50	b	19.97	–0.47	3.69	0.899
TPI 1h (pmol/L)	a	25.99	b	24.92	1.07	4.50	0.813
TPI 2h (pmol/L)	a	30.83	b	27.05	3.79	4.53	0.406
TPI 3h (pmol/L)	a	29.60	b	26.39	3.22	4.87	0.510
IPI 0h (pmol/L)	a	5.11	b	6.11	–0.99	1.23	0.421
IPI 0.5h (pmol/L)	a	5.62	b	7.28	–1.65	1.44	0.254
IPI 1h (pmol/L)	a	7.73	b	9.04	–1.31	1.83	0.476
IPI 2h (pmol/L)	a	10.61	b	9.28	1.34	2.24	0.552
IPI 3h (pmol/L)	a	9.69	b	9.35	0.34	2.05	0.869

Evaluated at covariates of sex=0.53, age=57.47 and BMI=23.00. a= SU responders (n=65); b=SU failures (n=34).

and Table 3 (<5 years *vs*. ≥ 5 years). Data relating to SI, IPI and TPI levels between the two groups with different disease durations are shown in Figure 1.

Discussion

In vitro study has found consistently that prolonged exposure to SU desensitises pancreatic β-cells and reduces insulin secretion.[3,9,11] In a previous *in vivo* study, the authors of the present study have demonstrated that OGTT in both SUr and SUf groups shows no marked difference in insulin related molecules level and a similar level of insulin deficiency.[12]

In the majority of SU-dependent patients, increasing disease duration means longer exposure to SU agents, and, according to the 'desensitisation' theory, this results in progressive insensitivity to SU agents. Consequently, disease duration could be an important factor in glucose control.

In the present study, it was expected results would demonstrate consistency with *in vitro* study which shows that patients exposed to SU agents ≥ 5 years would have lower SI secretory activity. Surprisingly, however, there was no evidence of time-related changes in SI level but a significantly lower proinsulin level at all time points. Furthermore, this group of patients was less able to reduce glucose level in response to a glucose load. Data were analysed for Homa IR index but no marked differences were found between groups. These finding indicated that IR was not a major causation in disease duration related glucose controlling ability, neither proinsulin secreting capacity. Results also suggest that the two groups of patients showed marked differences in proinsulin conversion, but that insensitivity to glucose stimulation occurs differently.

In responding to a glucose load, SU-treated patients in the present *in vivo* study showed time-related changes in proinsulin but not insulin levels; a finding that is inconsistent with other *in vitro* studies.[2–5] For years, studies into the cause of elevated proinsulin level in type 2 diabetes have concentrated on the exocytosis pathway,[1] and one hypothesis implicates dysfunction of proinsulin conversion machinery. However, if dysfunction of the converting machinery is responsible for elevation of proinsulin during OGTT, a decreased insulin level should reflect this. However, the results of the present study do not support this theory.

In a normal pancreatic islet, proinsulin is transferred in an energy-dependent manner from the rough endoplasmic reticulum to the Golgi apparatus for further processing.[1] Most (96%) proinsulin is cleaved into insulin and C-peptide, with the remainder comprising of intermediates and non-processed proinsulin. Approximately 98% is finally secreted into the circulation by exocytosis, while the remaining 2% proinsulin is secreted directly into the circulation from the Golgi apparatus through an unregulated constitutive secreting pathway.[1]

Proinsulin bioactivity reduces blood glucose and its effect is longer lasting than that of insulin.[15] Results of the present study support this, as patients who had higher proinsulin levels appeared to enjoy better glucose control, and unraveling of mechanisms behind the proinsulin-secreting pathway may prove in furthering knowledge of glucose control.

The present study investigated proinsulin-secreting capacity at various time points in two groups of patients selected to reflect early and late disease (i.e., disease duration of <5 years and ≥ 5 years) and to represent the effect of

Table 3. Estimated marginal mean and pairwise comparison between groups (disease duration <5 years *vs.* ≥ 5years).

Dependent variable	Estimated marginal mean*				Mean difference (a–b)*	Std error	Pairwise comparison *P*
BG 0h (mmol/L)	a	12.11	b	12.81	–0.70	0.83	0.397
BG0.5h (mmol/L)	a	17.20	b	17.88	–0.69	0.99	0.491
BG 1h (mmol/L)	a	20.77	b	22.70	–1.93	1.09	0.078
BG 2h (mmol/L)	a	22.75	b	25.31	–2.56	1.25	0.043
BG 3h (mmol/L)	a	19.47	b	22.20	–2.73	1.11	0.016
SI 0h (miu/L)	a	5.89	b	4.41	1.48	0.81	0.068
SI 0.5h (miu/L)	a	11.49	b	8.85	2.65	2.32	0.256
SI 1h (miu/L)	a	15.10	b	12.86	2.23	2.62	0.397
SI 2h (miu/L)	a	17.03	b	14.14	2.89	3.29	0.382
SI 3h (miu/L)	a	11.82	b	11.83	–0.01	2.12	0.997
TPI 0h (pmol/L)	a	20.75	b	13.52	7.23	3.14	0.023
TPI 0.5h (pmol/L)	a	24.98	b	14.48	10.51	3.61	0.004
TPI 1h (pmol/L)	a	2.34	b	18.57	13.77	4.40	0.002
TPI 2h (pmol/L)	a	36.99	b	20.89	16.09	4.44	0.000
TPI 3h (pmol/L)	a	33.40	b	22.59	10.82	4.76	0.025
IPI 0h (pmol/L)	a	6.91	b	4.31	2.60	1.21	0.033
IPI 0.5h (pmol/L)	a	8.37	b	4.53	3.84	1.41	0.008
IPI 1h (pmol/L)	a	10.63	b	6.14	4.50	1.79	0.014
IPI 2h (pmol/L)	a	12.69	b	7.20	5.50	2.19	0.014
IPI3h (pmol/L)	a	12.41	b	6.62	5.79	2.01	0.005

Evaluated at covariates of sex=0.53, age=57.47 and BMI=23.00. *a=disease duration <5years (n=62); b=disease duration ≥ 5 years (n=37).

progressive pathological changes. Based on the findings, it is proposed that i) proinsulin secretion decreased progressively in line with the loss of control of blood glucose in SU-treated type 2 diabetes; ii) duration of disease is closely related to progressive β-cell deterioration, and desensitisation to glucose stimulation is an evolving process that increases with time; and iii) proinsulin may play an important role in glucose control but it has less effect in SUf patients. Clearly, the mechanisms involved require further investigation. □

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