Secondary sulphonylurea failure: what pathogenesis is responsible?

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ABSTRACT

Sulphonylurea (SU) stimulates insulin secretion by pancreatic β-cells and is generally used as a first-line treatment for type 2 diabetes. However, after long-term SU treatment (six months or over), some patients begin to show an increase in blood glucose once again (secondary SU failure). Two theories have been put forward to explain this failure – dysfunction of the proinsulin conversion machinery or insulin resistance. However, the primary pathogenesis behind secondary SU failure still needs to be investigated. Using a reliable technique that specifically identifies intact proinsulin (IPI), total proinsulin (TPI) and specific insulin (SI), this study aims to discover if a defect in the proinsulin converting mechanism plays a role in SU failure. Three groups were recruited for this study: healthy controls (n=8), SU responders (n=38) and secondary SU failures (n= 46). Serum concentrations of insulin-related molecules released in response to a standard glucose challenge test were compared between the groups. It was found that total SI was lower in the patient groups ($P<0.05$ compared to the control group), while TPI and IPI showed no distinct difference between the three groups ($P>0.05$). TPI:SI ratio and IPI:SI ratio showed marked increases in the patient groups ($P<0.05$ compared to control group), with no obvious quantitative difference between SU responders and secondary SU failures ($P>0.05$). Similar results for the Homa Insulin Resistant Index were found between the two patient groups. Interestingly, blood glucose at 180 mins after glucose challenge was significantly higher in the secondary SU failure group ($P<0.05$), with no correlation to SI, while the SU responder group showed good correlation between the parameters ($P<0.05$). We conclude that type 2 diabetes is associated with obvious dysfunction in the proinsulin-converting process and shows severe SI deficiency in responding to glucose challenge. Dysfunction of the proinsulin conversion mechanism was not an extra cause responsible for SU failure.

KEY WORDS: Insulin. Islets of Langerhans. Proinsulin. Pancreatic beta cells. Sulfonylurea compounds.

Introduction

Sulphonylurea (SU) stimulates insulin secretion from pancreatic β-cells and is generally used as a first-line treatment in type 2 diabetes;[1] however, it does not always respond well to this oral antidiabetic agent. Some patients fail to show a significant decrease in blood glucose from the beginning of treatment (primary SU failure), while others show an increase in blood glucose once again (secondary SU failure) after longer-term (six months or over) treatment.

One of the disease-related factors of secondary failure is thought to be a process of increasing insulin deficiency due to a β-cell defect[1,2] that could result in a higher proportion of intact and split proinsulin, which are less biologically active than specific insulin.[3,4]

During the last decade, possible mechanisms of hyperproinsulinaemia have been reviewed in various groups of people, including those showing impaired glucose tolerance,[5] obese subjects,[6] type 2 diabetics[7] and patients on SU treatment.[8-11] These studies propose that dysfunction of the proinsulin conversion mechanism would result in increasing circulating proinsulin, probably with decreasing intermediates and insulin level.[3,6] However, whether or not defective β-cell function is to blame for SU failure remains unknown.[12,13] In addition, there is a controversial theory that insulin resistance, defined as normal concentrations of insulin producing a less than normal biological response,[14] is a characteristic of type 2 diabetes.

Which of these metabolic abnormalities – β-cell proinsulin conversion mechanism defect or insulin resistance – is the primary determinant of secondary SU failure, or do both exist concurrently?

Using a reliable technique that specifically identifies intact proinsulin (IPI), total proinsulin (TPI) and specific insulin (SI), the study reported here focuses on secondary SU failure to determine whether or not the proinsulin conversion mechanism defect plays an important role in it.

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Materials and methods

All participants in the study were Chinese and gave informed consent. Type 2 diabetes was diagnosed according to World Health Organization (WHO) guidelines (1999) at Guang Zhou Red Cross Hospital, and none of the patients had any clinical or biochemical evidence of cardiac, hepatic,

kidney or thyroid abnormalities during the year preceding the study. No subjects had received insulin treatment.

Two groups of patients were recruited initially: 50 patients went through a primary screen for SU responders; and 60 patients were screened for SU failure. Each of these patient groups was then subjected to a glibenclamide challenge test[15] to exclude unsuitable cases.

The control group comprised healthy volunteers with no family history of diabetes.

Glibenclamide challenge test

Glibenclamide (7.5 mg) was taken orally after a fasting glucose test, followed with a series of blood glucose tests at 60 mins, 120 mins and 180 mins. Glucose decrease rate (%) was calculated using the formula $(Glu_{0min}–Glu_{(x)min})/Glu_{0min}$ x 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 an SU responder, otherwise they were classified as primary SU failures (patients had not received SU before) or secondary SU failures (patients treated with SU for more than one year).

Study group details

SU responder group (n = 38, 24 males): Mean age 50 (range: 25-75) with one to two years of disease history. Out of the initial group, 12 patients were excluded (five cases confirmed as primary SU failure and seven were unsuitable due to sudden illness or inadequate blood samples).

Secondary SU failure group (n = 46, 20 males): Mean age 61.5 years (range: 38-82) with between one and 20 years of disease history. Out of the initial group, 14 patients were excluded (eight were confirmed as SU responders and six were unsuitable due to sudden illness or inadequate blood samples).

Control group (n = 8, thee males): Mean age 45 years (range: 32-58).

Blood sample collection

After overnight fast, all subjects had a standard 75 g oral glucose tolerance test (OGTT). Patients with secondary SU failure took their last dose of oral hypoglycaemic agent the day before the test. Five blood samples were collected (timed at zero mins, 30 min, 60 min, 120 min and 180 min) during the OGTT for TPI, IPI and SI analysis, and for blood glucose analysis. Sera were separated within 30 mins of collection. SI and glucose were analysed immediately after separation. Sera for TPI and IPI were stored frozen at –20^{0}C for later analysis.

Sample analysis

TPI and IPI were analysed on a Bio-Rad immunoassay system using enzyme-linked immunosorbent assay kits (Dako, Ely Cambridgeshire CB7 4ET, UK). Assay parameters were provided by the manufacture (detection limits: TPI 0.07 pmol/L, IPI 0.13 pmol/L). Inter- and intra-assay coefficients of variation were <10% for both TPI and IPI. Total imprecision was under 10% across the assay range. No cross-reactivity was detected when up to 3600 pmol/L and 8400 pmol/L of SI 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.

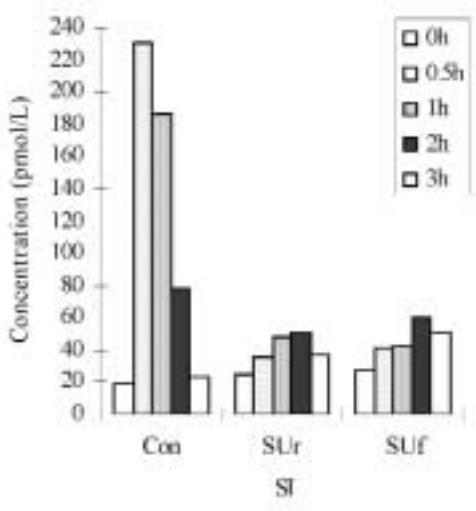

Fig. 1. Mean specific insulin (SI) concentration at different time points after a glucose tolerance test (Con = Control group, SUr=SU responder group, SUf=SU failure group).

SI was analysed on an Access chemiluminescence immunoassay system (Beckman, Cheska, MN 55318, USA) using supplied ultrasensitive insulin assay reagents. Analytical sensitivity (with 95% confidence) was 0.21 pmol/L, with a range of 0.21-2100 pmol//L. Total imprecision was <10% across the assay range. No cross reactivity was detected when up to 4000 pmol/L of proinsulin or 20,000 pmol/L of C-peptide were added. Reference fasting range used for routine service in our laboratory is 13.3-57.4 pmol/L.

Glucose were analysed on a Beckman CX5 autoanalyser using the glucose oxidase method.

Data analysis

SPSS software was used for data calculation and statistical analysis. Area under curve (AUC_{TPI}, AUC_{IPI} and AUC_{SI}) was calculated according to the trapezoidal rule to evaluate total quantity of insulin-related substance secreted during the glucose load test. Homa Insulin Resistant Index[16] was applied to analyse insulin resistance. Mann-Whitney U test and Spearman's correlation analysis were applied to test the difference and correlation between groups, respectively. Data are expressed as geometric means. $P<0.05$ was considered significant.

Results

Basal levels of glucose and insulin-related moleculesAs expected, fasting glucose levels were much higher in the two patient groups, with geometric means of 5.8 mmol/L (SE 0.08) from the healthy control group, 13.6 mmol/L (SE 0.49) from SU responder group and 13.7 mmol/L (SE 0.46) from SU failure group ($P=0.81$ between the two patient groups). No significant difference was found in fasting SI levels between the three groups.

As expected, fasting TPI and IPI levels in the two patient groups were significantly higher than in the control group ($P<0.001$ for both groups). However, there was no marked difference detected between the SU responder and secondary SU failure groups ($P=0.35$ for TPI and $P=0.78$ for IPI).

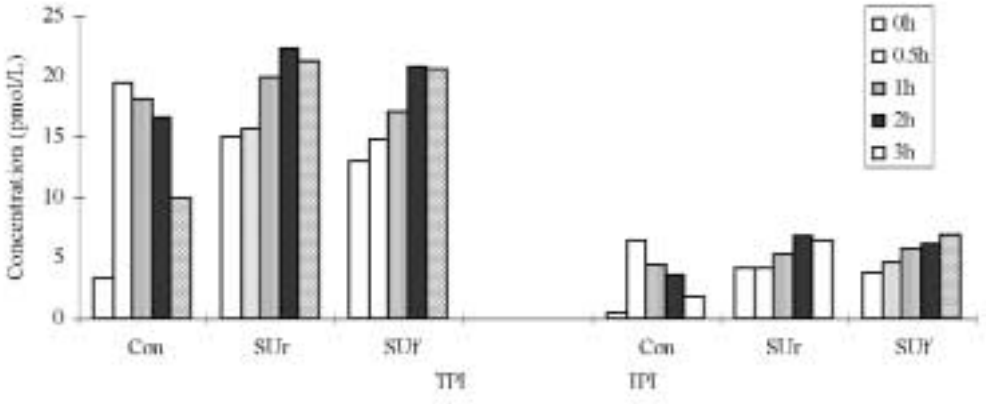

Fig. 2. Mean total proinsulin (TPI) and intact proinsulin (IPI) concentration at different time points after a glucose tolerance test.

Blood glucose and insulin-related molecules

In the control group, plasma glucose concentration returned to normal at 120 min and reached its lowest level at 180 min. In the patient groups, glucose stayed at higher levels throughout the 120-180 min period. The control group had obvious TI, TPI and IPI sharp peaks at 30-60 min, while the patient groups had a rather flattened peak appearing at 120 or 180 min.

Figures 1 and 2 illustrate the mean concentrations for all the insulin-related molecules at different time points during OGTT. It was found that the mean SI at 180 min, TPI at 180 min and IPI levels at 120 min and 180 min in most patients were higher than in the control group ($P<0.05$). These findings are in agreement with previous studies of first-phase insulin peak secretion during challenge testing in type 2 diabetes.[17]

Insulin-related molecules measured by area under curve

Table 1 shows the area under curve figures for SI, TPI and IPI in the three groups tested, and that the patient groups had much higher TPI:SI and IPI:SI ratios than the healthy control group. This indicated a dysfunction of the proinsulin converting process, resulting in disproportionate insulin-related molecules both in SU responder and secondary SU failure groups. No obvious difference was found in Homa IR between the two patient groups.

Significant differences between SU responder and secondary SU failure group

Most variables observed between the SU responders and SU failures were not obviously different (Table 1). However, Spearman's correlation analysis applied to the relationship between AUC_{SI} and the glucose level at 180 min showed a negative correlation in the SU responder group ($r = -0.57$, $P<0.001$) and no correlation in the secondary SU failure group ($r = -0.15$, $P = 0.318$). Scatter plots are shown in Figures 3 and 4.

Discussion

Secondary SU failure has been attributed to a variety of causes. Disease-related factors that have been proposed are increasing β-cell defect and increasing insulin resistance.[1] More recently, in discussing hypothetical causes of the β-cell dysfunction that might affect the proinsulin-processing,

Table 1. Statistical parameters among groups

Variables	Control group (n=8)	SU responders (n=38)	Secondary SU failures (n=46)	
	Geometric mean (SE)	Geometric mean (SE) and U test to controls	Geometric mean (SE) and U test to controls	U test between the two patient groups (P)
Glucose at 180 mins (mmol/L)	4.1 (0.26)	19.5 (0.74) P=0.002	22.8 (0.63) P=0.001	0.005
AUC_{SI}	601.8 (159.2)	166.6(22.8) P<0.001	235.4 (46.4) P=0.012	NS
AUC_{TPI} : AUC_{SI}	0.07 (0.009)	0.39 (0.14) P=0.001	0.28 (0.04) P=0.001	NS
AUC_{IPI} : AUC_{SI}	0.02 (0.004)	0.11 (0.05) P=0.001	0.09 (0.01) P=0.001	NS

AUC_{SI} Area under curve of specific insulin
AUC_{TPI} Area under curve of total proinsulin
AUC_{IPI} Area under curve of intact proinsulin
NS Not significant

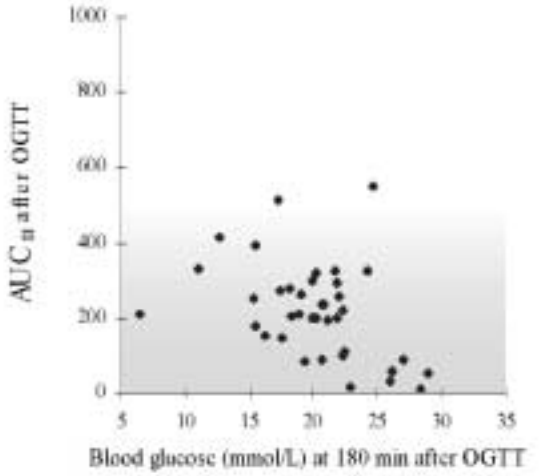

Fig. 3. Scatter diagram of blood glucose against AUC_{SI} in the sulphonylurea responder group (n=38, r = -0.57, P <0.001).

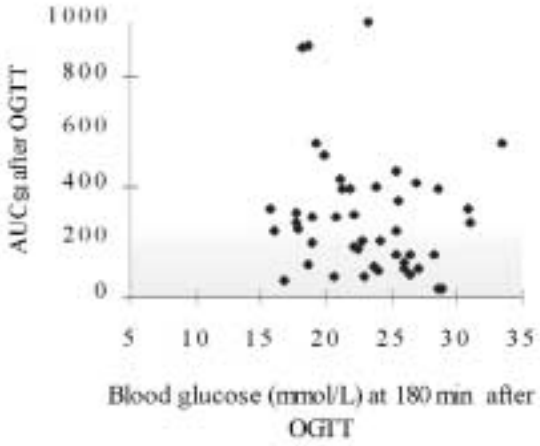

Fig. 4. Scatter diagram of blood glucose against AUC_{SI} in the secondary sulphonylurea failure group (n=46. r = -0.15, P = 0.318).

Rhodes *et al*.[3] listed several possible reasons which might lead to a proinsulin conversion mechanism dysfunction in β-cells, such as genetic variation in proinsulin conversion endopeptidases (PC2 and/or PC3) or a disproportionate ratio between PC3 and PC2. Whichever disorder exists in such patients, a high proportion of proinsulin with relatively low SI could be expected.

In the present study, we found both patient groups had much higher basal levels of proinsulin than the control group did, with a delayed peak of proinsulin appearing at 120-180 min after glucose load. We noticed that the difference in the total quantity of TPI and IPI (as AUC_{TPI} and AUC_{IPI}) secreted during the observation period were not markedly higher than in the healthy controls. In contrast to the proinsulin releasing pattern, having had a similar basal SI level among the groups, total SI (as AUC_{SI}) in the patient groups was much lower than in the control group.

No surprisingly, both patient groups had markedly higher AUC_{TPI}:AUC_{SI} and AUC_{IPI}:AUC_{SI} ratios than the control group did. A similar level of AUC_{TPI} accompanied by a much lower AUC_{SI} clearly indicates that the patients had problems in converting proinsulin into SI.

One can reason that if those with secondary SU failure had a dominant problem in the proinsulin conversion mechanism, the disproportate proinsulin to SI ratio must be more severe. However, we failed to detect any difference in this content (Table 1). This finding suggests that the proinsulin conversion mechanism dysfunction is not an extra dominant cause for SU failure.

As described earlier, we found no obvious quantitative difference in total SI released between the two patient groups after the glucose load, thus those with secondary SU failure whose glucose remained at a high level might have suffered from additional insensitivity to insulin. However, when Homa IR was calculated for the two patient groups, there was no firm evidence to suggest that the secondary SU failure group had more problems in insulin resistance.

Interestingly, mean glucose level at 180 min in the SU failure group was significantly higher than in the SU responder group. No doubt, those with SU failure had more problems in controlling glucose level than did the SU responders after a glucose load. Statistical analysis revealed that AUC_{SI} and glucose levels at 180 min were well correlated in the SU responder group (Figure 3) but not in SU failure group (Figure 4). This suggests that in responding to a glucose load, the series of actions between the secretion of SI and its biological action in the former group was relatively normal, but dysfunctional in the latter group.

A recent study has demonstrated that chronic hyperglycaemia in type 2 diabetes not only impaired β-cell function but also reduced the metabolic clearance rate of glucose.[18] Others have proposed that chronic hyperglycaemia may impair the absorption of SU.[12]

Although we have no direct proof from our study, most of those with secondary SU failure did have a longer disease history during which chronic hyperglycaemia was unsatisfactorily controlled. In our clinical practice, we found some patients with secondary SU failure showed improved insulin sensitivity and gained better control of hyperglycaemia after a period of insulin therapy. This outcome seems to agree with the above theory; however, other pathologies need to be investigated.

Whatever the outcome of further studies, some important conclusions may be drawn from this work. Firstly, in most cases of type 2 diabetes, first-phase secretion of insulin-like molecules disappeared after a glucose load. Secondly, type 2 diabetics had an obvious dysfunction in the proinsulin converting process and showed severe SI deficiency in responding to glucose challenge. Thirdly, proinsulin conversion mechanism dysfunction is not an extra dominant cause of secondary SU failure. Finally, those with secondary SU failure had an additional problem in that endogenous insulin failed to properly control blood glucose. □

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