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Sulfonylurea challenge test in subjects diagnosed with Type 1 diabetes mellitus

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Abstract

Patients with early onset diabetes due to defects in glucose-stimulated insulin secretion (GSIS) may respond better to sulfonylureas than insulin treatment. Such patients include those with monogenic disorders, who can be differentiated from autoimmune type 1 diabetes mellitus (T1DM) by genetic testing. Genetic testing is expensive and unknown defects in GSIS would not be diagnosed. We propose a sulfonylurea challenge test to identify patients that have been clinically diagnosed with T1DM, but who maintain a preferentially sulfonylurea-responsive insulin secretion. Three healthy controls, two neonatal diabetes (NDM) subjects, three antibody-positive (Ab+T1DM), and twelve antibody-negative (Ab-T1DM) subjects with Type 1 diabetes, were given an intravenous bolus of glucose followed by an oral dose of glipizide. Healthy controls showed a robust C-peptide increase after both glucose and glipizide, but NDM subjects showed a large increase in C-peptide only following glipizide. As expected, two of three Ab+T1DM, as well as eleven of twelve Ab-T1DM showed no response to either glucose or glipizide. However, one Ab-T1DM and one Ab+T1DM showed a small C-peptide response to glucose and a marked positive response to glipizide, suggesting defects in GSIS rather than typical autoimmune diabetes. These data demonstrate the feasibility of the sulfonylurea challenge test, and suggest that responder individuals may be identified. We propose that this sulfonylurea challenge test should be explored more extensively, as it may prove useful as a clinical and scientific tool.

Keywords

Neonatal diabetes; Type1 diabetes; Diabetes mellitus; Sulfonylurea; Treatment

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Introduction

The current understanding of T1DM is that autoimmune destruction of the β -cell leads to a permanent, insulin-requiring, disease. Accordingly, treatment is primarily focused on insulin replacement, with acute complications of hyper- and hypoglycemia being the primary barrier to tight glycemic control. T1DM has been associated with the presence of autoantibodies, including ICA-512, GAD-65 and IA-2; however, approximately 10% percent of patients diagnosed with T1DM are negative for autoimmune markers and 5% are persistently antibody-negative in follow up evaluations (1). The SEARCH for diabetes in youth study showed that 10% of 2,291 subjects have non-autoimmune diabetes and suggested genetic mutations that affect β -cell function as a possible etiology of diabetes in these patients (2). Accordingly, the ISPAD clinical practice guidelines now recommend genetic testing in very early onset and antibody-negative childhood diagnoses (3). A higher incidence of HNF1A mutations and variants that are highly associated with Type 2 diabetes, have been identified in the persistently antibody-negative group (1). We and others have demonstrated that at least one form of insulin-deficient diabetes, Neonatal Diabetes Mellitus (NDM), is primarily due to defects in the glucose metabolism and excitation-dependent insulin secretion pathway (4–10). In such patients, β -cell mass is not initially lost, and insulin therapy is not necessarily appropriate. Indeed, most NDM patients who have mutations in one of the two subunits of the ATP sensitive K^+ (K_{ATP}) channel (Kir6.2 or SUR1) will robustly and repeatedly respond to sulfonylureas, which circumvents the insulin secretory defect (7, 11–14).

Restoring the insulin secretory response in patients who still have functional yet silent β -cells not only improves glycemic control but also often results in a reduction in the incidence of hypoglycemia. It is conceivable that our understanding of the mechanistic basis of the diabetes in some individuals carrying the diagnosis of T1DM is not correct, and that defects in the pathway of GSIS are at fault. The dramatic demonstration of sulfonylurea dependent insulin secretion in NDM (11), as well as in carriers of HNF1A mutations (15) makes a compelling argument for further investigation of the potential action of these drugs in relevant groups of patients with diabetes. Sulfonylureas may provide not only a suitable tool for identification of such individuals but also a potentially appropriate therapy, as is clear in humans and mice with NDM (4, 11–14, 16, 17). The pathway of glucose-stimulated insulin secretion is complex and defects at any step could result in failure of insulin secretion (reviewed by (6, 18)). We hypothesize that some people diagnosed with T1DM, particularly those that are autoantibody negative, may actually suffer from defects in GSIS and still have viable β -cells that do not secrete in response to glucose, but will still secrete insulin in response to sulfonylureas. In this study, we therefore attempted to develop an approach to evaluate endogenous insulin secretion in response to glucose- and sulfonylurea-challenge in a cohort of patients previously diagnosed as Type 1 diabetes.

Methods

Subjects

All procedures were approved by the Washington University Human Research Protection Office's Institutional Review Board. Subjects of any age and gender with a clinical diagnosis

of T1DM of at least two years duration (based on criteria established by the American Diabetes Association (19)) were recruited from Endocrinology and Diabetes clinics at St. Louis Children's Hospital and Barnes-Jewish Hospital at Washington University in St. Louis between September 2012 and May 2014. A total of 45 T1DM subjects, 27 male (61%) and 17 female (39%), ranging in age from 3 to 50 years old were enrolled. Informed consent was obtained from all subjects. At the initial visit of the research study, ICA-512 and GAD-65 antibody levels were obtained and analyzed at Quest Diagnostics (Quest Diagnostics, 3 Giralda Farms, Madison, NJ). ICA-512 levels >1.25 JDRF units and GAD-65 antibody levels > 0.02 nmol/L were considered to be positive.

Subjects with liver, renal, neurologic, or cardiac disease were excluded. Three healthy subjects without diabetes or other chronic disease were included as normal controls for the sulfonylurea challenge test. Two subjects diagnosed with NDM and known to be responsive to sulfonylureas were further included as positive controls. One was diagnosed with TNDM with recurrence in adulthood (subject TNDM). This subject had previous negative sequencing for mutations of KCNJ11 (Kir6.2) and ABCC8 (SUR1) and was being treated with insulin, but had developed hypoglycemia on sulfonylurea therapy in the past. She consented to whole exome sequencing (as a follow up to this study) and was found to have uniparental disomy on chromosome 6, known to be associated with transient neonatal diabetes (20, 21). In addition, she had a homozygous R242H variant of the MEPIA gene, of unknown relevance. The other permanent NDM (PNDM) subject was known to have an R201H mutation of KCNJ11 (from genetic sequencing as an adult) and was on both glyburide (sulfonylurea) and insulin therapies for diabetes management. Three subjects diagnosed with type-1 diabetes mellitus (T1DM) with positive autoantibodies were included as presumed negative controls (but see Results). Twelve subjects previously diagnosed with T1DM who had negative ICA and GAD antibody levels were included as test subjects.

Sulfonylurea Challenge

Three control subjects without diabetes (Subjects C1, C2, C3), 3 subjects with antibody-positive Type 1 diabetes (Subjects Ab+T1DM:1, Ab+T1DM:2, and Ab+T1DM:3), 2 subjects with known NDM (Subjects PNDM and TNDM) and 12 subjects found to have antibody-negative Type 1 diabetes (Subjects Ab-T1DM:1 through 12) participated in the sulfonylurea challenge test. We measured C-peptide response to a single bolus dose of glucose followed by an oral dose of sulfonylurea. Glipizide was chosen as the sulfonylurea based on its relatively short half-life.

Subjects arrived fasted in the morning after omitting their usual morning insulin or glyburide dose. Basal insulin therapy was continued. Urine ketone and blood glucose levels were measured, and human chorionic gonadotropin testing (for pregnancy) was performed on arrival. If ketone levels were high or the pregnancy test was positive, further testing was cancelled. Fasting insulin, C-peptide, and glucose levels were obtained at 20 minutes, 10 minutes and 0 minutes prior to 0.5 g/kg i.v. dextrose (maximum 20 g dextrose), and again at 1, 3, 5, 10, and 20 minutes after i.v. dextrose. If the glucose level on arrival was greater than 300 mg/dL, no dextrose was given but blood samples were obtained with the same time sequence. A single oral dose of glipizide was given 20 minutes after i.v. dextrose; insulin, C-

peptide and glucose levels were obtained over the next 180 minutes. Subjects were then given lunch and dosed with insulin as per their usual regimen at lunchtime. Controls C1, C2, and C3 were adults and were given 5, 15, and 40 mg of glipizide, respectively. Ab+T1DM:1, 2 and 3, and PNDM were adults over 50 kg and were given 40 mg glipizide. Ab-T1DM:1-12 and TNDM, between 3 and 45 years old, were given 0.3 mg/kg up to max of 15 mg glipizide for the sulfonylurea challenge test.

Results

Parameter setting in control subjects

C1, C2, and C3, all healthy subjects who had tested negative for ICA and GAD antibodies (Table 1), showed C-peptide rising in response to glucose and rising again after glipizide (Fig. 1). A small increase in C-peptide levels was found in C1's response to 5 mg glipizide (Fig. 1A), but there was a vigorous response in C2 and C3 given 15 or 40 mg glipizide, respectively, with corresponding hypoglycemia (Fig. 1B, C). The rise in C-peptide after glipizide is in contrast to the continuing fall in insulin expected by 20 minutes after a single bolus dose of glucose (22). Based on this result, a dose of 15 mg glipizide was chosen as sufficient to elicit a C-peptide response but minimize hypoglycemia in normal subjects, and was given in initial studies in T1DM subjects. However, once it became evident that most T1DM subjects failed to demonstrate any hypoglycemia with a 15 mg dose, a dose of 40 mg was used thereafter.

Figure 2 shows the results from the two subjects with neonatal diabetes. Subject PNDM underwent sulfonylurea challenge with 20 g i.v. dextrose and 40 mg glipizide. Dextrose administration resulted in hyperglycemia up to serum glucose levels of 450 mg/dl with only a modest (1.55 ng/ml) increase in C-peptide, reflecting the expected impaired glucose-mediated insulin secretion in individuals with KCNJ11 gain-of-function mutations (18). Sulfonylurea challenge elicited a greater increase in C-peptide levels, with a peak at 1.85 ng/ml, even as glucose levels decreased (Fig. 2A). Patient TNDM similarly showed a small increase in C-peptide after 20 g i.v. dextrose, and a marked C-peptide release after 15 mg glipizide (Fig. 2B).

Two of the antibody positive T1DM subjects (Ab+T1DM:1 and 2) (Table 1) showed the anticipated failure to increase C-peptide levels in response to either dextrose infusion or sulfonylurea injection (Fig. 2C,D). However, Ab+T1DM:3 showed a small C-peptide response to dextrose, and an unexpectedly higher increase in C-peptide levels after a single dose of 40 mg glipizide (Fig. 2E). Ab+T1DM:3 was a 20 year old woman diagnosed with Type 1 diabetes at age 15 when she presented with a hemoglobin A1c >14% without ketoacidosis with positive GAD-65 antibodies (10 U/ml) and ICA512 antibodies (3.2 U/ml). She was poorly controlled on subcutaneous insulin and presented twice with moderate ketonuria, once after apparently taking little or no insulin for several months. She became pregnant soon after this study result became available and is currently lost to follow up.

Sulfonylurea challenge in antibody-negative T1DM subjects

Twenty nine of the additional 41 diagnosed T1DM subjects tested positive for at least one of the three tested antibodies and were not studied further (Ab screen only, Table 1). The twelve antibody-negative T1DM subjects (Ab-T1DM, Table 1) underwent the glucose and sulfonylurea challenge. Of these, eleven also showed no increase in C-peptide levels after either 20 g i.v. dextrose or glipizide (Fig. 3A). Interestingly, however, one Ab-T1DM subject (Ab-T1DM:12) did respond preferentially to glipizide (Fig. 3B). Ab-T1DM:12 was a 16 year old boy who presented at age 14 with diabetes with hemoglobin A1c of 11.5%, mild acidosis (pH of 7.32), and has maintained good control on subcutaneous insulin. GAD65 and ICA512 antibody levels were undetectable.

Discussion

Can Type 1 diabetes be sub-stratified?

It is often assumed that young, non-obese, patients with diabetes have T1DM, without potential for therapy other than injected insulin. Thus, most children and young adults who appear to fit the phenotype for T1DM do not undergo testing for monogenic diabetes. The volume of patients and perceived low yield and high cost of genetic testing is a barrier, as is the large number of genes now known to cause neonatal diabetes and MODY diabetes. However, the dramatic improvement in lifestyle and potentially in glycemic control afforded by the identification of a sulfonylurea-responsive form of diabetes would make identification worthwhile, if an appropriate test could be delineated. Low levels of detectable C-peptide have been documented in multiple patients with T1DM who have a long duration of diabetes (23–25) and our limited data presented here support these findings in a younger population than previously described. The mechanistic basis is unclear, but such findings raise the possibility that stimulation of meaningful levels of endogenous insulin secretion by sulfonylurea medications might be possible in such patients. Genetic testing is currently the only strategy in clinical practice for definitive determination of alternate causes of diabetes and with the potential to introduce therapies other than insulin. We suggest that testing for a differential C-peptide response to glucose challenge vs. sulfonylurea may not only assist in categorizing residual β -cell function and provide cost-effective supporting evidence for sending genetic evaluation for certain forms of sulfonylurea-responsive MODY, but might also result in alternate therapeutic options for responsive individuals.

Sulfonylurea challenge as a clinical test?

Clinical response to sulfonylureas varies in type 2 diabetes, and even in neonatal diabetes with the same underlying genetic mutations in the sulfonylurea target K_{ATP} channel genes ABCC8 and KCNJ11 (11). We found low glucose-stimulated C-peptide production in most subjects diagnosed with T1DM, consistent with the presumption of near complete loss of β -cells, whether antibodies were positive or negative. However, two of twelve T1DM subjects, one antibody positive and one antibody negative, responded robustly to a single dose of glipizide even though both subjects had a poor C-peptide response to glucose infusion (Figures 2,3). This differential response to glucose versus sulfonylurea suggests that these subjects are not simply typical T1DM patients with residual β -cells in a prolonged “honeymoon” period. Instead, it suggests they are patients with a defect in glucose-

stimulated insulin secretion that can be circumvented by sulfonylurea. Patients with typical autoimmune destruction of β -cells may show a small C-peptide response to sulfonylurea if there are β -cells remaining, particularly during the so-called “honeymoon” phase of the illness, but such patients would be expected to show a similar small C-peptide response to hyperglycemia. A finding of a poor C-peptide response to glucose but a more robust C-peptide response to sulfonylurea implies a defect in glucose metabolism-secretion coupling.

To address this issue quantitatively, we propose that the test be administered so as to directly compare the relative response to sulfonylurea and to glucose. Figure 4 illustrates a proposed analysis. The C-peptide area under the curve (AUC) in response to dextrose (AUCDex) can be estimated by assuming an exponential decay following injection, and then subtracted from the total AUC to obtain the glipizide response (AUCGlip, Figure 4A). Alternatively, administering the glucose challenge and the drug challenge on two separate occasions would allow the glucose AUC to be obtained directly. Using the first approach, we show that the AUCGlip for all responders and positive control subjects is in the range 50–500ng/L.min, but is below the detection limit of ~10ng/mL.min for the non-responders (Figure 4B, above). Notably, the ratio AUCGlip/AUCDex (Figure 4B, below) does appear to distinguish responders (AUCGlip/AUCDex >6) from control subjects who respond to both glucose and SU (AUCGlip/AUCDex <2).

The study found a higher number of antibody negative subjects than naively expected. We suggest that is likely due to the antibody screens not having being made at the time of diagnosis. Although our subject group is very small the finding of sulfonylurea responsivity in both one autoantibody positive and one autoantibody negative patient is suggestive that positive antibody testing may not necessarily rule out alternative causes. When genetic testing is obtained in the clinical setting, typically only individual candidate genes are examined, and any novel genetic causes will be missed. Thus any individuals without a known genetic mutation would miss the opportunity to explore sulfonylureas as a therapeutic option. Since the interpretation of genetic testing is best correlated with clinical status, the protocol we present could provide additional information to clinical providers and to patients regarding residual β -cell function and stimulatory C-peptide response. We would note that a robust C-peptide response to sulfonylurea in this challenge test still does not necessarily mean that the subject has a form of diabetes that will have a good outcome with long-term sulfonylurea therapy, and clearly far more extensive follow-up studies are needed.

Conclusions

In conclusion, the sulfonylurea challenge test that we present is a promising, relatively low-cost, low risk method of identifying which patients with newly diagnosed diabetes may have a specific defect in metabolism-excitation-secretion coupling rather than autoimmune T1DM. Such patients may then benefit from further genetic analysis for known and unknown causes of diabetes. The test could potentially identify those who could benefit from sulfonylurea therapy. The sentinel example of NDM caused by mutations in the K_{ATP} channel has proven the principle that even long-standing diabetes with such defects, who otherwise exhibit no evidence of C-peptide response to glucose can respond robustly to sulfonylureas (7, 11, 14). The ability of sulfonylurea drugs to stimulate C-peptide production

has not been well characterized in broader cohorts of patients diagnosed with T1DM. The protocol we present allows objective measurement of C-peptide response to sulfonylurea as an assessment of β -cell function in the identification of sulfonylurea-responsive subjects with diabetes. The feasibility we demonstrate, together with positive findings in even such a small cohort, therefore warrants more extensive implementation to determine the usefulness of the test, both as a potential guide to the choice of most appropriate therapy and genetic testing strategies for patients, as well as for identification of novel genetic causes of diabetes.

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Figure 1.

Effect of glucose and glipizide on glucose (triangles) and C-peptide (circles) concentrations in healthy control subjects. Control subjects: C1 (A), given 20 g i.v. dextrose at 20 minutes and 5 mg oral glipizide at 40 minutes; C2 (B), given 20 g i.v. dextrose at 20 minutes and 15 mg glipizide at 40 minutes; and C3 (C), given 20 g i.v. dextrose at 20 minutes and 40 mg glipizide.

Figure 2.

Effect of glucose and glipizide on glucose (triangles) and C peptide (circles) concentrations in presumed positive responders (known NDM patients) and presumed non-responders (Antibody-positive T1DM patients). Note different scale axes as compared with Fig. 2. C-peptide levels and glucose response in subject PNDM (A) and TNDM (B) were tested with 20 g i.v. dextrose at 20 minutes and 40 mg oral glipizide at 40 minutes (C,D) Antibody-positive T1DM subjects (Ab+T1DM:1 and Ab+T1DM:2) show no change in C-peptide concentration after glucose or 40 mg glipizide challenge. In D, no glucose was given as the

blood glucose already exceeded 300 mg/dl. E: Subject Ab+T1DM:3 shows increased C-peptide concentration after both glucose and 40 mg glipizide challenge.

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Figure 3.

Effect of glucose and glipizide on glucose (triangles) and C-peptide (circles) in antibody-negative T1DM subjects. (A) Averaged results ($\pm$ SEM) from eleven subjects with antibody negative diabetes, all of whom showed no significant increase in C-peptide after 20 g i.v. dextrose and C-peptide release after 15 mg glipizide. Note that scale of C-peptide is different than figure 4. (B) One subject with antibody negative diabetes, Ab-T1DM:12, showed some C-peptide response (circles) to glucose and glipizide.

Figure 4.

Analysis of SU responsivity versus glucose responsivity. (A) Method of analysis illustrated for data from control Subject 1. C-peptide response to glucose was assumed to decline exponentially from initial peak to baseline (mean of 4 measurements before dextrose injection). The area under the resultant curve (AUCDex, blue) was then subtracted from the area under the curve (up to 200 minutes) to estimate the AUCGlip. (B) AUCGlip (above) and the ratio of AUCGlip/AUCDex (below). For healthy control subjects, PNDM, and TNDM, as well as the two responder T1DM patients (Ab+T1DM:3, Ab-T1DM:12),

AUCGlip was >50 ng/ml.min, but was <10 ng/ml.min, for all non-responder Ab+T1DM and Ab-T1DM patients. AUCGlip/AUCDex was <3 for healthy control subjects, but was >6 for responder NDM controls and test subjects.

Table 1

GAD and ICA antibody test results in control and diabetic subjects.

Subject ID	Age	gender	GAD antibody	ICA antibody
C1	18	f	0	0
C2	30	m	0	0
C3	54	f	0	0
PNDM	31	f	Not tested	Not tested
TNDM	35	f	0	0
Ab+T1DM:1	19	m	0.27	0
Ab+T1DM:2	23	f	1.28	0
Ab+T1DM:3	22	f	2.39	0
Ab-T1DM:1	4	m	0	0
Ab-T1DM:2	18	f	0	0
Ab-T1DM:3	17	f	0	0
Ab-T1DM:4	14	m	0	0
Ab-T1DM:5	13	m	0	0
Ab-T1DM:6	12	m	0	0
Ab-T1DM:7	17	m	0	0
Ab-T1DM:8	16	f	0	0
Ab-T1DM:9	14	f	0	0
Ab-T1DM:10	18	f	0	0
Ab-T1DM:11	46	f	0	0
Ab-T1DM:12	16	m	0	0
Ab screen only	20	m	0.1	0
Ab screen only	22	m	5	4.03
Ab screen only	21	m	0	0
Ab screen only	22	f	0	0
Ab screen only	21	m	0	40
Ab screen only	14	m	0.02	0
Ab screen only	18	m	1.85	0
Ab screen only	20	m	0	0
Ab screen only	17	m	0	0
Ab screen only	16	m	2.56	0
Ab screen only	18	f	1.93	0
Ab screen only	17	m	0	0
Ab screen only	15	m	0.07	0
Ab screen only	16	m	2.38	0
Ab screen only	8	f	0	0
Ab screen only	15	m	0	0
Ab screen only	18	f	1.07	0

Subject ID	Age	gender	GAD antibody	ICA antibody
Ab screen only	9	f	0.06	0
Ab screen only	15	f	0.07	0
Ab screen only	15	m	0.12	0
Ab screen only	16	m	0.07	0
Ab screen only	8	m	0	0
Ab screen only	9	m	0	10
Ab screen only	14	m	0	0
Ab screen only	19	f	0.05	0
Ab screen only	9	m	1.03	0
Ab screen only	30	m	0.05	0
Ab screen only	6	f	0	63
Ab screen only	14	f	0	0

Table 2

Response to glucose infusion or to glipizide in control and diabetic subjects.

Subject ID	C peptide response to glucose	C peptide response to glipizide
C1	large	large
C2	large	large
C3	large	large
PNDM	small	large
TNDM	small	large
Ab+T1DM:1	none	none
Ab+T1DM:2	none	none
Ab+T1DM:3	small	large
Ab-T1DM:1	none	none
Ab-T1DM:2	none	none
Ab-T1DM:3	none	none
Ab-T1DM:4	none	none
Ab-T1DM:5	none	none
Ab-T1DM:6	none	none
Ab-T1DM:7	none	none
Ab-T1DM:8	none	none
Ab-T1DM:9	none	none
Ab-T1DM:10	none	none
Ab-T1DM:11	none	none
Ab-T1DM:12	small	large