



The Effect of DPT-1 Intravenous Insulin Infusion and Daily Subcutaneous Insulin on Endogenous Insulin Secretion and Postprandial Glucose Tolerance

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OBJECTIVE

To investigate the effect of parenteral insulin therapy on endogenous insulin secretion in the Diabetes Prevention Trial–Type 1 (DPT-1).

RESEARCH DESIGN AND METHODS

In the parenteral insulin arm of DPT-1, subjects without diabetes at high risk of future type 1 diabetes randomized to active treatment received a yearly 4-day intravenous insulin infusion (IV-I) and daily subcutaneous insulin (SC-I). To examine the effects of these insulin therapies on endogenous insulin secretion, C-peptide and glucose levels were compared during oral glucose tolerance tests (OGTTs) performed on and off IV-I and SC-I. Forty-six paired OGTTs were performed in 30 subjects from DPT-1 to determine the effect of IV-I. Twenty paired OGTTs were performed in 15 subjects from DPT-1 to determine the effect of SC-I.

RESULTS

IV-I suppressed fasting and OGTT-stimulated C-peptide (62% and 40%, respectively), and it significantly lowered fasting glucose (67.4 ± 4.5 mg/dL during IV-I vs. 90.9 ± 1.8 mg/dL off insulin; $P < 0.05$). By contrast, post-OGTT glucose levels were significantly higher during IV-I: Glucose during IV-I versus off insulin at 120 min was 203.9 ± 15.1 vs. 151.6 ± 10.2 mg/dL, respectively ($P < 0.05$); 49% of OGTTs became transiently diabetic (>200 mg/dL at 120 min) when receiving IV-I. Fasting glucose was significantly lower when receiving SC-I versus when off insulin (85 ± 3 vs. 94 ± 2 mg/dL, respectively; $P < 0.05$), but SC-I did not significantly alter fasting or OGTT-stimulated C-peptide compared with being off insulin.

CONCLUSIONS

These data demonstrate that the IV-I used in the DPT-1 markedly suppressed endogenous insulin secretion, which was frequently associated with postprandial glucose intolerance. SC-I, however, did not.

Type 1 (insulin-dependent) diabetes results from immune-mediated destruction of pancreatic islet insulin-secreting β -cells in genetically predisposed individuals. The onset of clinical symptoms of diabetes represents the critical turning point in a progressive decline in β -cell function and appears only when β -cell secretory function is severely impaired. Although type 1 diabetes develops insidiously, often years after

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the induction of the pathogenic immune-mediated destructive process, it can be predicted by using immunological markers and testing insulin secretion (1–4). The Diabetes Prevention Trial–Type 1 (DPT-1) was designed to test whether intervention during the prodromal period of the disease can prevent or delay the clinical onset of type 1 diabetes.

The DPT-1 was a randomized, national, multicenter trial designed to determine whether insulin therapy of high-risk individuals without diabetes could prevent or delay subsequent type 1 diabetes (5). In the parenteral arm of the DPT-1, insulin was administered intravenously (IV-I) for 4 days each year and subcutaneously (SC-I) each day to those subjects randomized to active treatment. One proposed mechanism whereby parenteral insulin might confer protection against subsequent type 1 diabetes was inhibition of endogenous insulin secretion (sometimes called β -cell rest). The aim of this ancillary study was to determine whether IV-I and/or SC-I suppressed endogenous insulin secretion.

RESEARCH DESIGN AND METHODS

In the DPT-1, a combination of autoantibodies (islet cell antibodies and insulin autoantibodies), genetic (absence of HLA-DQA1*0102, HLA-DQB1*0602), and metabolic markers (impaired first-phase insulin response based on intravenous glucose tolerance test and/or impaired glucose tolerance during an oral glucose tolerance test [OGTT]) were used to identify relatives of patients with type 1 diabetes who did not have diabetes but were at high risk of future type 1 diabetes ($\geq 50\%$ within 5 years). After informed consent, high-risk subjects were randomized to parenteral insulin or a closely monitored control group. Active treatment consisted of yearly IV-I for 4 days, performed at a general clinical research center or an equivalent facility, and daily SC-I. The IV-I used regular human insulin (Humulin R; Eli Lilly) and was initiated at a basal infusion rate of 0.015 units/kg/h, with frequent dose adjustments to maintain whole-blood glucose level 60–80 mg/dL. During the rest of the year, subjects received subcutaneous ultralente human insulin (Humulin U; Eli Lilly) given twice daily (a total daily dose of 0.25 units/kg) (5).

To detect the development of type 1 diabetes, OGTTs were performed every 6 months after subjects had been off all insulin for 3 days (off-I). Oral glucose was administered in a dose of 75 g in adults or 1.75 g/kg body weight (maximum 75 g) in children as a solution in flavored water that was consumed within 5 min. Two baseline samples were obtained at 10 and 0 min before the glucose was consumed and then at 30, 60, 90, and 120 min after glucose consumption was completed. Glucose and C-peptide were measured at all time points in the DPT-1 Beta Cell Function Core Laboratory (Seattle, WA).

To determine the effect of IV-I and SC-I on endogenous β -cell function, fasting glucose, and glucose tolerance, the DPT-1 OGTTs performed off-I for 3 days per DPT-1 protocol were compared with OGTTs performed on IV-I and on SC-I in this DPT-1 ancillary study. The OGTT while on IV-I was performed on the fourth day of the insulin infusion, with the infusion still continuing, and was done within a week after the DPT-1 OGTTs (off SC-I). The OGTT on SC-I was performed with subcutaneous ultralente insulin uninterrupted and was done either before or after the DPT-1 OGTTs (off SC-I) within 3 months. Five clinical centers at the University of Washington, University of Minnesota, Children's Hospital of Los Angeles, Stanford University, and University of Miami participated in this ancillary study of DPT-1.

For the IV-I versus off-I comparison, 46 paired OGTTs were performed in 30 individuals. Fasting C-peptide and glucose were available for all 46 pairs, but complete glucose and C-peptide measurements were available for 37 paired OGTTs from 22 subjects. For the SC-I versus off-I comparison, glucose and

C-peptide were available for 20 and 19 paired OGTTs, respectively, in 15 individuals. The demographics of the subjects in both the IV-I and SC-I groups are summarized in Table 1.

Statistical Analysis

Mean glucose and C-peptide values were calculated at times related to OGTT testing performed while receiving insulin and off-I, adjusted for subjects with multiple testing. Data are expressed as mean \pm SE. Differences in means were tested using ANOVA, adjusting for subjects who had more than one test. Differences in categorical variables were tested using the χ^2 statistic and Fisher exact test. The relationship between the obtained glucose and C-peptide results was examined using both Pearson correlation coefficients and stepwise linear regression, adjusted for subjects with multiple tests. All tests of significance were two-tailed. Statistical analyses were performed using SAS software (SAS Institute, Cary, NC) and conducted by the DPT-1 Data Monitoring Unit of the University of South Florida, Tampa, FL.

RESULTS

The Effect of IV-I and SC-I on Basal and OGTT-Stimulated Insulin Secretion (C-Peptide)

IV-I suppressed fasting C-peptide approximately 62% (mean C-peptide \pm SE during IV-I vs. off-I 0.35 ± 0.07 vs. 0.93 ± 0.11 ng/mL, respectively [0.12 ± 0.02 vs. 0.31 ± 0.04 nmol/L, respectively] [$n = 46$]) and suppressed post-OGTT C-peptide approximately 40% (mean C-peptide \pm SE during IV-I vs. off-I at 30 min (1.86 ± 0.23 vs. 3.11 ± 0.33 ng/mL, respectively [0.62 ± 0.07 vs. 1.04 ± 0.11 nmol/L, respectively] [$n = 37$]; $P < 0.05$) and at 60 min

Table 1—Demographics of the subjects in each group

	IV-I group ($n = 30$)	SC-I group ($n = 15$)
Age at randomization (years)	16.5 \pm 12.4	20.1 \pm 12.9
BMI	18.7 \pm 4.2	20.4 \pm 4.8
Sex		
Male	17 (56.7)	9 (60)
Female	13 (43.3)	6 (40)
Race/ethnicity		
Non-Hispanic white	29 (96.7)	15 (100)
Unknown	1 (3.3)	
Impaired glucose tolerance	12 (40)	6 (40)

Values are mean \pm SD or n (%).

(2.66 ± 0.26 vs. 4.34 ± 0.43 ng/mL, respectively [0.89 ± 0.09 vs. 1.45 ± 0.14 nmol/L, respectively] [$n = 37$]; $P < 0.05$) (Fig. 1). In the paired OGTTs, 43 of 46 (93.5%) had lower fasting C-peptide during IV-I versus off-I; 21 of 37 (56.8%) had lower mean glucose-stimulated C-peptide (mean C-peptide levels at 30, 60, 90, and 120 min – mean basal C-peptide levels at –10 and 0 min during fasting) based on OGTTs during IV-I versus off-I. There was no significant relationship between age, sex, BMI, or fasting and peak C-peptide off-I with the degree of suppression of fasting or stimulated C-peptide while receiving insulin. In addition, the results of IV-I on C-peptide levels were similar when only one paired test per subject (first pair) was used in the analysis.

The correlation between fasting C-peptide and oral glucose-induced peak C-peptide was analyzed during the control and on insulin infusion OGTTs. There was a positive correlation between fasting and peak C-peptide in the OGTTs when off-I ($R = 0.4614$; $P < 0.05$), but no significant correlation was found in OGTTs during IV-I ($R = 0.2160$; $P = 0.19$) (Fig. 2).

Fasting and stimulated C-peptide levels during OGTTs while receiving and off SC-I were similar; the only significant difference occurred at 120 min. The mean C-peptide level at 120 min was higher during SC-I versus off-I (4.64 ± 1.36 vs. 4.16 ± 1.32 ng/mL, respectively [1.55 ± 0.45 vs. 1.39 ± 0.44 nmol/L, respectively] [$n = 19$]; $P < 0.05$).

The Effect of IV-I and SC-I on Fasting Glucose and Oral Glucose Tolerance

Fasting glucose was significantly lower in OGTTs during IV-I versus those off-I (67.4 ± 4.5 vs. 90.9 ± 1.8 mg/dL, respectively [$n = 46$]). By contrast, post-OGTT glucose levels were higher during IV-I (mean glucose for IV-I vs. off-I at 90 min 216.8 ± 12.7 vs. 165.2 ± 10.7 mg/dL, respectively [$n = 37$]; at 120 min 203.9 ± 15.1 vs. 151.6 ± 10.2 mg/dL, respectively [$n = 37$]; all $P < 0.05$) (Fig. 3). In 33 of the 37 paired OGTTs the 2-h glucose was higher during IV-I compared with off-I, and in 18 of 37 (48.7%) the IV-I OGTT was transiently diabetic (i.e., >200 mg/dL at 120 min) (Fig. 4). There was no significant difference in the 2-h glucose levels between those subjects with diabetic OGTTs and those with non-diabetic OGTTs during IV-I when they

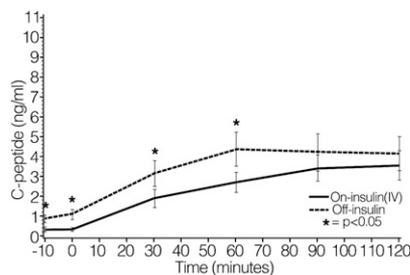


Figure 1—C-peptide during OGTTs performed on and off IV-I therapy. Values are adjusted mean C-peptide \pm standard errors of the mean ($n = 37$ paired OGTTs).

were off-I (134.8 ± 47 vs. 139.7 ± 39.9 mg/dL; $P = 0.7339$). There was no significant relationship between age, sex, BMI, or fasting and peak C-peptide off-I with the degree of hyperglycemia while receiving insulin. The effect of IV-I on glucose levels showed similar results when all the data and only one test per subject (the first test) was analyzed.

Correlation between the percentage change of C-peptide and the percentage change of glucose per subject in OGTTs during IV-I and off-I was analyzed. There was no correlation between the percentage change of C-peptide and the percentage change of glucose in the OGTTs at 0, 30, 60, and 120 min, except for a relatively weak correlation between the percentage change of C-peptide at 120 min and the percentage change of glucose at 60 min ($R = 0.4696$; $P < 0.05$). In addition, there was no significant relationship of the

delta 0–30 min C-peptide change during OGTTs between IV-I and off-I ($R = 0.2109$; $P > 0.05$).

Fasting glucose was significantly lower during SC-I versus off-I (85 ± 3 vs. 94 ± 2 mg/dL, respectively [$n = 20$]; $P < 0.05$). There were, however, no significant differences in mean glucose levels while receiving and while off SC-I at the 30-, 60-, 90-, and 120-min OGTT time points (data not shown).

CONCLUSIONS

We report in this study that the 4-day IV-I used in the DPT-1 inhibits endogenous insulin secretion and that the administration of oral glucose partially overcomes this inhibition. This suppression of endogenous insulin secretion is frequently associated with postprandial glucose intolerance. By contrast, daily SC-I, as used in the DPT-1, does not alter endogenous insulin secretion.

Parenteral insulin therapy has been shown to protect against type 1 diabetes in both the BB rat and the NOD mouse—animal models of type 1 diabetes in humans. Repeated injections of insulin in young, prediabetic BB rats (6–12) or NOD mice (13–16) from early life inhibited the development of diabetes and reduced the severity of islet inflammation (insulinitis). Evidence supporting both metabolic and immunologic mechanisms has been reported (9,11,15,16).

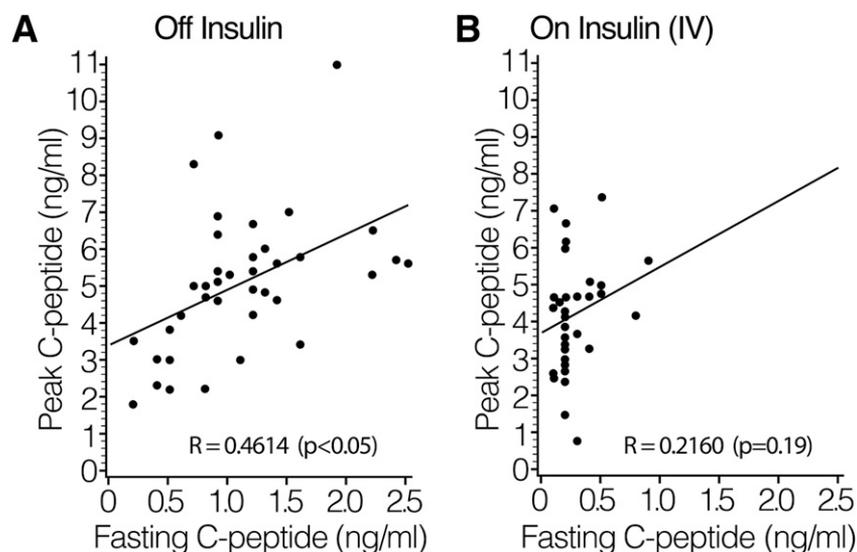


Figure 2—Relationship between fasting and stimulated C-peptide (nanograms per milliliter) off insulin (panel A) and on IV-I (panel B) ($n = 37$ paired OGTTs).

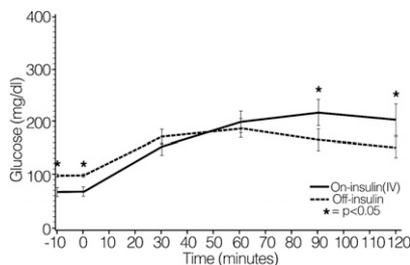


Figure 3—Glucose during OGTTs performed on and off IV-I therapy. Values are adjusted mean glucose \pm standard errors of the mean ($n = 37$ paired OGTTs).

In humans, early and aggressive insulin treatment was suggested to be beneficial in newly diagnosed patients with type 1 diabetes in the earlier studies (17–19). Intensive insulin therapy in newly diagnosed patients with type 1 diabetes, involving 2 weeks of treatment with intravenous insulin therapy via an artificial pancreas, preserved β -cell function at 1-year follow-up; the patients treated with the artificial pancreas had significantly higher (nearly twofold) stimulated C-peptide compared with the conventionally treated patients (NPH insulin, 1 unit/kg/day in two divided doses). During the 2-week period of treatment with the artificial pancreas, endogenous insulin secretion seemed to be suppressed with low urinary C-peptide in these patients (one-seventh that of the conventionally treated patients) (17). Another study comparing intensive insulin therapy (four daily subcutaneous insulin injections: rapid-acting insulin before three meals and long-acting insulin at bedtime) with continuous intravenous insulin infusion for 2 weeks in patients with newly diagnosed type 1 diabetes showed preserved β -cell function during the first year of insulin therapy (18). High-dose insulin therapy also resulted in long-term

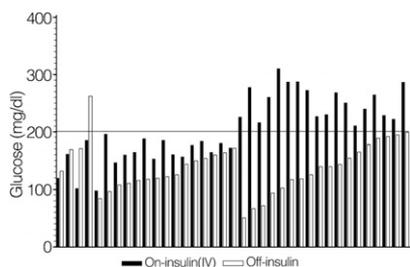


Figure 4—Comparison of 2-h OGTT glucose levels off and on IV-I in each individual ($n = 37$ paired OGTTs).

survival of β -cells and the disappearance of islet cell antibodies in type 1 diabetes (19). However, a recent clinical trial using inpatient hybrid closed-loop control for about 3 days followed by outpatient therapy with a sensor-augmented pump initiated within 7 days of the diagnosis of type 1 diabetes did not provide any benefit in preserving β -cell function compared with multiple daily injections or insulin pump therapy after 1 year (20). The lack of benefit may be related to the similar achievement of good glycemic control over the course of 1 year in the two groups; the few days of hybrid closed-loop control treatment at onset of the disease did not produce any additive effect.

Evidence from the Diabetes Control and Complications Trial (DCCT) further suggests that intensive parenteral insulin may slow or inhibit the type 1 diabetes disease process. At entry into the DCCT, a subset of patients with type 1 diabetes had Sustacal-stimulated C-peptide levels of 0.20–0.50 nmol/L (called C-peptide responders vs. C-peptide nonresponders with stimulated C-peptide <0.20 nmol/L). When these patients were randomized to intensive therapy, they maintained higher levels of C-peptide for a longer period of time and had a lower likelihood of becoming nonresponsive than when randomized to conventional therapy. This observation was initially reported for the first 2 years of the DCCT (21) and further confirmed by the final DCCT results (22). Although patients in both the intensive and conventional treatment groups subcutaneously received similar doses and types of insulin, an immune mechanism may also have been operative since intensive insulin therapy with pumps or multiple daily injections induces more insulin antibodies than conventional insulin therapy (23). The lower glucose levels in the intensively treated subjects most likely resulted in less β -cell stimulation (β -cell rest), making the β -cells less vulnerable to immune attacks by decreasing β -cell antigen expression and cytokine production, which may have resulted in relative β -cell protection from an immune attack compared with patients in the conventional treatment group.

Prophylactic parenteral insulin therapy was reported to delay the clinical onset of diabetes in subjects at high risk of type 1 diabetes in small studies

(24,25); however, the benefit of this intervention was not found in other studies (26,27). The DPT-1 was a randomized, multicenter trial that enrolled approximately 340 high-risk subjects without diabetes in the parenteral insulin treatment arm. The treatment protocol included IV-I for 4 days on a yearly basis, with SC-I given twice per day. The outcome of the DPT-1 failed to demonstrate that insulin therapy was protective against future type 1 diabetes (5), which resolved the controversies regarding the benefit of prophylactic parental insulin therapy from the pilot studies. While clinical benefit was not demonstrated, we found in this ancillary study that the DPT-1 IV-I suppressed endogenous insulin secretion. In a separate study we also found that the DPT-1 IV-I causes temporary inhibition of β -cell secretion in normal human subjects (28); the suppressed β -cell function returned to baseline levels after IV-I was stopped for 3 days in normal subjects. Therefore, it is likely that the IV-I-induced inhibition of β -cell function does not have a prolonged effect in DPT-1 subjects. In addition, we found in this ancillary study that daily SC-I did not alter endogenous β -cell function, which could be related to the lower dose of SC-I used in the study. The lack of a clinical benefit in the DPT-1 could be related to the only temporary inhibition of β -cell secretion with IV-I, the lack of an effect of SC-I on β -cell function, or a combination of both.

Although β -cell dysfunction and the destruction of type 1 diabetes is mediated by immunologic effector mechanisms, the functional state of the β -cells may profoundly influence their interaction with the immune system. We and others demonstrated that the expression of several β -cell antigens is increased when β -cells are stimulated and decreased when β -cells are less active (29–33). β -Cell cytotoxicity induced by several mechanisms also seems to be variable, depending on the functional state of the β -cells (34,35). Diazoxide, a K^+ channel opener that inhibits insulin secretion, reduced autoantigen expression in vitro (32) and in vivo (33) and protected against diabetes in BB rats (9). In clinical trials, both diazoxide and octreotide, a long-acting somatostatin analog, induced β -cell rest in patients with type 1 diabetes (36); treating

patients with new-onset type 1 diabetes with either of the two drugs improved residual β -cell function (37,38). For type 1 diabetes, the disease process might be enhanced by conditions resulting in β -cell stimulation. Conversely, β -cell inhibition might offer protection against the type 1 diabetes disease process. Consistent with the finding in this study that IV-I inhibits endogenous insulin secretion, we previously demonstrated that parenteral insulin used in DPT-1 suppressed T-cell proliferation to islet antigens, indicating that IV-I alters immune responses (39). The effect of IV-I on the immune response was only temporary, however, lasting only a few months, which could also contribute to the negative result of the DPT-1 study.

To our surprise, IV-I was frequently associated with postprandial glucose intolerance. The underlying potential mechanism(s) may be related to suppression of portal insulin, lack of acute-phase insulin release, or induction of insulin resistance in peripheral tissues (40). However, our data do not support the lack of acute-phase insulin release, resulting in postprandial glucose intolerance, since there was no significant difference of the delta 0–30 min C-peptide change between the control and on insulin infusion OGTTs. The lack of an effect of IV-I on acute-phase insulin release could also be a result of the DPT-1 subjects having impaired first-phase insulin response when randomized in the study (one of the eligibility requirements for the study), thereby making it difficult to detect an effect of IV-I on acute-phase insulin release in these subjects. In addition, we found that IV-I had more effect on the inhibition of basal β -cell function than stimulated β -cell function. Consequently, the expected relationship between fasting C-peptide and stimulated C-peptide observed in OGTTs when off-I was lost during IV-I (Fig. 2).

If the suppression of endogenous β -cell function (β -cell rest) indeed has any protective effect against the development of type 1 diabetes, future studies may be needed to determine what aspects of β -cell function need to be suppressed to achieve a protective effect. Interventions that selectively suppress only some specific aspects of β -cell function could potentially be developed, resulting in protection against

type 1 diabetes while maintaining insulin secretion and avoiding the postprandial hyperglycemia we observed.

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