The Therapeutic Efficacy of Human Insulin (recombinant DNA) in Patients with Insulin-dependent Diabetes Mellitus: A Comparative Study with Purified Porcine Insulin

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The therapeutic efficacy of human insulin (recombinant DNA) was compared with that of purified porcine insulin (PPI) in seven male subjects with previously treated insulin-dependent diabetes mellitus. In a random crossover design the patients received either PPI or human insulin during one of two consecutive 7-day periods of intensive insulin therapy. Control was evaluated on days 6 and 13. Tissue sensitivity and responsiveness to the study insulins were determined by insulin dose-response studies performed using the euglycemic glucose clamp on days 7 and 14. Insulin dose and all measures of control on days 6 and 13 were not statistically different between treatments. When the insulin dose-response studies during each treatment were compared there were no differences between them. Thus, in previously treated patients with insulin-dependent diabetes, undergoing brief but intensive insulin therapy with continuous subcutaneous insulin infusion, human insulin is as clinically efficacious as PPI. Furthermore, insulin sensitivity and responsiveness, as assessed by dose-response studies during the euglycemic glucose clamp were equivalent for both insulins. DIABETES CARE 5 (SUPPL. 2): 73–77, 1982.

Recombinant DNA technology has made it possible to manufacture human insulin by inserting the genetic code for human insulin into bacteria. The biosynthesis of a homologous insulin might be expected to possess the following theoretical advantages over other insulin preparations: (1) minimal antigenicity, (2) maximum biologic potency in man, and (3) unlimited supply.

Human insulin, whether produced by human pancreas, recombinant DNA technology, or semisynthetically,[1] differs from porcine insulin by only one amino acid. Human insulin prepared by recombinant DNA technology has been shown in normal volunteers and in acute studies in patients with insulin-dependent diabetes mellitus to be equivalent to porcine insulin during studies using the glucose clamp technique.[2–5] Although this small difference in structure has been proven not to be of importance in normal subjects, its effects during the management of diabetes mellitus and in the presence of insulin antibodies remain unclear.

We hypothesized that human insulin (recombinant DNA), because of its structural and chemical homology to endogenous insulin, might be more efficacious than purified porcine insulin (PPI) in previously treated patients with insulin-dependent diabetes. To assess the efficacy of human insulin and PPI, we felt it necessary to achieve similar blood glucose control with each insulin. We used a randomized crossover design, then compared the dose required to achieve this control, the metabolic consequences, and the tissue sensitivity and responsiveness to each insulin at the end of each treatment.

METHODS

Patient selection. Seven healthy male patients with insulin-dependent diabetes mellitus were recruited from the diabetes clinics of the Lilly Laboratory for Clinical Research and Indiana University School of Medicine. The patients' characteristics are summarized in Table 1. All body fat determinations were performed by measuring the sum of skinfold thickness at four sites—biceps, triceps, subscapular, and suprailiac—with a Lange caliper (Cambridge Scientific Industries, Inc., Cambridge, Maryland) and estimating percentage of body fat from the tables of Durnin.[6] Hemoglobin A_{1c} was determined by a micromodification of the Trivelli method[7] in a constant temperature chamber (23°C). Coefficient of variation is less than 2.5%. An internal standard is included with each run to insure proper quality control.

Informed consent was obtained from all patients, and the studies were performed in accordance with the Helsinki Declaration of 1975.

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TABLE 1
Patient characteristics

Characteristics	Means	Range
Age	33.7 ± 6.5	14–68
Weight (kg)	75.5 ± 2.7	68–85
Height (cm)	179.0 ± 4.0	173–189
Body fat (%)	18.1 ± 1.4	12.9–21.5
HbA_{1c} (%)	9.2 ± 0.4	8.8–9.6 (normal 5.5–8.5)
Duration of insulin therapy (yr)	12.1 ± 3.7	2–31
	Insulin secretion during the OGTT	
	Fasting	2-h postprandial
Glucose (mg/dl)	132.6 ± 16.8	424.7 ± 117.8
Free insulin (μU/ml)	27.8 ± 5.3	19.4 ± 5.3*
Total insulin (μU/ml)	497.5 ± 181.9	410.7 ± 50.2

*Not statistically different by paired *t* test.

MATERIALS

Human insulin produced by combining A and B chains synthesized by recombinant DNA technology and PPI were selected for identical concentration and activity.[8] All preparations were provided in concentrations of 40 U/ml. The radiotracer used for the measurement of hepatic glucose production was 3-$^{3}_{1}$H-D-glucose (New England Nuclear, Boston, Massachusetts).

PROTOCOL

In a random cross-over design (Figure 1) the patients received either PPI or human insulin during one of two consecutive 7-day periods. During the study the patients received a weight-maintenance diet consisting of 30 cal/kg with a caloric distribution of 50% carbohydrate, 30% fat, and 20% protein. Calories were distributed throughout the day at 0800, 1200, 1700, and 2100 h in portions of 20%, 30%, 30%, and 20%, respectively. On day 1, normoglycemia was obtained with a Biostator (Life Sciences Instruments, Miles Laboratories, Elkhart, Indiana). On day 2 the insulin infusion provided by the Biostator was discontinued for 1 h and then a 2-h oral glucose tolerance test (OGTT) was performed with 75 g of glucose. After the OGTT, Biostator control was reinstituted and the patient brought back to normoglycemia. On days 2–6 and 8–13, control was maintained with continuous subcutaneous insulin infusion (CSII) via an Auto-Syringe Model 2C infusion device (Auto-Syringe, Inc., Hookset, New Hampshire). The initial insulin dose was determined from the amount of insulin delivered by the Biostator. Insulin dose was adjusted daily (except on days 6 and 13), based on measurements of capillary blood glucose performed on an Ames Dextrometer (Ames Division, Miles Laboratories).

On days 6 and 13, the metabolic effects of therapy were monitored by obtaining plasma for lactate, glycerol, pyruvate, acetoacetate, beta-hydroxybutyrate, branched chain amino acids, and alanine in the fasting state at 0800 h. Glycemic control was assessed by summed glucose concentrations (sum of fasting and 90-min postprandial glucose concentrations at each meal, "glucose brackets"[9]) and mean fasting and postprandial glucose concentrations. Free insulin levels were obtained in the fasting state and at 1 and 2 h after meals. "Mean fasting" levels of glucose and insulin were calculated by obtaining the mean of all the premeal levels at each of the four meals. "Mean postprandial" levels were calculated in a similar manner.

On days 7 and 14 tissue sensitivity and responsiveness to the study insulins were evaluated by administering an insulin dose-response study using the glucose clamp technique combined with isotopically determined hepatic glucose production.

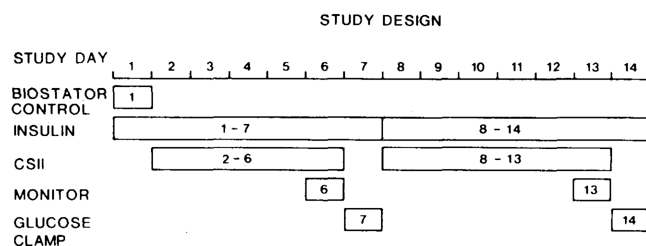

FIG. 1. Protocol for randomized crossover study.

BIOCHEMICAL ANALYSIS

Glucose and other substrates. Plasma glucose concentrations were determined by a glucose-oxidase method using batch analysis.[10] Enzymatic analysis of glycerol, lactate, pyruvate, acetoacetate, and beta-hydroxybutyrate used methods adapted from Bergemeyer.[11] Plasma free fatty acids were determined using an adaptation of Novak's method.[12] The measurements of branch chain amino acids and alanine were performed by gas liquid chromatography using the trifluoroacetyl-n-butyl esters of the amino acids.[13]

Insulin immunoassay. Measurement of free and total insulin was performed in aliquots of sera using PEG to precipitate anti-insulin antibodies.[14] Supernatant insulin was measured by the method of Heding[15] using ^{125}I porcine insulin, prepared by a lactoperoxidase method, monoiodinated at A-14, and purified by HPLC.[16] An anti-porcine insulin antibody that does not discern between porcine and human insulin was used. Fifty percent displacement of bound tracer occurs with a standard of 50 μU/ml. Interassay variation is 15%.

Glucose clamp technique and hepatic glucose production. The glucose clamp studies were performed using the Biostator and the glucose clamp technique as modified from DeFronzo[17] by Rizza.[18] After a 120-min baseline period during which basal 3-$^{3}_{1}$H-D-glucose turnover data were obtained, the insulin

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TABLE 2
Comparison of the metabolic control obtained using CSII with either human insulin or purified porcine insulin

Meal (mg/dl)	Glucose brackets*	
	Human insulin	PPI
1	297.4 ± 46.3	245.8 ± 26.2
2	214.6 ± 20.2	209.7 ± 21.9
3	255.2 ± 39.7	206.3 ± 22.0
4	242.9 ± 20.7	295.7 ± 50.6

*By two-way analysis of variance there is no significant difference between types of insulin for comparison of glucose brackets.

dose-response study began with a glucose target of 95 ± 5 mg/dl. Three sequential infusions of study insulin were administered at rates of 0.5, 1.0, and 5.0 mU/kg/min for 120 min each. Additional dextrose was provided by a variable speed syringe pump. The last 40 min of data from each period were used for analysis and calculations. Rates of glucose turnover were determined in five of seven subjects by the method of Steele[19] as modified by DeBodo[20] with the infusion of 3-_1^3H-D-glucose.

Specific activity was determined every 10 min during the last 40 min of each dose of insulin, including the baseline period. Plasma was dried in an oven to remove tritiated water, rehydrated, then dissolved in Protosol (New England Nuclear), and suspended in Aquasol II (New England Nuclear), resulting in 35% efficiency and no quenching based on the internal standards method. Hepatic glucose production was obtained by subtracting the amount of glucose provided by the Biostator and the syringe pump from the isotopically derived total glucose appearance rate.[18]

STATISTICAL ANALYSIS

All data were analyzed by analysis of variance or paired *t* tests. All data are expressed as the mean ± SEM.

TABLE 3
Comparison of the metabolic control obtained using CSII with either human insulin or purified porcine insulin

Glucose and insulin	Human insulin	PPI	P*
MFI (μU/ml)	55.8 ± 8.6	55.8 ± 10.0	NS
MFG (mg/dl)	102.2 ± 11.3	90.4 ± 13.0	NS
MPPI-1 (μU/ml)	64.2 ± 9.5	59.4 ± 11.4	NS
MPPI-2 (μU/ml)	61.9 ± 10.9	54.1 ± 11.2	NS
MPPG-2 (mg/dl)	137.7 ± 14.2	139.9 ± 14.8	NS
Insulin dose (U)	47.8 ± 4.8	50.5 ± 5.4	NS

Abbreviations: MFI = mean fasting insulin; MPPI-1, -2 = mean postprandial insulin at 1 and 2 h; MFG = mean fasting glucose; and MPPG-2 = mean postprandial glucose at 2 h.
*Paired *t* test.

RESULTS

As seen in Table 1, the patients were a diverse group in regard to age and duration of diabetes; they were quite comparable in regard to weight (all ± 10% of ideal body weight[21]), height, percent body fat, HbA_{1C}, and insulin secretion. No subject had major complications of diabetes, i.e., cardiovascular disease, nephropathy, or retinopathy. All patients used less than 60 U of mixed beef-pork insulin before the study. Four subjects received human insulin first and three received PPI first.

The results of the comparison on monitor days (days 6 and 13) are listed in Tables 2 and 3. As shown in Table 3, the insulin dose on monitor days was not statistically different between treatments. Glycemic control and free insulin concentrations were also compared. No differences could be elicited when glycemic control was compared using summed glucose concentrations ("glucose brackets," Table 2) or mean fasting or postprandial concentrations (Table 3). When serum free insulin concentrations were compared using mean fasting and 1- and 2-h postprandial levels, no differences between human insulin and PPI were noted (Table 3). Note should be made of the lack of the expected postprandial increment in free insulin. Premeal boluses were given 30 min before meals and, as a result, the 1- and 2-h postprandial levels are 1.5 and 2.5 h after the premeal boluses.

Amino acids and several other substrates were measured in the fasting state at 0800 h on monitor days (days 6 and 13) and listed for comparison in Table 4. Again, no statistical difference between treatments is noted.

At the end of each treatment on days 7 and 14 (Figure 1), insulin dose-response studies were performed using the

TABLE 4
Comparison of the metabolic control obtained using CSII with either human insulin or PPI

Amino acids and other substrates	Basal fasting levels at 0800 h		
	Human insulin	PPI	P*
Ala†	256 ± 13	246 ± 17	NS
Val†	220 ± 23	218 ± 10	NS
Leu†	132 ± 27	114 ± 11	NS
Ileu†	72 ± 21	50 ± 5	NS
Lac†	1170 ± 440	835 ± 67	NS
Pyr†	34 ± 7	28 ± 8	NS
BOHB†	119 ± 80	99 ± 47	NS
AcAc†	83 ± 27	60 ± 9	NS
Glyc†	72 ± 15	71 ± 15	NS
FFA†	610 ± 111	650 ± 60	NS

Abbreviations: Ala = alanine; Val = valine; Leu = leucine; Ileu = isoleucine; Lac = lactate; Pyr = pyruvate; BOHB = beta hydroxybutyrate; AcAc = acetoacetate; Glyc = glycerol; and FFA = free fatty acids.
*Paired *t* test.
†μmol/L.

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TABLE 5
Comparison of the insulin dose-response studies performed by the glucose clamp technique at the end of each treatment period

Dose	Glucose utilization (mg/kg/min)*		Free insulin concentration (μU/ml)	
	Human insulin	PPI	Human insulin	PPI
I	2.7 ± 0.3	2.9 ± 0.6	45.6 ± 7.2	43.5 ± 9.2
II	7.9 ± 0.7	7.1 ± 0.7	73.4 ± 8.7	85.9 ± 10.8
III	14.2 ± 1.0	14.4 ± 0.8	539.0 ± 36.5	570.3 ± 5.1

By two-way analysis of variance both free insulin and glucose utilization increase with dose ($P < 0.001$) and there are no differences between types of insulin.
*Defined as the amount of glucose infused to maintain the subject in the euglycemic state at each dose.

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insulin with which the subjects had been treated for the preceding 6 days. The results of these studies are presented in Tables 5 and 6. In Table 5, the infusion of 0.5, 1.0, and 5.0 mU/kg/min generated a highly significant dose-response relationship between free insulin concentrations and glucose utilization (glucose infused to maintain euglycemia). There was no difference between insulins, at any dose, for either free insulin concentration or glucose utilization.

In Table 6 the levels of amino acids and several other substrates are compared during the insulin dose-response studies, and no statistical difference in the metabolic effects of the two insulins can be seen.

Hepatic glucose production was suppressed to an equal degree by both insulins. In the basal state hepatic glucose production was 2.7 ± 1.1 mg/kg/min before the human insulin dose-response studies and 1.8 ± 0.5 mg/kg/min before the PPI dose-response studies. After dose 1, hepatic glucose production was suppressed to 0.1 ± 0.5 mg/kg/min and 0.1 ± 0.6 mg/kg/min for human insulin and PPI, respectively. No hepatic production of glucose could be detected after dose 1. There was no statistical difference between human insulin and PPI in the basal state or at dose 1 using the paired *t* test for analysis.

DISCUSSION

During this random crossover study comparing human insulin (recombinant DNA) and purified porcine insulin, we were unable to demonstrate any statistically significant difference between treatments as measured by glucose control, insulin dose, and effects on amino acids and other substrates. Furthermore, tissue sensitivity and responsiveness and suppression of hepatic glucose production as measured by the insulin dose-response studies were equivalent for both insulins.

All patients had insulin antibodies as demonstrated by the discrepancy between free and total insulin in the plasma (Table 1). No patient had a suggestion of immunologic resistance since all required less than 70 U/day before and during the study. In spite of the presence of antibodies, the metabolic response to the insulin dose-response studies was quite comparable to that reported in the literature for normal volunteers.[17,18] It is quite possible, however, that if antibodies fall in response to treatment with human insulin a difference in efficacy may emerge.

We conclude from this study that in previously treated patients with diabetes mellitus undergoing brief but intensive

TABLE 6
Comparison of the dose-response relationships of amino acids and other substrates obtained during the insulin dose-response studies with human insulin and PPI at the end of each treatment period*

Dose	Human insulin			PPI		
	I	II	III	I	II	III
Ala†	250 ± 17	283 ± 28	256 ± 19	232 ± 14	263 ± 11	172 ± 35
Val†	172 ± 9	140 ± 6	109 ± 6	176 ± 11	145 ± 11	80 ± 10
Leu†	73 ± 5	52 ± 7	33 ± 5	85 ± 5	52 ± 6	23 ± 4
Ileu†	27 ± 8	14 ± 5	6 ± 2	25 ± 7	15 ± 5	5 ± 2
Lac†	747 ± 103	1128 ± 158	1526 ± 211	725 ± 73	1088 ± 111	1741 ± 205
Pyr†	26 ± 7	39 ± 10	51 ± 12	17 ± 2	33 ± 14	91 ± 47
BOHB†	15 ± 10	16 ± 13	21 ± 14	43 ± 22	17 ± 11	89 ± 74
AcAc†	28 ± 2	21 ± 1	19 ± 2	42 ± 10	27 ± 3	70 ± 50
Glyc†	25 ± 4	23 ± 4	30 ± 5	29 ± 6	27 ± 6	29 ± 7
FFA†	172 ± 23	113 ± 13	118 ± 26	193 ± 22	139 ± 22	124 ± 20

*By two-way analysis of variance there is no difference between treatments.
†μmol/L.

therapy with continuous subcutaneous insulin infusion, human insulin is as clinically efficacious as purified porcine insulin.

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