Crossover Study with Human Insulin (recombinant DNA) in Type I Diabetic Subjects

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The activity of three combinations of regular and NPH human insulin (recombinant DNA) has been compared to that of an intermediate-acting pork insulin in a crossover study in type I diabetic subjects. After a prephase, the patients injected each insulin subcutaneously for 1 wk in different order. During the 5-wk period, daily glucose profiles were self-monitored, except at the end of each week, when blood was sampled for the determination of glucose, HbA_1, glycosylated albumin, C-peptide, glucagon, and routine laboratory parameters. Fasting as well as mean daily glucose and mean amplitude of glucose excursions were similar during the treatment with pork and the various human insulin preparations. There was also no significant difference in C-peptide, glucagon, HbA_1 or the routine laboratory parameters with each insulin tested. Glycosylated albumin, however, was significantly lower during the test period with intermediate-acting pork insulin and human insulin (25:75 regular:NPH), when compared with the prephase. We conclude that in type I diabetic subjects human insulin in special galenic preparations shows very similar metabolic activity to pork insulin. DIABETES CARE 5 (SUPPL. 2): 39–42, 1982.

Since its first application in humans,[1] human insulin produced by recombinant DNA technology has been tested in numerous studies for its biological efficacy and immunogenicity in humans (for review, see ref. 2). Human NPH insulin seems to have a more rapid onset and a shorter duration of action, when compared with highly purified pork NPH insulin, as reported repeatedly during the Symposium on Human Insulin of Recombinant DNA Origin and published in this volume. The purpose of the cross-over study presented was to find an optimal combination of regular and NPH human insulin for the treatment of type I diabetic subjects. Therefore three different combinations of regular human and NPH insulin were compared with an intermediate-acting, purified pork insulin preparation (Depot Insulin CS) after subcutaneous injection.

SUBJECTS, MATERIALS AND METHODS

Subjects. Eight otherwise healthy type I diabetic subjects gave their written, informed consent. Important clinical data of these patients are listed in Table 1.

Materials. Human insulin produced by recombining A- and B-chains synthesized by genetically modified strains of *E. coli* was provided by Dr. F. Enzmann (Eli Lilly and Company, Bad Homburg, West Germany) at a concentration of 40 U/ml at pH 7.3.

The three combinations of regular and NPH insulin had the following compositions: A (XA 2080 AMX; 20:80 regular:NPH); B (XA 2120 AMX; 30:70 regular:NPH); C (XA 2100 AMX; 25:75 regular:NPH). These insulins were compared with commercially available highly purified pork intermediate-acting surfen insulin (Depot Insulin CA; Ch.B. 098W079) from Hoechst. Instead of protamine, surfen (aminochinaldyl urea) is used to prolong insulin absorption.

Protocol. The patients were trained in home-monitoring of blood glucose, using the Ames glucometer. Blood glucose levels had to be tested each day during the 5-wk study in the fasting state, 1 h after breakfast, 1 h before lunch, immediately before dinner and the second insulin injection, and 2 h after dinner. Each Saturday blood glucose profiles, including fasting blood glucose levels, were obtained in the hospital's metabolic ward. On these occasions additional blood was sampled in the fasting state for routine laboratory examinations as well as for HbA_1 (labile and stable form), glycosylated albumin, C-peptide, and glucagon.

The eight patients were divided into four subgroups, and human insulin or pork insulin applied (s.c.) in a cross-over,

TABLE 1
Clinical data of diabetic patients

Patient	Sex	Age (yr)	Duration of IDDM (yr)	% ideal body weight	Diabetic complications*
I.B.	F	29	14	100	R
N.D.	M	49	10	113	R, Neu
M.G.	F	58	29	102	R, Neu
E.H.	M	38	13	100	R, Neu
E.K.	F	22	11	108	—
A.L.	M	27	10	94	R
A.S.	M	38	7	107	—
E.W.	M	41	24	111	R, Neu, N
$\bar{X}$ ± SEM		37 ± 4	14.8 ± 2.7	104 ± 2	

*R = retinopathy, Neu = neuropathy, N = nephropathy

double-blind manner, depicted in Figure 1, each for 1 wk, beginning on Sunday. The time interval between the insulin injection and the start of the meal was 30 min. The patients had normal daily life activities, even on the days at the metabolic ward, and the diet was kept constant during the period of the study.

Methods. Blood glucose was measured with Dextrostix using the glucometer (Ames) and the hexokinase method.[3] HbA_1 was determined with the microcolumn technique (Isolab, Akron), with and without aldimine eliminator.[4] Glycosylated albumin (HMF) was determined using a modification of the method of Dolhofer and Wieland.[5] C-peptide was measured using the radioimmunoassay procedure from Byk-Mallinckrodt (Dietzenbach). Glucagon was first extracted from plasma by ethanol and subsequently measured by a radioimmunoassay, using a pancreas-specific antiserum (K5563), using the method of Heding.[6] All other laboratory parameters were determined with established and well-controlled methods normally used in routine clinical chemistry.

Calculations. Mean blood glucose (MBG) and mean amplitudes of blood glucose excursions (MAGE) were calculated according to Service et al.[7] The results are expressed as mean ± SEM, and comparisons have been made using the unpaired Student's *t* test.

Results. After acute and long-term (daily during 3 wk) s.c. administration of human insulin, no abnormal skin or systemic reactions could be observed. The data in Table 2 demonstrate that no significant changes were noted in blood cells, important serum parameters of kidney and liver function, or of electrolyte and lipid metabolism during the periods of treatment with human insulin, in comparison with the times of purified pork insulin administration. The blood glucose values measured at home and those analyzed during the 1-day stay at the metabolic ward were not significantly different, demonstrating first the accuracy and reliability of home monitoring of blood glucose,[8] and secondly, that the blood

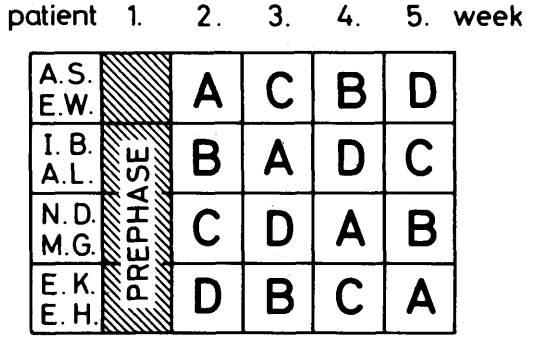

FIG. 1. Design of the study. In the prephase the patients injected purified pork insulins, which they used prior to the study (combinations of rapid and intermediate-acting insulins). The insulin preparations A–D are defined in MATERIALS.

TABLE 2
Laboratory findings in fasting diabetics during pork and human insulin treatment. Mean ± SEM of 8 patients determined twice with pork and three times with human insulin treatment.

Parameter	Pork insulin	Human insulin
platelets × 10^3	190 ± 18	209 ± 15
leucocytes × 10^3	6.7 ± 0.1	6.6 ± 0.3
erythrocytes × 10^3	4.8 ± 0.1	4.6 ± 0.1
hemoglobin (g/dl)	15.2 ± 0.2	14.5 ± 0.1
hematocrit (%)	45 ± 1	46 ± 1
Na^+ (mval/L)	139 ± 1	137 ± 1
K^+ (mval/L)	4.3 ± 0.1	4.4 ± 0.1
Ca^{2+} (mval/L)	4.8 ± 0.1	4.7 ± 0.1
phosphate (mg/dl)	3.9 ± 0.2	3.8 ± 0.2
uric acid (mg/dl)	4.0 ± 0.3	3.7 ± 0.3
creatinine (mg/dl)	0.9 ± 0.03	0.8 ± 0.06
alkaline phosphatase (U/L)	100 ± 12	117 ± 7
GOT (U/L)	11 ± 1	12 ± 1
GPT (U/L)	11 ± 1	11 ± 1
GGT (U/L)	13 ± 2	11 ± 1
serum protein (g/dl)	6.7 ± 0.4	6.0 ± 0.6
triglycerides (mg/dl)	83 ± 14	92 ± 12
cholesterol (mg/dl)	248 ± 10	240 ± 9
HDL cholesterol (mg/dl)	60 ± 8	56 ± 6

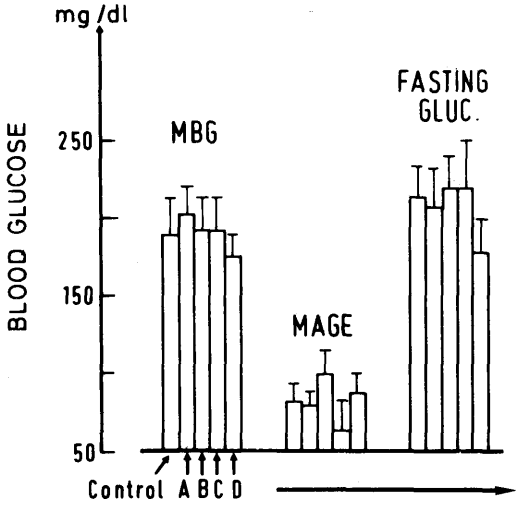

FIG. 2. Fasting and mean daily blood glucose (MBG) and mean amplitude of blood glucose excursions (MAGE) measured at the end of each test period (N = 8).

glucose levels measured under our clinical observation were representative for the metabolic control during the various treatment schedules. Fasting blood glucose, MBG, and MAGE, a parameter of stability of glucose metabolism, were very similar during the control period and the various periods on human insulin (Figure 2). These results emphasize that the purified pork insulin used in this study and human insulin in three different combinations of regular and NPH insulin have very similar characteristics in lowering blood glucose. The rate of hypoglycemic attacks was not more frequent under human insulin treatment. Stable HbA_1, a parameter of long-term glucose control,[9-11] was significantly higher ($P < 0.0005$) in the diabetic subjects than in healthy controls (6.13 ± 0.16%; N = 19). The mean HbA_1 of about 10% in our diabetic patients remained unchanged throughout the various treatment phases (Figure 3). The same was true for the labile HbA_1, the rapid reversible aldimine form of glycosylated hemoglobin (Figure 3). The mean labile HbA_1 in normals was 0.30 ± 0.08%; (N = 19).

Glycosylated albumin was also significantly higher in diabetic than in nondiabetic patients (137 ± 7 versus 72 ± 3 μmol HMF/mmol albumin; $P < 0.0005$). During treatment with pork insulin (Depot CS) and human insulin (25:75 regular:NPH) the glycosylated albumin was significantly lower ($P < 0.025$ and $P < 0.05$), when compared with the control period; the other differences were not significant.

Serum C-peptide and plasma glucagon levels are shown in Figure 4. The values were not significantly different during the various insulin regimens.

DISCUSSION

The plasma glucose–lowering potency of human insulin in the special galenic preparations used is similar to that of highly purified intermediate-acting pork insulin. These results confirm and expand data obtained under clinical conditions with a glucose-controlled insulin infusion system.[12-14] No significant differences were noted in the action of various combinations of rapid and intermediate-acting human insulin.

For reason of comparison, the insulin amount in the different combinations was kept constant during the test period in each individual, resulting in fairly bad control of glucose metabolism in most of the diabetic volunteers, as judged by glucose levels, HbA_1 and glycosylated albumin. These results underline again the importance of individual adaptation of insulin with respect to dosage and combination of rapid and intermediate-ating forms.

Because of its proven efficacy and the absence of toxicity, human insulin will be important in the future for the treatment of insulin-dependent diabetic patients and short-term indication for insulin adminstration. Further long-term studies with various galenic preparations of human insulin will

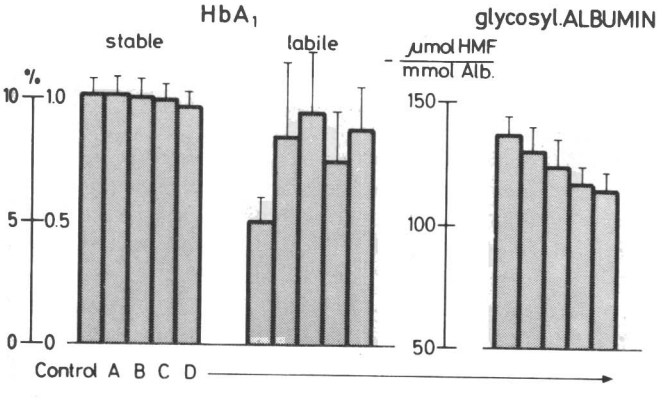

FIG. 3. Glycosylated hemoglobin (stable and labile form) and glycosylated albumin levels measured at the end of each test period in the fasting state (N = 8).

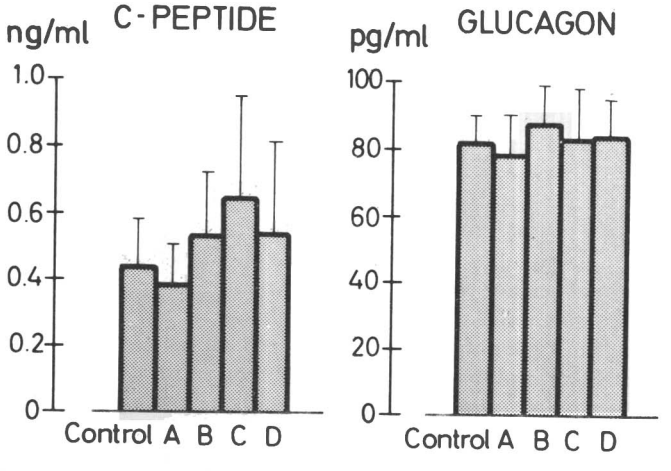

FIG. 4. Fasting C-peptide and glucagon levels measured at the end of each test period (N = 8).

provide more information abut the antigenicity of this insulin.

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