Henry Ford Hospital Medical Journal

Volume 31 | Number 2 Article 3

6-1983

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Henry Ford Hosp Med J
Vol 31, No 2, 1983

Experience with Biosynthetic Human Insulin in Diabetes†

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Thirty diabetic patients new to insulin were entered in an open label prospective study of biosynthetic human insulin (BHI). All patients experienced symptomatic control of diabetes attributable to dietary and BHI insulin therapy. Detailed six-month evaluation data were reviewed in 19 patients. A significant drop in fasting plasma glucose and glycosylated hemoglobin was noted at two months, and a further modest decrease occurred at six months. E. coli polypeptide antibodies were unchanged from baseline at six months, indicating that no bacterial protein contamination of BHI occurred. Percent binding of serum antibodies to human insulin measured in 19 patients at baseline and at six months showed a statistically significant increase in mean value without accompanying clinical symptoms. Clinical hypoglycemia did not differ from that seen in patients who received animal insulin. Biosynthetic human insulin appears comparable in clinical efficacy and safety to purified pork insulin. Ongoing studies will be required to determine whether BHI is less immunogenic than purified pork insulin.

Research on the chemical analysis of insulin was first reported in 1959 when Sanger described the amino acid sequence of animal insulin (1). One year later, Nicol and Smith reported on the amino acid sequence of human insulin (2). In the next decade, investigators in the United States, West Germany, and China chemically synthesized insulin. While the primary amino acid structure of human, beef, and pork insulins varies only slightly from each other (Table I), this difference is sufficient to produce a significant immunogenic effect, especially with beef insulin, in which the amino acid sequence varies from human insulin by three amino acids. Although human and pork insulins are nearly identical, an antibody response will occur when pork insulin is injected into diabetic humans. Nevertheless, highly purified, unmodified pork insulin is the least immunogenic of animal insulins. Reasonably, human insulin might be without antigenic effect when injected into a homologous host. Except for the lack of automatic influence on changing blood glucose levels, injected human insulin should be the best therapy for the insulin-requiring diabetic patient.

Human insulin has been produced both by a recombinant DNA process (3) and by a semisynthetic technique. In the latter technique, by enzymatic transpeptidation, the amino acid threonine is substituted for alanine at the B30 position of the insulin molecule (4). The chemical and biologic properties of biosynthetic human insulin (BHI) are identical to those of pancreatic human insulin (5). Pharmacologically pure, BHI matches animal insulin in biologic action, yielding a prompt fall in blood glucose after intravenous or subcutaneous injection (6).

TABLE I

COMPARATIVE PARTIAL AMINO ACID STRUCTURE OF ANIMAL AND HUMAN INSULIN

Insulin*	A chain			B chain
Position	8	9	10	30
Beef	Ala	Ser	Val	Ala
Pork	Thr	Ser	Ilu	Ala
Human	Thr	Ser	Ilu	Thr

*All other amino acids on A and B chain are identical between species.

Submitted for publication: April 21, 1983
Accepted for publication: May 5, 1983
†These studies are supported by a grant from the Eli Lilly Company, Indianapolis, Indiana.
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TABLE II

DEMOGRAPHIC DATA ON 30 DIABETIC PATIENTS

Age: 21-85 years (mean: 56 years)		
Sex: Male:	11	
Female:	19	
Race: White:	17	
Black:	13	
Type of Diabetes: Insulin-dependent:		1
Non-insulin-dependent:		27
Secondary:		2
Duration of Diabetes: 0-26 years (mean: 5.6 years)		
Percent of Ideal Body Weight: At onset: 134 ± 28		
At six months: 143 ± 31		

Immunologically, however, BHI presented a conundrum before it was in clinical use for human diabetes. Would a homologous protein be immunogenic? Since endogenous human insulin secreted into the pancreatic venous effluent lacks antigenicity, does the manufacturing process necessary to produce BHI create immunogenicity? Would human insulin injected subcutaneously stimulate antibody production?

To explore the clinical efficacy and safety of human insulin, to investigate its immunogenic potential, and as part of a multi-center clinical trial, we initiated an open label prospective study of BHI in insulin-requiring diabetic patients in 1981.

Materials and Methods

Thirty diabetic patients new to insulin were recruited over a six-month period (Table II). All patients had symptoms and signs of uncontrolled diabetes when therapy began. The serum of each patient was free of proinsulin antibodies, substantiating the absence of prior use of exogenous insulin. Before admission to the study, the patient or a family member signed a detailed informed consent form.

Baseline studies included analyses on the state of metabolic imbalance (blood and urine for glucose, serum C-peptide levels, total glycosylated hemoglobin); immunologic responsiveness (islet cell antibodies and immune complexes, circulating insulin antibodies [7], E. coli polypeptide antibodies [8], and proinsulin antibodies); and parenchymal organ function (complete blood count and multichannel serum chemistry tests). As described elsewhere, intradermal testing was performed at baseline and after six months of treatment with BHI. The double-blind test kit consisted of human insulin in increasing concentrations plus positive and negative controls (9).

After insulin therapy began, patients were checked monthly for two months, then bimonthly thereafter up to the present (26 months). The diabetes in these patients was managed with a dietary prescription appropriate to weight, physical activity, and sufficient insulin to establish and maintain metabolic control. Study patients received either human neutral regular insulin (NRI), human isophane (NPH) insulin, or both as individually required.

At each clinic visit, a thorough history was taken and a physical assessment was made. To monitor metabolic control of the diabetes, each patient regularly performed self-testing of blood glucose using an Ames dextrometer. Fasting and stimulated blood glucose levels and glycosylated hemoglobin values were measured at each visit.

We have six-month evaluation data on 19 of 30 patients in the study. Ten of the 19 received NRI daily along with NPH insulin. Other patients have received NRI from time to time to correct isolated episodes of hyperglycemia.

Four patients were dropped from the study. Two inadvertently received animal insulin, one patient chose to stop insulin because of hypoglycemia, and a fourth patient died of natural causes.

Results

All patients experienced symptomatic control of diabetes attributable to dietary therapy and insulin replacement. Mean weight increased significantly over a six-month period from 134% to 143% of ideal weight (Table II).

Complete blood counts, blood chemistry tests, and urinalyses were unchanged except for glycosuria and ketonuria. While 18 of 19 patients had fasting glycosuria, and 11 of 19 had fasting ketonuria at baseline, no patient had either fasting glycosuria or ketonuria when examined six months later. No data are available on serum lipids.

Plasma glucose concentration was significantly reduced within two months after insulin was started. A further modest decrease was noted at six months (Table III). Mean fasting C-peptide levels in 19 patients declined from 0.84 pmoles/ml at baseline to 0.79 pmoles/ml at latest assessment. Islet cell antibodies were negative in 15 of 16 patients, although slight fluorescence was noted in one patient. No significant immune complexes were demonstrated in these 16 patients. E. coli polypeptide antibodies were unchanged from baseline when patients were reexamined at six months (101 ± 44 vs 103 ± 76 counts/minute; p value = 0.90). No changes occurred in intradermal tests.

Percent binding of serum antibodies to human insulin measured in 19 patients at baseline and at six months

showed a statistically significant increase in mean value (1.48 ± 1.39% vs 5.61 ± 6.54%; p value = 0.020). At six months, only five patients had significant levels of antibody binding (over 4.3%, mean plus two standard deviations). Three of these values were 11.6%, 21.7%, and 23.5%.

Discussion

Our open label study of 30 diabetic patients who received biosynthetic human insulin for up to 26 months indicates that it is safe and efficacious. After six months of therapy, the daily insulin dose in our patients averaged 42 ± 20 units, a finding compatible with previous experience with animal insulin. Galloway and co-workers in short-term studies found daily insulin dosages unchanged when crossover experiments between BHI, pork, and beef-pork insulin were carried out (9). Double-blind, short-term crossover studies in established diabetic patients using beef, pork, and biosynthetic human insulin have found only subtle differences between blood glucose responses to the three insulin preparations (10). No local reactions to BHI at the site of injection occurred in our patients; nor have we identified patients with subcutaneous insulin lipohypertrophy or lipoatrophy.

Hypoglycemic reactions were the same as those following the use of animal insulin and did not appear with greater or less frequency than expected, except that some patients experienced a more rapid onset of blood glucose lowering effect of NPH-BHI manifested by mid-morning or pre-lunch hypoglycemia. In addition, these patients at times required two injections of NPH-BHI to insure normoglycemia before breakfast. Pharmacokinetic studies (11) and clinical studies (12,13) have suggested a time-action of NPH-BHI that is faster in onset and shorter than that of beef-NPH insulin, an observation that agrees with our findings. However, this particular time action of NPH-BHI was not seen in most of our patients, was easily dealt with, and probably relates to the ratio of insulin to protamine in the NPH preparations.

Initial antibody data impute minimal immunogenicity to BHI. Because it is homologous, it has been suggested that antibodies might not develop. Fineberg and colleagues have reported a decrease in qualitative and quantitative insulin antibody binding when patients previously taking mixed beef-pork insulin have been changed either to purified pork insulin (PPI) or to BHI (7). These investigators also noted small but significant decreases in antibody binding in patients changed from PPI to BHI. Short-term crossover studies in established diabetic patients have suggested little difference between PPI and BHI in the concentration of insulin antibodies (14). On the other hand, significant differences do occur in antibody concentration when beef insulin is changed to human insulin, an expected finding because of the greater immunogenicity of beef insulin. In longer-term studies with BHI, Fineberg and colleagues found lower antibody levels to insulin in patients on BHI when compared to patients on PPI (15). They noted that no increase in antibodies to BHI occurred after six months of therapy. Data available from 19 of our patients receiving BHI showed a significant rise in human insulin antibody binding in five patients at six months. None of these

TABLE III

FASTING PLASMA GLUCOSE AND GLYCOSYLATED HEMOGLOBIN VALUES AT BASELINE, AT SIX MONTHS, AND AT LATEST MEASUREMENT

	Fasting plasma glucose (mg/dl)	Glycosylated hemoglobin* (%)
Baseline (30)**	363 ± 134	15.2 ± 4.2
Six months (26)	161 ± 48	9.5 ± 2.4
Latest (16)	181 ± 70	10.5 ± 2.4

*Normal values (6.0-8.8%)
**Number of patients tested

TABLE IV

SERUM INSULIN ANTIBODIES (% BOUND/TOTAL) AFTER BHI USE

Patient Number	Actual Baseline Value	Time on study (days post-therapy) 15-45	91-150	151-210
501	2.9	0.0	0.0	7.2
503	1.4	0.0	0.2	1.2
504	1.4	0.0	0.7	4.0
505	0.2	0.0	0.0	1.3
506	3.3	0.7	1.9	3.9
507	0.8	1.3	2.4	11.6
508	0.0	0.8	1.9	21.7
509	2.1	0.2	0.5	3.5
510	0.4	0.0	0.0	1.1
511	5.2		2.1	4.3
512	2.0	0.3	1.5	2.4
513	0.5	0.0	2.1	4.5
514	2.3	0.2	0.0	3.8
515	2.7*	2.4	0.2	0.9
516	0.2	0.0	1.5	23.5
517	0.6	0.0	0.0	2.7
518	0.2	0.0	0.0	0.0
519	0.0	0.0	3.8	3.7
523	2.0	0.0	3.0	5.8

*10 days post-therapy

Summation

No. of Patients	Mean Baseline	Last Assessment	p Value
19	1.48 ± 1.39	5.61 ± 6.54	0.020

patients manifested clinical findings compatible with insulin allergy or resistance. Statistically significant insulin antibody binding ranged from 5.8% to 23.5%. Raw data for each patient and summation data are charted in Table IV. Comparable species-specific insulin antibody binding in patients receiving pork or mixed beef-pork insulin could average two-to-eight-fold higher. Serum antibody binding in patients with immunologic insulin resistance will approach 80-90% (Fineberg, personal communication). Longer follow-up on these patients and others with similar findings will be required before the clinical significance can be ascertained. Comparing BHI immunogenicity with that of PPI remains under study; however, longer-term studies suggest that BHI may be less immunogenic than PPI (15). The clinical significance of this observation remains to be elucidated.

Careful analysis for a possible effect of E. coli antigens was carried out in all patients who received BHI. No evidence of E. coli polypeptide antibodies was identified. Baseline and six month levels were unchanged. Accordingly, concern that a bacterial protein might be present as a result of the fermentation process and introduced during BHI therapy can be allayed. These proteins are eliminated during the insulin purification processes (9).

Biosynthetic human insulin controls the symptoms and lowers the plasma glucose of patients with insulin-requiring diabetes. It is used similarly to the purified animal insulins in comparable daily dosages. The time action of NPH-BHI appears to be slightly shorter and to have a quicker onset than occurs with NPH derived from animal insulin. In the patients we have treated, we have not identified any with insulin allergy or insulin resistance. Although we have not treated patients with pre-existing insulin allergies, others have successfully treated such patients (16,17). In our experience, BHI is safe to use for the insulin-requiring diabetic patient.

Current indications for the use of human insulin include: 1) patients who are allergic to insulin or have chronic immunologic insulin resistance; 2) initial insulin administration to young, insulin-dependent diabetic patients; 3) patients who require insulin therapy intermittently; 4) patients with insulin lipoatrophy who fail to respond to treatment with purified pork insulin; 5) as a substitute for purified pork insulin; and 6) patient or physician preference.

If the long-term use of human insulin yields the lowest levels of antibody achievable, human insulin should be standard therapy for young insulin-dependent diabetic patients. Theoretically, minimal immunogenicity might extend the remission phase of diabetes by protecting residual islet cell function. This possibility requires clinical documentation. Furthermore, significantly lower insulin antibody levels might reduce the metabolic lability that characterizes patients with insulin-dependent diabetes. Data on this question remain inconclusive. Human insulin should be tried in patients who demonstrate erratic subcutaneous insulin absorption, although the mechanism of this problem remains unclear and may be multifactorial. Finally, the availability of BHI relieves the growing fear of insufficient insulin supply, since human insulin from recombinant DNA sources does not depend on the availability of animal pancreas.

We have no experience with semisynthetic human insulin, but we expect no differences between human insulin of biosynthetic and semisynthetic sources. Longer-term studies will teach us whether human insulin is preferred to PPI. Human insulin will increase in use just because it is an homologous insulin as long as its cost to the patient is not prohibitively high.

Acknowledgments

We are grateful to our many colleagues who referred their patients to us for this study.

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Diabetic Neuropathic Arthropathy: Charcot Joint

- Afflicts fewer than 0.5% of diabetic patients
- Diabetes more frequent cause than leprosy or lues
- Progressive subluxation and destruction of traumatized joint
- Ninety percent in foot and ankle
- Arterial circulation generally good
- Mid-foot swelling and collapse of longitudinal arch
- Characteristic radiological changes
  - joint distortion
  - fractures
  - bony fragmentation
  - osteoporosis
  - new bone formation

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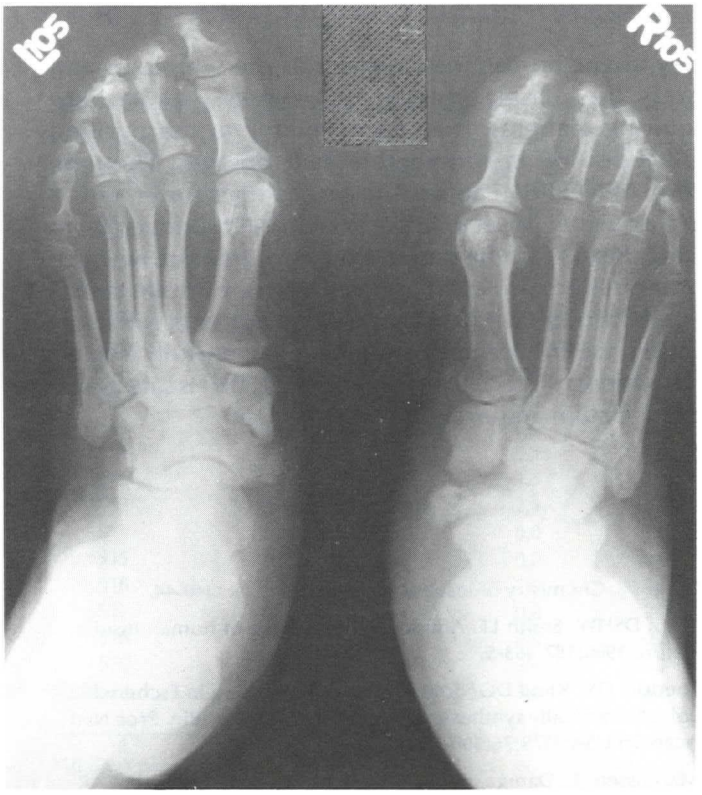

Charcot Joint: Radiograph of a case of bilateral Charcot with marked destruction of tarsal bones.