FINAL CLINICAL REPORT OF THE EXCLUSIVE
USE OF NEUTRAL REGULAR HUMAN INSULIN (rDNA) IN
THE TREATMENT OF INSULIN-DEPENDENT DIABETES MELLITUS
IN PATIENTS WHO HAVE NEVER RECEIVED INSULIN

Protocol B5K-MC-IBAI
Humulin

Multi-Clinical Trial Conducted by:
For Lilly Research Laboratories
Eli Lilly and Company
Lilly Corporate Center
Indianapolis, Indiana 46285

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1. INTRODUCTION:

This is a unique study which examines the use of neutral regular human insulin (rDNA) in the treatment of diabetes mellitus. Studies which have been carried out previously indicate that human insulin is the least complication-prone insulin therapy in man. However, no studies to date have been carried out using exclusively neutral regular human insulin. As this might prove to be even more complication free than intermediate duration human insulin + regular, the effects of this therapy was studied in insulin-treated diabetic patients who had never been treated with insulin prior to this study.

2. OBJECTIVES:

The objectives of this study are to determine the immunogenicity and effectiveness of neutral regular human insulin in the treatment of insulin-requiring individuals with diabetes mellitus.

3. INVESTIGATORS:

4. INVESTIGATIONAL PLAN:

4.1. The study was an open label, multi-clinic, noncomparative study.

4.2. All subjects were assigned to multiple regular human insulin injection therapy, either administered by multiple injections or use of a continuous subcutaneous insulin infusion pump.

4.3. This study was not blinded.

4.4. Concomitant medications were allowed.

5. STUDY POPULATION:

5.1.1. Selection was limited to diabetics 5 years of age or above, either male or female who had not previously received insulin and who required this form of therapy in the judgment of their personal physicians. Criteria for requiring insulin were overnight fasting, plasma glucose concentrations in excess of 200 mg/deciliter and/or 2 hour postprandial glucose levels in excess of 300 mg/deciliter with or without symptoms and signs of diabetes mellitus. Pregnant patients could be included in the study.

5.1.2. Exclusion criteria: Exclusion criteria included previous insulin therapy, life expectancy of less than 3 years, cancer of all types, renal disease as indicated by serum creatinine over 2.0 mg%, and advanced cardiovascular disease.

5.2. Disease diagnostic criteria: See above.
5.3. Sample size: Sample size in this study was intended to include a total of 100-130 patients with diabetes not previously treated with insulin.

6. DOSAGE AND ADMINISTRATION:

6.1. Human insulin therapy dosage was adjusted in accordance with metabolic needs of the patient. Metabolic needs of the patient were determined by use of home glucose monitoring using an Ames Glucometer or Chemstrip BG.

6.2. Materials and supplies: Insulin used for this study was Humulin R (Humulin insulin rDNA-neutral regular). Supplies for home glucose monitoring and insulin were provided by the sponsor, Eli Lilly and Company.

7. PROCEDURES AND METHODS:

7.1. Study procedures.

7.1.1. Pre-therapy phase: Written informed consent was obtained from all patients or parents or guardians of minors prior to enrollment in the study. Signed forms are kept on file by the investigator. Within 30 days prior to treatment with HI, the following occurred: 1. a history and physical examination, and 2. overnight fasting and 1 hour postprandial blood samples collected for analyses indicated in Appendix A (Master Schedule of Events). For children the volume of blood drawn was a maximum of 2.5 ml per pound of body weight.

7.2. Laboratory procedures: Laboratory procedures were performed at the visits shown in Appendix A. — The last injection of regular insulin was at least 2 hours prior to the fasting specimen for serum glucose and C-peptide. Patients on pumps turned off these devices at least 1 hour prior to the fasting specimen. Insulin therapy was to be administered immediately after the 1 hour post-Sustacal sample was drawn. Laboratory procedures are indicated in the following table:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>In the Laboratory of:</th>
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<tbody>
<tr>
<td>Serum glucose, on overnight fasting and 1-hour postprandial samples</td>
<td>Eli Lilly and Company</td>
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<tr>
<td>(Sustacal 1 ml, 1 calorie, for each calorie of the usual breakfast meal</td>
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<td>of the patient).</td>
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<tr>
<td>Urinalysis for glucose and acetone.</td>
<td>Investigator</td>
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<tr>
<td>Serum insulin antibody titers (will be performed twice prior to initiation</td>
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<td>of HI therapy). (See Appendix C).</td>
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</table>
Human C-peptide, on overnight fasting and 1-hour post-prandial samples (Sustacal 1 ml, 1 calorie, for each calorie of the usual breakfast meal of the patient).

Glycohemoglobins

Tests for antibodies to E. coli polypeptides—an immunologic study performed on serum.

2). To be performed within 30 days prior to study-drug therapy and thereafter every 12 months:

**Procedure**

**In the Laboratory of:**

**CBC:**
- hemoglobin
- hematocrit
- white blood cell count
- and differential
- platelet count

**Blood chemistry:**
- calcium
- inorganic phosphorus
- glucose
- BUN
- uric acid
- cholesterol
- total protein
- albumin
- total bilirubin
- alkaline phosphatase
- LDH
- SGOT
- serum creatinine
- SGPT
- albumin-globulin (A/G) ratio
- sodium
- potassium
chloride
CO₂

Routine urinalysis:

specific gravity
pH
albumin
glucose
white and red blood cells
casts

Results from laboratory tests done by were mailed promptly to the investigator and to Eli Lilly and Company. If a markedly abnormal test result occurred for SGOT, creatinine, bilirubin or serum potassium, telephoned the monitor and the monitor contacted the investigator immediately. When abnormal values were present, the investigator indicated his opinion as to whether the abnormality was study-drug related, and the test was repeated as clinically indicated.

Results from tests done at the investigator's laboratory were recorded in the comment section of the case report forms provided by the sponsor, which was signed by the investigator. When abnormal values were present, the investigator indicated his opinion as to whether the abnormality was study-drug related. The investigator sent the sponsor the standardization procedures employed by the laboratory he was using, the name and location of the laboratory, and a list of normal ranges for all tests that were done.

7.3. and 7.4. Appropriateness and consistency of measurements and quality assurance were arranged with each individual laboratory and such criteria were submitted to the sponsor.

8. PATIENT DISPOSITION CRITERIA:

8.1. Terminations: HI therapy could be discontinued if during the study the patient was judged by the physician as no longer needing insulin therapy or the patient experienced a serious adverse reaction warranting discontinuation of HI therapy. In such instances, the patient was followed according to protocol as if on drug therapy. Studies could also be terminated at the request of the patient or parent or guardian. Also patients were terminated if the patient received nonstudy insulin, lack of efficacy was demonstrated within 6 months on HI, or it could be demonstrated that the patient's antibody levels at the initial visit were consistent with previous insulin therapy.

8.2. Qualification for analysis: Patients were considered evaluable if they had at least 6 evaluable visits to cover a span of at least 12 months from visit 1. Evaluable visits satisfied the requirement that no nonstudy insulin was taken between entry to the study and that visit and satisfy the appropriate time requirements. The rules for visits included visit 1 being evaluable if the patient's control antibody titers were compatible with never taking insulin,
visits 8 and 14 were required to be evaluable, subsequent visits occurred 2 months ± 4 weeks after the previous visit unless the patient was hospitalized for control of his diabetes at the time a visit was scheduled. In such instances, future evaluable visits were determined by consultation with the investigator.

8.3. Study extensions: An additional year of study was available.

9. EFFICACY CRITERIA:

Efficacy was judged on the basis of hemoglobin A₁ values and fasting glucose concentrations on the day of visit.

10. DATA ANALYSIS METHODS:

Data were analyzed by

There were 77 evaluable patients of whom 69 completed a second year of study. Data are given as mean ± standard deviation unless otherwise indicated. All measurements were examined for normality and it was found that both bound insulin and Z bound levels were not normally distributed. A logarithmic transformation to the base 10 was used to normalize the data. One was added to all values before transformation to enable values of 0 to remain as such following transformation. These data are reported as the mean ± s.d. of the transformed values and the untransformed mean (geometric mean).

Patients were categorized as IDDM or NIDDM based on their age at onset of diabetes and body mass index (BMI=weight/height²). Patients with age of onset less than or equal to 40 years and BMI less than or equal to 23.4 for males and 27.0 for females were classified as IDDM. All others were called NIDDM. These BMIs correspond to 115% of ideal body weight.

Continuous variables were compared between IDDM and NIDDM by repeated measures analysis of variance (RMANOVA). This type of analysis provides three tests of significance - type (a test comparing IDDM and NIDDM overall levels, time (a test showing if values change over the course of the two years of the study) and type by time interaction (a test indicating if the changes over time are parallel in the IDDM and NIDDM patients). Since this analysis requires that patients included have no missing data and many patients were missing c-peptide values, t-tests were also done on c-peptide values at baseline, 12 and 24 months. Measures of insulin binding were examined in two ways. Chi-square tests were used to compare the incidence of significant levels of binding at any time in either the first year or both years. For subjects who had significant levels of binding at some point in the study, RMANOVA was done to compare levels in IDDMs and NIDDMs. Maximum binding levels were computed in all subjects who had significant levels of binding at any time during the study. Only subjects who completed the two years are included in these analyses. Levels were compared in the two groups using t-tests.

11. RESULTS:

11.1 Population description (Table 1 and Table 2):
A total of 77 individuals, 19 IDDM and 58 NIDDM participated. Most were Caucasian with a slight preponderance of male patients. Ages ranged from 4 to 76 years. Body mass index varied widely. Thirty individuals have received no therapy prior to this study, whereas 47 had been treated with sulfonylureas.

11.2 Insulin dosage, frequency, and therapeutic complications (Tables 2 and 3):

Insulin dosage increased slightly from 2 months to 2 years, 29.4 to 36.5 U/day, however at both time points, there were patients who did not require insulin, 3 and 8 individuals respectively. No incidents of lipoatrophy were observed, however, 3 individuals sustained lipohypertrophy. Only 1 individual had an episode of local allergy (injection site limited) and 1 individual had an episode of transient systemic dermal reactions. Neither individual required a cessation of therapy. No individual remained on CSII. In the 61 individuals remaining on insulin at 2 years, the majority were on 2 shots, whereas 12 were on 4 shots per day.

11.3 C-peptide concentrations (Table 4):

Forty-eight individuals had complete 24 month fasting c-peptide concentrations, whereas 34 had complete post-prandial data. A gradual fall in fasting c-peptide levels were seen in both groups whereas in NIDDM, 1 hr p.c. c-peptide levels were higher at 2 months than at 18 and 24 months and levels at 6 months also exceeded those at 24 months. Post-prandial levels were available in only 3 individuals with IDDM. C-peptide levels as expected were greatest in patients with NIDDM. In comparing individuals with data available at baseline, 12 and 24 months, fasting c-peptide levels were higher in NIDDM than IDDM. In both NIDDM and IDDM, mean post-prandial levels differed little throughout.

11.4 Occurrence and levels of significant % bound insulin:

In our assay, % B/T minus control % B/T is significant if this difference exceeds 2 times the maximum coefficient variation for control serum binding, that is 4%. In table 5, we contrasted the occurrence of significant % binding which was seen throughout 1 and 2 years for patients with IDDM and NIDDM. The 1-year data includes all individuals with complete 1-year results and the 2-year data includes all individuals with complete 2-year results. Between 1 and 2 years, 5 out of 19 patients with IDDM and 10 of 58 patients with NIDDM dropped out of the study. As can be noted, drop-outs did not affect the significance of differences shown between IDDM and NIDDM. At both time points, significant binding occurred much more frequently in IDDM than NIDDM. However, in both groups there were individuals with nonsignificant levels of binding, at 2 years in IDDM, 26% vs 80% in NIDDM.

In table 6, the log transformed levels of % binding for individuals whose % binding exceeded 4% in at least one visit are
contrasted over 24 months. In IDDM, the geometric mean of % bound increased to 10.4% at 6 months and fell to 2.4% at 24 months. In contrast, the geometric mean of % bound in NIDDM increased to 3% at 6 months and fell to 2.5% at 24 months. The increase with time was significant. The maximum significant % bound seen in IDDM patients was 16%, whereas in NIDDM the level was 6.6%, p<.003.

11.5 Occurrence and levels of significant bound insulin:

Bound insulin levels were defined as significant if total insulin minus 2 s.d. exceeded free insulin plus 2 s.d. after correction for recovery. The occurrence of significant bound insulin (Table 7) is virtually identical to the % bound data throughout 1 and 2 years. Again, the occurrence was greatest in IDDM patients. The geometric mean of bound insulin peaked between 4 and 6 months (Table 8) for IDDM and was much lower and delayed for NIDDM (6 to 16 months, 1.3 to 2.9 μU/ml). However, there was not a significant time effect when contrasting groups. Levels in IDDM significantly exceeded NIDDM throughout.

11.6 Insulin efficacy and body weight:

Insulin dosages are shown in table 9, mean dosages did not change over time, nor did doses in patients with IDDM differ from NIDDM. However, HbA1c levels were lower in NIDDM (Table 10) whereas body weight was much greater in NIDDM than IDDM (Table 11). Insulin dose/kg is contrasted in Table 12. Dose/kg in NIDDM was less than IDDM but the p was = .054. These results contrast with previous reports in which insulin dose/kg are increased in type 2 vs type 1. Thus considering HbA1c values, enhanced efficacy of regular insulin in type 2 is suggested. Therefore an additional contrast was made as shown in Table 13, that is dose per M² of surface area. Correction for surface area obviates dose differences between types of patients.

12. SUMMARY AND CONCLUSIONS:

In this open label study, patients with IDDM and NIDDM were treated de novo only with regular human insulin. Seventy-seven individuals were evaluable at 1 year and 69 individuals completed 2 years of the study. Lipoatrophy was not observed, whereas transient dermal reactions were seen in 2 individuals. As in previous studies, human insulin proved to be quite low in immunogenicity in both types of diabetic individuals. The occurrence of significant levels of % bound and bound insulin levels was quite low in NIDDM (25% at 1 year and 35% at 2 years), whereas most individuals with IDDM developed antibodies to insulin (71% at 1 year and 73% at 2 years).

Surprisingly, efficacy data revealed that HbA1c levels were lower in patients with NIDDM (i.e. 9.7 vs 11.5% at 2 years) despite their significantly greater body weights. A comparison of dose/kg also revealed that doses per weight were somewhat lower in NIDDM than IDDM (p=.054). When normalized to surface area, type differences in dose were not significant. Thus multiple dose regular human insulin dosage may represent a highly efficacious therapeutic approach in NIDDM, but dosage should probably be estimated on the basis of surface area.
In summary, multiple dosage regular human insulin therapy is efficacious and low in side effects. Such therapy may be of increased advantage in NIDDM.