Human insulin: study of safety and efficacy in man

D R OWENS, M K JONES, T M HAYES, L G HEDING, K G M M ALBERTI, P D HOME, J M BURRIN, R G NEWCOMBE

Abstract

The safety and efficacy of a new highly purified neutral soluble human insulin produced by conversion of porcine insulin was compared with a highly purified neutral soluble porcine insulin in six normal men. The insulins were administered by subcutaneous injection at a dose of 0.075 U/kg body weight. Somatostatin was infused during the experiment to suppress endogenous insulin secretion. No difference was found in the plasma glucose, insulin, or metabolite responses. Thus the potency, onset, and duration of effect were identical with the two insulins. No short-term side effects to either insulin were observed.

Highly purified, semi-synthetic human insulin offers a safe and effective means to explore the possible advantages of homologous human insulin in the management of diabetes mellitus.

Introduction

Both porcine and bovine insulins are used to treat diabetes mellitus. These insulins differ in their structure from human insulin by one and three amino-acids respectively.¹ We therefore need to know whether human insulin has any advantages over the other insulins in the treatment of diabetes.

Human insulin may be prepared from human pancreas,² by total chemical synthesis,³ by semi-synthesis from porcine insulin,^{4,5} and by recombinant DNA technology using *Escherichia coli*.⁶ Pancreatic human insulin has been available for some time, primarily for allergy testing in diabetics⁷; and fully synthetic human insulin has been used for only short-term treatment of a few patients.⁸ *E coli* human insulin has recently been compared with porcine insulin in healthy men.⁹

We decided to compare the plasma glucose, insulin, and intermediary metabolite responses to subcutaneously administered highly purified semi-synthetic human and porcine neutral soluble insulins in normal men. Somatostatin was infused intravenously to suppress endogenous beta-cell secretion^{10,11} and allow the plasma insulin profiles to represent the absorption and clearance of the administered insulins more precisely.

Subjects, materials, and methods

We studied six healthy men aged 27-38 years who were within 10% of ideal body weight¹² and had no family history of diabetes mellitus. All gave informed consent, and the study was approved by the local ethical committee. None of the subjects had received insulin before, and none had a history of atopy or allergy or was taking any medication at the time of the study.

The neutral soluble human insulin was manufactured by Novo Industri A/S, Copenhagen, to monocomponent specifications. The process for producing semi-synthetic human insulin employs porcine insulin as the basic raw material, which is converted into human insulin by replacing a single amino-acid in the B chain (B30 alanine) with threonine by an enzymatically catalysed process that preserves the disulphide bonds. The crude product is then purified to monocomponent specifications by chromatographic procedures. The equivalent porcine insulin, Actrapid MC, was used as the comparative insulin preparation, and the diluent contained in Actrapid MC was used for the control studies.

Each volunteer was studied on three separate occasions at weekly intervals. At 0800, after a 10-hour fast (time -120 min), a cannula was inserted into an antecubital vein kept patent by a slow-running infusion of saline (0.154 mmol/l; 0.9 mg/100 ml). Blood samples were taken via a three-way tap attached to the intravenous cannula. A second intravenous cannula was sited in the opposite antecubital vein, and 30 minutes later (time -90 min) a somatostatin infusion (100 µg/h) was begun using an infusion pump and continued throughout.

Human insulin, porcine insulin, and diluent were administered at random by subcutaneous bolus injection (time 0 min) using a Plastipak SFP 1 ml syringe. Insulins were given at a dose of 0.075 U/kg body weight. The injection site was 10 cm from the umbilicus towards the anterior superior iliac spine.

Venous blood samples were obtained at -120, -105, -90, -60, -30, 0, 10, 20, 30, 40, 50, 60, and every 30 minutes up to 360 minutes. Aliquots were taken into heparin for measurement of immunoreactive insulin (IRI)¹³ and C-peptide¹⁴ concentrations, into fluoride for plasma glucose determination (hexokinase), and (0.5 ml) into 2 ml 3% (w/v) perchloric acid for estimation of lactate, alanine, and 3-hydroxybutyrate concentrations.¹⁵ Samples were centrifuged within five minutes and the supernatants stored at -20°C until assay. The detection limit of immunoreactive insulin was 0.0062 nmol/l (0.036 ng/ml) and the sensitivity (SD within-assay) 0.0025 nmol/l (0.015 ng/ml). The detection limit of C-peptide was 0.01 nmol/l (0.03 ng/ml) and the sensitivity 0.004 nmol/l (0.012 ng/ml).

For each individual subject all samples were measured in one assay. Human and porcine insulins reacted identically in the insulin radioimmunoassay.

Statistical—Results are expressed as means ± SE except where stated otherwise. Concentrations of 3-hydroxybutyrate were log transformed to normalise distribution.¹⁶ Individual pairs of treatments were compared using paired analysis of variance with time 0 as a covariate. The computer program used was the Statistical Package for the Social Sciences.

Results

No adverse reactions to either insulin were observed. Figure 1 shows the plasma glucose, C-peptide, and immunoreactive insulin values in the six subjects when infused with somatostatin and given subcutaneous boluses of diluent (control preparation), human insulin, and porcine insulin. Plasma glucose concentrations were similar in the three study periods before subcutaneous injection. During the period preceding injection of the comparative preparations somatostatin caused a fall in plasma glucose of approximately 0.4 mmol/l (7.2 mg/100 ml). In the control study there was then a rise to 5.0-5.8 mmol/l (90-105 mg/100 ml) from time 0 to the end of the study. Thirty minutes after administration of each insulin the plasma glucose value showed a prompt fall, reaching a nadir of about 3.0 mmol/l

Welsh National School of Medicine, Cardiff CF4 4XW

D R OWENS, MB, CHB, honorary lecturer in medicine
M K JONES, MRCP, senior registrar in medicine
T M HAYES, FRCP, senior lecturer and consultant physician
R G NEWCOMBE, MA, PHD, lecturer in medical statistics

Novo Research Institute, Novo Alle, DK-2880 Bagsvaerd, Denmark
L G HEDING, PHD, head of medical department

Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP

P D HOME, DPHIL, MRCP, research associate, department of clinical biochemistry and metabolic medicine
K G M M ALBERTI, DPHIL, FRCP, professor of clinical biochemistry and metabolic medicine
J M BURRIN, PHD, research associate, department of clinical biochemistry and metabolic medicine

(54 mg/100 ml) between 90 and 150 minutes and remaining about 1.5 mmol/l lower than the control value at six hours. From 30 minutes after injection the glucose concentrations during the two insulin studies were significantly different from control values ($0.005 > p > 0.001$). There was no significant difference between the glucose responses to human and porcine insulins at any time (figs 1 and 2).

FIG 1—Effect of highly purified human and porcine insulins on plasma glucose, insulin (IRI), and C-peptide concentrations (mean $\pm$ SEM) in six normal men. Somatostatin infused from -90 to +360 min. Insulin (0.075 U/kg) or diluent (control) given subcutaneously at time 0.

Conversion: SI to traditional units—Glucose: 1 mmol/l $\approx$ 18 mg/100 ml. C-peptide: 1 nmol/l $\approx$ 3.02 ng/ml. IRI: 1 nmol/l $\approx$ 5.8 ng/ml.

The mean plasma C-peptide concentration decreased from 0.45 nmol/l to 0.15 nmol/l (1.36 ng/ml to 0.45 ng/ml) during the first 90 minutes of somatostatin infusion (fig 1). After injection of diluent C-peptide concentrations tended to rise after two hours, reaching roughly 75% of preinfusion values by the end of the experiment. C-peptide concentrations after insulin were significantly lower than after diluent—from 30 minutes after porcine insulin and from 120 minutes after human insulin. From two to five and a half hours after both insulins C-peptide values remained between 0.04 and 0.07 nmol/l (0.12 and 0.21 ng/ml) (fig 1). During the experiment no difference was seen between the values after human and porcine insulins (fig 2).

Initial plasma insulin concentrations of approximately 0.045 nmol/l (0.26 ng/ml) fell to 0.024 nmol/l (0.14 ng/ml) after 90 minutes of somatostatin. Values remained low after diluent, rising slightly at four hours. Subcutaneous injection of human insulin led to a rapid rise in plasma insulin concentrations, reaching a peak after 40 to 90 minutes and being significantly higher than control values from 10 minutes after

injection (fig 1). After porcine insulin peak plasma insulin concentrations were reached 40 to 120 minutes after injection, values again being significantly higher than control values from 10 minutes. Peak insulin concentrations after the human and porcine insulins were 0.13 ± 0.02 nmol/l (0.75 ± 0.12 ng/ml) at 90 minutes and 0.12 ± 0.03 nmol/l (0.70 ± 0.17 ng/ml) at 60 minutes, respectively. A significant difference in insulin values was observed only at six hours, with a higher concentration ($p < 0.05$) after human insulin (figs 1 and 2).

FIG 2—Comparison of effect of human and porcine insulins on plasma glucose, insulin (IRI), and C-peptide concentrations (mean $\pm$ SEM) in six normal men. Somatostatin infused from -90 to +360 min. Insulin (0.075 U/kg) or diluent (control) given subcutaneously at time 0. (Results shown as means of differences between responses to two insulins (porcine minus human) for six subjects. Value of 0 implies no difference in response. Outer lines represent ± 1 SE of mean difference.)

Conversion: SI to traditional units—Glucose: 1 mmol/l $\approx$ 18 mg/100 ml. C-peptide: 1 nmol/l $\approx$ 3.02 ng/ml. IRI: 1 nmol/l $\approx$ 5.8 ng/ml.

There was considerable intraindividual and interindividual variation in intermediary metabolite values. In general, values rose during somatostatin infusion, before insulin injection. After diluent 3-hydroxybutyrate concentrations tended to rise, whereas after both insulins concentrations promptly fell, reaching a nadir at about three hours (table); there was no consistent difference after either insulin. Blood glycerol concentrations also rose before administration of the comparative insulin preparations. After porcine and human insulins the values quickly fell, reaching a nadir at 40-90 minutes and 60-180 minutes respectively. No pronounced posthypoglycaemic rise was observed, however, as was seen for 3-hydroxybutyrate (table).

Metabolite values in normal men given highly purified human and porcine insulins ($n=6$)

		3-Hydroxybutyrate (mmol/l)		Glycerol (mmol/l)	
		Porcine	Human	Porcine	Human
Basal	Mean	0.020	0.065	0.045	0.088
	Range	0.005-0.055	0.020-0.175	0.005-0.085	0.010-0.225
Preinjection	Mean	0.170	0.305	0.115	0.185
	Range	0.025-0.390	0.045-0.735	0.050-0.165	0.130-0.325
Nadir*	Mean	0.020	0.075	0.045	0.055
	Range	0.005-0.050	0.020-0.20	0.020-0.090	0.030-0.085
6 h	Mean	0.395	0.370	0.115	0.095
	Range	0.045-1.060	0.080-0.615	0.075-0.170	0.055-0.140

*3-Hydroxybutyrate: porcine insulin 150-180 min, human insulin 180 min; glycerol: porcine insulin 49-90 min, human insulin 60-180 min.

Conversion: SI to traditional units—3-Hydroxybutyrate: 1 mmol/l $\approx$ 10.3 mg/100 ml. Glycerol: 1 mmol/l $\approx$ 9.2 mg/100 ml.

Discussion

Both insulins were well tolerated, and no adverse effects were observed. No differences were found between human and porcine insulin in their effect on blood glucose, insulin, C-peptide, and intermediary metabolite concentrations.

The experimental model used to assess the response to human insulin included a low-dose (100 µg/h) somatostatin infusion to suppress endogenous insulin secretion.¹⁰ The very low C-peptide values show that suppression was achieved (fig 1). C-peptide values were lower after insulin injection than after diluent, suggesting either that both insulins suppressed insulin secretion direct or, more likely, that endogenous insulin secretion was additionally decreased by the accompanying hypoglycaemia. Using somatostatin allowed us to compare the plasma insulin profiles. After subcutaneous injection insulin concentrations increased significantly from 10 minutes and reached a plateau at 40 to 120 minutes. The two insulins produced profiles suggesting similar rates of absorption and local and systemic clearance. This combined with the identical effects on blood glucose shows that within the limits of the experimental protocol no distinction could be drawn between the two insulins. No firm physiological conclusions were possible, since somatostatin inhibits the secretion of other hormones¹⁷ and, more important, decreases splanchnic blood flow,¹⁸ which may slow insulin degradation. The comparative results, however, remain valid. Keen *et al.*,⁹ using *E coli*-derived human insulin in normal men, also failed to detect any real differences between human and porcine insulins but did not measure insulin concentrations.

The human insulin used in our study was made with porcine insulin as the starting material. In view of possible shortages of insulin derived from conventional sources—namely, bovine and especially porcine pancreas¹⁹—bacterially produced insulin offers an attractive alternative. Major long-term studies, however, are required to prove that this insulin is totally free of *E coli*-derived trace contaminants.

We have shown that the hypoglycaemic potency, timing of effect, and short-term safety of human insulin is similar to an equivalent pharmaceutical formulation of a currently available, highly purified porcine insulin. This human insulin therefore provides the opportunity to explore the possible benefits of homologous insulin for diabetes mellitus.

This study was conducted with technical help from Sister A J Rochester (clinical investigations unit, WNSM); Miss E J Ashley and Miss R Fox (medical students, WNSM); Mrs Marianne Heyden, Bente Hansen, Lisbet Pederson, and Birgit Jensen (NRI); and the department of medical biochemistry (WNSM). We are grateful to Professor R Hall (WNSM) and Dr J Schlichtkrull (NRI) for con-

structive criticism and advice, and to Mr A Simpson for the illustrations.

Requests for reprints should be sent to Dr D R Owens.

References

- Nicol DSHW, Smith LF. Amino acid sequence of human insulin. *Nature* 1960;187:483-5.
- Mirsky IA, Jinks R, Perisutti G. The isolation and crystallisation of human insulin. *J Clin Invest* 1963;42:1869-72.
- Sieber von P, Kamber B, Hartmann A, Jöhl A, Riniker B, Rittel W. Totalsynthese von humaninsulin unter gezielter bildung der disulfidbindungen. *Helvetica Chimica Acta* 1974;57:2617-21.
- Moriyama K, Oka T, Tsuzuki H. Semi-synthesis of human insulin by trypsin catalysed replacement of Ala-B30 by Thr in porcine insulin. *Nature* 1979;280:412-3.
- Gattner HG, Danho W, Naithani VK. Enzyme-catalysed semi-synthesis with insulin derivatives. In: *Proceedings of second international insulin symposium, Aachen, Germany, September 1970*. Berlin: Walter de Gruyter, 1980:117-23.
- Chance RE, Kroeff EP, Hoffmann JA. Chemical, physical and biological properties of recombinant human insulin. In: *FDA conference on insulin and growth hormone, NIH, 1980*. (In press.)
- Kreines K. The use of various insulins in insulin allergy. *Arch Intern Med* 1965;116:167-71.
- Teuscher A. Die biologische wirkung von voll synthetischem humanem insulin bei patienten mit Diabetes Mellitus. *Schweiz Med Wochenschr* 1979;109:743-7.
- Keen H, Pickup JC, Billous RW, *et al.* Human insulin produced by recombinant DNA technology: safety and hypoglycaemic potency in healthy men. *Lancet* 1980;ii:398-401.
- Alberti KGMM, Christensen SE, Iversen J, *et al.* Inhibition of insulin secretion by somatostatin. *Lancet* 1973;ii:1299-301.
- Home PD, Pickup JC, Keen H, Alberti KGMM. Continuous subcutaneous insulin infusion. Comparison of plasma insulin profiles after infusion or bolus injection of the mealtime dose. *Metabolism* (in press).
- Metropolitan Life Insurance Company. *Statistics bulletin*. New York, 1960;41:6 (Feb), 1 (Mar).
- Heding LG. Determination of total serum insulin (IRI) in insulin-treated diabetic patients. *Diabetologia* 1972;8:260-6.
- Heding LG. Radioimmunochemical determination of human C-peptide in serum. *Diabetologia* 1975;11:541-8.
- Lloyd B, Burrin J, Smythe P, Alberti KGMM. Enzymatic, fluorimetric continuous-flow assays for blood glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate. *Clin Chem* 1978;24:1724-9.
- Foster KJ, Alberti KGMM, Hinks L, *et al.* Blood intermediary metabolites and insulin concentrations after an overnight fast: reference ranges for adults and interrelations. *Clin Chem* 1978;24:1568-72.
- Assan R. La somatostatine: une nouvelle hormone? *Diabete Metab* 1976;2:135-46.
- Felig P, Wahren J. Somatostatin and diabetes: suppression of glucose absorption rather than stimulation of glucose disposal. *Metabolism* 1976;25:1509-11.
- National Diabetes Advisory Board. *A study of insulin supply and demand*. Washington DC: US Department of Health, Education and Welfare, 1978. (NIH-78-1588.)

(Accepted 12 February 1981)

ONE HUNDRED YEARS AGO We hear from Durham news, or rather rumours, of a serious character. Durham has tended more and more to occupy of late an honourable and promising position among medical universities of England, partly making up the shortcomings of other English Universities. Until a few years ago, all candidates for the medical degrees there were obliged, before going on to the degree examinations, to pass an examination equivalent to that for the B.A. at Durham. Recently, a more liberal regulation was introduced, by which candidates were permitted to take the Preliminary Fellowship of the Royal College of Surgeons of England, in place of the Durham examination. In consequence of the change so introduced, the medical department has taken on a new development, and the medical degrees have been rendered practicable of attainment by the profession generally. The sudden abolition of the Preliminary Fellowship by the College of Surgeons has created an unexpected complication. Failing the Preliminary Fellowship, there is no alternative examination open to intending candidates, unless they have entered at or graduated at a university, except the above-mentioned slightly modified B.A. examination at Durham. This examination, it is rumoured, the Senate of the University intend to uphold, or, at all events, to replace only by an examination of so high a standard as to be

next to impracticable. The effect of such a resolution on the part of the Senate would be practically the closure of the only door to graduation open to the majority of those who, having intended at the commencement of their course of study to obtain simply a medical or a surgical qualification, find, later on, an university degree very desirable. Such a result would not only involve great loss to the University itself, and especially to the medical department, which is in a fair way to develop indefinitely, but be very regrettable as a loss to the country at large, at a time when university education and extension are being more and more sought after. As in these directions the Senate of the Durham University are bestirring themselves, it is to be hoped that they will formulate some liberal measure by which the present difficulty may be tidied over. At Cambridge, the recent modification in the regulations, by which the previous examination is fixed as the only Arts examination required as a preliminary before admission to the medical course and degrees, is being followed by the most satisfactory results. Similar results would undoubtedly follow, if a similar change were made at Durham. It is of vital importance to the future extension and usefulness of the University, and of great importance to English students of medicine, that a liberal course should be taken in this matter. (*British Medical Journal*, 1881.)