Study of porcine and human isophane (NPH) insulins in normal subjects

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Summary. The plasma glucose, C-peptide and insulin responses to subcutaneously administered highly purified porcine, 'semi-synthetic' and 'biosynthetic' human isophane (NPH) insulin and diluting medium as control in normal male subjects were evaluated. Porcine and semi-synthetic human NPH insulins were administered at two dose levels of 0.15 and 0.30 U/kg body weight and biosynthetic human NPH at 0.15 U/kg body weight only. At the low dose level the three insulin preparations resulted in a similar maximal hypoglycaemic effect within 3–5 h after administration. However, over the remainder of the 11 h post-injection period, the plasma glucose level was lower after semi-synthetic human insulin. In contrast, at the 0.30 U/kg dose level, there was no difference

in the early or late hypoglycaemic response between porcine and semi-synthetic human NPH insulins of equivalent pharmaceutical formulation. The clinical relevance of these findings needs further evaluation. The data suggest that for the 'intermediate-acting' NPH insulin preparations, both the species of insulin, nature and quantity of the retarding protein and their subsequent interaction may determine their time-action characteristics.

Key words: Porcine NPH insulin, semi-synthetic and biosynthetic human NPH insulin, pharmacokinetics, pharmacodynamics, normal subjects.

In 1946 a neutral crystalline suspension of protamine insulin (NPH – Neutral Protamine Hagedorn), otherwise known as 'isophane insulin', was introduced [1]. This insulin preparation became popular as a once or twice daily insulin used alone or admixed with soluble insulin as required [2, 3]. The advent of human insulin prepared either from porcine insulin by enzymatic conversion [4] or by employing recombinant DNA technology [5] has resulted in human NPH insulin becoming available for clinical use.

The purpose of this study was to compare the pharmacokinetics and pharmacodynamics of three NPH insulin preparations – porcine NPH insulin and both semi-synthetic and biosynthetic human NPH insulin following subcutaneous administration.

Subjects, materials and methods

Subjects

Six healthy normal male subjects, aged 24–38 years, within 10% of their ideal body weight [6] and with no family history of diabetes mellitus were studied. The study was approved by the local Ethical Committee and informed consent was obtained from each of the volunteers.

Materials

Three different neutral NPH insulin preparations (U-40) were studied: (1) porcine NPH insulin (Protaphane MC, Novo Industri, Copenhagen, Batch No. 501*2, expiry date: 01.02.83); (2) 'semi-synthetic' human NPH insulin produced by transpeptidation of porcine insulin (Protaphane HM, Novo, Batch No. 103*2, expiry date: 01.09.83) and (3) 'biosynthetic' human NPH insulin produced by recombinant DNA technology (Humulin I, Eli Lilly, Indianapolis, USA, Batch No. X290A20A, expiry date: 01.09.84). Diluting medium (Novo, Batch No. 7881, expiry date: 01.09.83) was used in the control studies. Determinations of insulin content of the three insulin preparations were carried out using a quantitative high pressure liquid chromatographic method: gel permeation chromatography method with porcine MC insulin as a standard. The calculated potency for porcine, semi-synthetic and biosynthetic human NPH insulins used in the study was 39.6, 40.9 and 40.5 IU/ml respectively. Other details relating to the protamine sulphate content and quantities of excipients in the three preparations and diluting medium studied are included in Table 1A and B.

Methods

All the subjects recruited were involved on six separate study days 1–2 weeks apart. Each study period commenced at 08:00 h after a 10 h fast (time – 60 min) with the siting of an IV cannula into an antecubital fossa vein connected to a slow running saline (0.154 mmol/l) infusion (approximately 1 litre/8 h). Frequent blood samples were obtained via a three-way tap. Following a basal period of 1 h, the

Table 1A. Contents of insulin preparations (1 ml U40)

	Human NPH insulins		Porcine NPH insulin
	Semi-synthetic	Biosynthetic	
Insulin ^a (IU/ml)	40.9	40.5	39.6
Protamine sulphate (mg)	0.142	0.144	0.145
Glycerol (mg)	16	16	16
Metacresol (mg)	1.5	1.6	1.5
Phenol (mg)	0.65	0.72	0.65

^a Determined by quantitative high pressure liquid chromatography gel permeation chromatography method with monocomponent porcine insulin as standard

Table 1B. Contents of diluting medium

	Diluting medium
Sodium acetate (mg)	1.4
Sodium chloride (mg)	7.0
Methyl parahydroxybenzoate (mg)	1.0

comparative insulins or diluting medium (control) were administered in a randomized fashion by SC injection into the anterior abdominal wall mid-way between the umbilicus and anterior superior iliac spine. A Plastipak SFP 1 ml syringe (Becton-Dickinson, Brooklyn, New York, USA) was employed. The insulin preparations (1) and (2) were administered at 0.15 and 0.30 U/kg and (3) at 0.15 U/kg body weight.

During the basal (pre-injection) period, three venous blood samples were taken. Following SC injection, blood samples were taken every 30 min for 180 min and hourly thereafter up to 660 min. The blood was aliquoted into fluoride for plasma glucose determination (hexokinase), heparin for immunoreactive insulin [7] and C-peptide measurements [8]. The blood was centrifuged within 5 min of sampling and the supernatants stored at -20°C until assay. The detection limit of immunoreactive insulin was 0.022 pmol/ml and the sensitivity (SD within assay) was 0.009 pmol/ml. The detection limit for C-peptide was 0.035 pmol/ml and the sensitivity 0.015 pmol/ml. (Conversion: SI to traditional units: glucose: 1 mmol/l $\approx$ 18 mg/dl; IRI: 1 nmol/l $\approx$ 5.8 ng/ml; C-peptide: 1 nmol/l $\approx$ 3.02 ng/ml). For each individual subject all samples were measured in one assay. Human and porcine insulins reacted identically in the insulin radioimmunoassay.

During each 12 h study period, the subjects remained supine and rested in a metabolic unit; they were monitored clinically and the site of injection inspected. No smoking was allowed during the study period, exercise was limited and standardised as far as possible and the room temperature was constant at 22°C .

Statistical analysis

The results are expressed as mean $\pm$ SEM, and the comparisons were carried out using the paired Student's *t*-test. This method was preferred to analysis of variance as it does not require assumptions about variance homogeneity. The analyses were conducted on the absolute values and on the data after adjusting for the corresponding control values and pre-injection (basal) concentrations. In order to take into account the changes observed during the control day, the control day values for each subject have been subtracted from those obtained during the insulin test days. Also, to correct for the within and between subject variations during the 1 h pre-injection basal period, the resulting values were then adjusted for these differences using the mean of the three pre-injection levels for each study period.

Results

During the control day the plasma glucose concentration fell from 5.3 ± 0.12 mmol/l at -1 h to 4.6 ± 0.13 mmol/l at 11 h. Similarly, endogenous β -cell secretion was reduced over the 12 h study period as depicted by a lowering in plasma C-peptide from 0.42 ± 0.05 to 0.21 ± 0.05 pmol/ml and insulin level from 0.055 ± 0.007 to 0.028 ± 0.003 pmol/ml.

After the injection of porcine and semi-synthetic human NPH insulin at 0.15 U/kg body weight, the plasma glucose was lowered from a pre-injection level of 5.4 ± 0.1 mmol/l to a nadir of 4.3 ± 0.1 mmol/l at 4 h and 4.1 ± 0.2 mmol/l at 3 h respectively (Fig. 1A). Subsequently a short-lived recovery in plasma glucose was observed ($+0.2$ – 0.3 mmol/l) over the ensuing 2 h period, although by 11 h the plasma glucose values had reverted to 4.4 ± 0.2 and 4.1 ± 0.2 mmol/l for porcine and human insulin, respectively. There was no significant difference in the observed hypoglycaemic response to the two species of monocomponent NPH insulins (Fig. 1A). Comparison of the adjusted values, however, demonstrated a significantly greater hypoglycaemic response with semi-synthetic human NPH insulin at 6 h ($p < 0.05$) and 8 h ($p < 0.01$; Fig. 2A). Both insulins resulted in a similar reduction in the mean plasma C-peptide concentration to approximately 20% of the basal level by 5–8 h and which remained essentially unchanged up to the end of the study period (Fig. 1A). With porcine NPH insulin the plasma insulin levels increased from 0.057 ± 0.006 pmol/ml to a peak of 0.091 ± 0.011 pmol/ml at 4 h. Following semi-synthetic human NPH insulin with a similar pre-injection plasma insulin level of 0.055 ± 0.007 pmol/ml, a slightly earlier and higher mean peak level was observed at 3 h of 0.099 ± 0.010 pmol/ml (Fig. 1A). Comparison of the absolute values did not reveal any difference between porcine and human insulin, whereas the incremental insulin values were significantly higher with human insulin at 8 h ($p < 0.05$; Fig. 2A). By 6–8 h for both insulins, the mean plasma insulin concentrations had returned to basal levels.

In comparison with the low dose level the administration of both porcine and semi-synthetic human NPH insulins at the higher dose of 0.30 U/kg body weight resulted in a more rapid and greater fall in plasma glucose concentration (Fig. 1A and B). Starting from a similar pre-injection level of 5.2 ± 0.1 mmol/l, porcine and human insulin promptly lowered the plasma glucose reaching a nadir of approximately 3.5 mmol/l at 3 and 4 h, respectively (Fig. 1B). Subsequently, with both insulins, there was a small short-lived recovery in plasma glucose. There was no significant difference between the two insulins in their hypoglycaemic effect at 0.30 U/kg (Figs. 1B and 2B). A similar reduction in plasma C-peptide levels was observed with porcine and semi-synthetic human NPH insulin, with values reaching 0.05 pmol/ml by 4 h post-injection, i.e. 10% of basal

Fig. 1A–C. The mean $\pm$ SEM plasma glucose, C-peptide and insulin levels in six normal male subjects following SC porcine (Δ --- Δ) and semi-synthetic human ($\circ$ — $\circ$) NPH insulin at A 0.15 and B 0.30 U/kg body weight; C semi-synthetic ($\circ$ — $\circ$) and biosynthetic ($\bullet$ --- $\bullet$) human NPH insulin at 0.15 U/kg body weight. Diluting medium was used for control studies. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Fig. 2. The mean $\pm$ SEM plasma glucose, C-peptide and insulin concentrations in six normal male subjects. Values adjusted for corresponding control day and basal (pre-injection) values (Δ) following SC porcine (Δ --- Δ) and semi-synthetic human ($\circ$ — $\circ$) NPH insulin at A 0.15 and B 0.30 U/kg body weight; C semi-synthetic ($\circ$ — $\circ$) and biosynthetic ($\bullet$ --- $\bullet$) human NPH insulin at 0.15 U/kg body weight. * $p < 0.05$; ** $p < 0.01$

levels (Figs. 1 B and 2 B). Similar mean peak insulin levels were achieved following SC porcine and human insulin of 0.132 ± 0.026 and 0.133 ± 0.023 pmol/ml at 2 and 4 h, respectively. Thereafter the insulin levels fell more rapidly with porcine insulin reaching a value of 0.067 ± 0.006 pmol/ml by the end of the study. The mean plasma insulin concentration with human insulin at 11 h post-administration was higher at 0.085 ± 0.012 pmol/ml.

Although there was a trend towards higher plasma insulin levels following human insulin from 4 h onwards there was no significant difference in the absolute or adjusted values (Figs. 1 B and 2 B).

There was a significant dose-related effect on plasma glucose, C-peptide and insulin levels with both porcine and semi-synthetic human NPH insulin.

Biosynthetic human NPH insulin at the 0.15 U/kg dose level lowered the plasma glucose from a pre-injection level of 5.3 ± 0.1 to 4.3 ± 0.2 mmol/l by 4 h, with the values remaining between 4.4 ± 0.1 and 4.5 ± 0.1 mmol/l until the end of the study (Fig. 1 C). The plasma glucose lowering effect of biosynthetic human NPH insulin was significantly less than for semi-synthetic human insulin at 4 and 8 h ($p < 0.05$; Fig. 1 C). This tendency was confirmed in the comparison of the adjusted data, demonstrating a significantly greater hypoglycaemic response with the semi-synthetic human NPH insulin at 8 h ($p < 0.01$) and 9 h ($p < 0.05$; Fig. 2 C).

The plasma C-peptide level was reduced from 0.43 ± 0.005 pmol/l to a nadir of 0.12 ± 0.02 pmol/ml at 7 h after injection of biosynthetic human NPH insulin. The levels observed were significantly higher than after semi-synthetic human insulin from 5–10 h ($p < 0.05$ – 0.001 ; Fig. 1 C), although the comparison of the adjusted data did not confirm this difference (Fig. 2 C). Following the administration of biosynthetic human NPH insulin at 0.15 U/kg, the plasma insulin levels increased from 0.057 ± 0.006 pmol/ml at time 0 h to a peak mean level of 0.099 ± 0.055 pmol/ml at 2.5 h (Fig. 1 C). By 9 h after injection, the mean insulin concentration had reached pre-injection levels. The plasma insulin profile was similar for both human insulin preparations although the level was significantly higher ($p < 0.05$) with semi-synthetic human NPH insulin at the 10 h timepoint only (Fig. 1 C). Comparison of the adjusted data revealed that a significantly higher insulin level was achieved with biosynthetic human NPH insulin at 2.5 h ($p < 0.05$; Fig. 2 C). Thereafter from 4 h onwards the mean incremental values were higher with semi-synthetic human NPH insulin, the difference reaching significance at 10 h ($p < 0.05$; Fig. 2 C).

Discussion

It is only in the last decade that limited data on plasma insulin levels following the subcutaneous administration of NPH (isophane) insulin preparations have be-

come available [9–14]. The use of plasma insulin measurements to characterize the pharmacokinetics of NPH insulins assumes that no protamine-zinc-insulin complexes are absorbed into the circulation. Bauman and Yalow have however demonstrated, using gel filtration, that some complexed insulin is in fact absorbed into the circulation [15].

Galloway et al. [14] did not demonstrate a difference in the hypoglycaemic response to purified porcine NPH and biosynthetic human NPH insulin in normal subjects at 0.15 U/kg body weight when administered subcutaneously in the deltoid area. The only difference observed was a higher serum insulin concentration following human insulin at 4 h after injection [16].

Analysis of the observed glucose response to porcine and semi-synthetic human NPH insulin in our study agrees with this impression. In contrast to previous investigation, our study design also allows the results to be corrected for the effect of prolonged fasting as seen during the control day. Using the corrected values and deriving the difference from the basal pre-injection concentrations demonstrates a significantly greater hypoglycaemic effect of human insulin between 6 and 8 h after administration at the 0.15 U/kg dose level. This was associated with a significantly higher plasma insulin level at 8 h with human insulin. There was however, no difference in the degree of C-peptide suppression following the two insulins. Weinges et al. [17] using the Gerritzen test method in normal subjects observed a tendency towards a faster hypoglycaemic effect with biosynthetic human NPH insulin compared to purified porcine NPH insulin at a dose of 20 U subcutaneously. In our study at a higher dose level of 0.30 U/kg (22–24 U), the hypoglycaemic response to porcine and semi-synthetic human insulin was identical. There was however a trend for higher insulin levels with human insulin from 3 h after administration to the end of the study period. Bottermann et al. [18] using the euglycaemic clamp technique also demonstrated a significantly higher plasma insulin level and dextrose infusion rate with human compared to porcine insulin. The evidence therefore suggests better bioavailability of human than porcine insulin from subcutaneous depot, possibly due to the greater hydrophilicity of human insulin [4, 5, 19].

Interestingly, comparing semi-synthetic and biosynthetic human NPH insulins at 0.15 U/kg, we observed a significantly greater hypoglycaemic effect with the former. Whilst a similar glucose nadir was achieved with both insulins at 3 h after administration, a quicker recovery towards normoglycaemia was seen with biosynthetic human NPH insulin. An extended observation period would be necessary to see if this trend persisted. The small differences in the protamine content is an unlikely explanation for the differences observed between the two human NPH insulins. The proportion of protamine insulin complexes absorbed may however have been different between the insulins, although this was not measured in this study. Recent clinical findings

also suggest a trend towards a relatively shorter duration of blood glucose lowering effect with biosynthetic human NPH insulin in contrast to porcine or bovine NPH insulin preparations [20].

Our findings underline the importance of both insulin species and pharmaceutical formulation on the pharmacokinetic and pharmacodynamic profile of the NPH (isophane) insulin preparations examined.

According to the experimental procedure adopted involving normal fasting subjects the onset of hypoglycaemic action of the NPH insulins was evident within the first hour, the maximum effect observed between 3 and 4 h with a duration in excess of 11 h. However, such idealized time-action characterization can only be of limited value in the clinical situation. Critical clinical evaluation is necessary to assess the relevance of the small differences observed between semi-synthetic and biosynthetic human NPH insulin.

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