PHARMACOKINETICS AND DISPOSITION

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Pharmacokinetics and pharmacodynamics of a premixed formulation of soluble and protamine-retarded insulin aspart

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Abstract Objective: With the aim to obtain a premixed rapid-acting insulin with a serum insulin profile more closely resembling the endogenous meal-stimulated serum insulin profiles, a 30/70 (rapid/intermediate-acting) premixed suspension of the rapid-acting insulin analogue insulin aspart (BIAsp30) was compared with a similar premixed suspension of biphasic human insulin 30/70 (BHI30) after a single subcutaneous injection.

Methods: The study had a randomised, double-blind, two-period crossover design. Twenty-four healthy male subjects received a single subcutaneous dose of either 0.2 U·kg⁻¹ bodyweight of BIAsp30 or BHI30 on two study days.

Results: BIAsp30 was absorbed faster than BHI30, as reflected in the area under the insulin concentration-time curve from 0 to 90 min after dosing [AUC(0-90 min)]. This was significantly larger for BIAsp30 than for BHI30 (1403 ± 372 versus 752 ± 191 mU·l⁻¹·min⁻¹ [mean ± SD]; P < 0.0001). Furthermore, the time to maximum serum insulin concentration (t_max) of BIAsp30 was approximately half the t_max of BHI30 (60 [45–70] versus 110 [90–180] min [median, interquartile range]; P=0.0001) and the maximum insulin concentration (C_max) was significantly higher for BIAsp30 than for BHI30 (23.4 ± 5.3 versus 15.5 ± 3.7 mU·l⁻¹ [mean ± SD]; P < 0.0001). The serum glucose profiles showed a significantly earlier onset of the glucose-lowering effect following BIAsp30 than following BHI30.

Conclusions: The improved absorption properties of soluble insulin aspart in its premixed formulation provide a basis for a more efficient meal-related glucose control and immediate pre-meal delivery when compared with a similar human premixed insulin in the treatment of diabetes mellitus.

Key words Biphasic insulin aspart 30 · Premix · Insulin analogue

Introduction

Premixed insulins are widely used for the treatment of both type-1 and type-2 diabetes mellitus. The main advantage with a premixed insulin is that fewer injections are required per day for reasonably effective diabetic control in patients who will not or cannot be treated with more complex dosing regimens. A premixed human insulin containing 30% soluble insulin and 70% protamine-retarded or NPH insulin [30/70 premixed insulin or biphasic human insulin 30 (BHI30)] has empirically been found to control blood glucose throughout the day when administered before the morning and evening meals.

Following subcutaneous injection, absorption of soluble human insulin is delayed due to the slow dissociation of insulin hexamers into dimers and monomers, and maximum insulin concentrations are reached within 1.5–2 h. This does not mimic the normal physiological insulin response to a meal [1–4], and it is therefore recommended that soluble human insulin (administered alone or as a premixed insulin) is administered approximately 30 min before a meal to compensate for the delay.

In insulin aspart, proline at position 28 of the B chain of the insulin molecule is replaced by aspartate. This substitution facilitates the dissociation of the hexameric insulin complexes at high concentrations [5]. Preclinical and clinical studies have shown that soluble insulin aspart is twice as fast as unmodified soluble human insulin and has a shorter duration of action [5–11].

A premixed insulin aspart was developed with a soluble fraction of 30% and a protamine-crystallised fraction of 70% [biphasic insulin aspart 30 (BIAsp30)]. Incorporation of insulin aspart into a premixed formulation should combine the advantages of the rapid-acting analogue with the advantages of a premixed
formulation, i.e. a combination of a rapidly absorbed insulin and a fraction with a longer duration of action. Therefore, the pharmacokinetics and pharmacodynamics of BIAsp30 were compared with a similar premix of human insulin (BHI30) in healthy volunteers.

Subjects and methods

Subjects

Twenty-four subjects took part in this single-centre, randomised, double-blind, two-period crossover trial carried out at the Covance Clinical Research Unit, Leeds, England. Subjects were all healthy, non-smoking males aged 21–38 years with a body mass index of 27 kg·m⁻² or less. Twenty-three were Caucasian and one was Asian. Subjects were excluded if they took any concomitant medication or suffered any concurrent illness and if they had a first-degree relative with diabetes mellitus. All subjects gave written informed consent.

The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice and was approved by the local ethics review board.

Trial procedure

After an initial screening visit, subjects eligible for the trial were randomly assigned to a trial drug sequence. Block randomisation with a block size of four was used. On each of the two study days, 4–10 days apart, each subject received a single subcutaneous injection of BIAsp30 (0.2 U·kg⁻¹ bodyweight) or BHI30 (0.2 IU·kg⁻¹ bodyweight). Both insulins were 100 I.U·mL⁻¹ and were administered using a NovoPen 1.5 (Novo Nordisk A/S, Copenhagen, Denmark).

Following a high-carbohydrate meal at 2100 hours on the day before dose administration, subjects fasted until the end of the 24-h blood sampling period. One of the two test insulins was administered on the morning of the study day. Blood samples were collected for the determination of serum insulin, C-peptide and glucose profiles at the following time points: 30 min before dosing, immediately before dosing and at 15, 30, 45, 60, 70, 80, 90, 100, 110, 120, 150, 180, 210, 240, 270, 300, 360, 420, 450, 540, 600, 720, 840, 960, 1200 and 1440 min after dosing. A follow-up visit was performed 4–10 days after the second study day.

Pharmacokinetic and pharmacodynamic assessments

The endpoints chosen represented the soluble fractions of the insulin (i.e. the early part of the profile) and the protamine-retarded fraction (i.e. the later part of the profile). The primary endpoint was the area under the insulin concentration curve for the first 90 min after injection [AUC(0–90 min)]. Other endpoints included the maximum insulin concentration (C_max), time of maximum insulin concentration (t_max) and AUC from 6–24 h after dosing [AUC(6–24 h)]. AUC(0–90 min) and AUC(6–24 h) were calculated using the trapezoidal rule [12].

Serum insulin and C-peptide levels were measured by Medi-Lab A/S (Copenhagen, Denmark). Insulin was measured by standard radiimmunoassay using the Pharmacia insulinRIA 100 kit (Pharmacia, Uppsala, Sweden). C-peptide was measured using the DAKO C-peptide enzyme-linked immunosorbent assay (ELISA). The C-peptide levels were used to correct for endogenous insulin using the basal insulin:C-peptide ratio to obtain a corrected exogenous insulin profile:

I_ex(t) = I_ex(t) (1 – (C(t) I_t < 0) / C(t) < 0)

where I_ex(t) is the exogenous insulin concentration at time t post-injection; I_ex(t) is the total insulin concentration at time t post-

injection; C(t) is the C-peptide concentration at time t; I_t < 0 is the initial endogenous insulin concentration; and C(t) < 0 is initial C-peptide concentration [13].

The Pharmacia insulin assay kit does not completely cross-react with insulin aspart. Therefore, after extensive validation (data unpublished), the following correction formula was applied to calculate the corrected insulin aspart concentration [10]:

Insulin aspart_{corrected} = F \times (1503 \times \text{insulin aspart}_{fraction}) / (1398 – \text{insulin aspart}_{fraction})

where F denotes the dilution factor and insulin aspart_{fraction} is in picomoles per litre as is the diluted assay result.

The endpoints derived from the serum glucose profile were the minimum glucose concentration in the first 6 h [C_{min(0–6 h)}] and the time of minimum glucose concentration [t_{min(0–6 h)}]. Serum glucose levels were measured by Medi-Lab A/S using the glucose oxidase method.

Safety assessments

Adverse events were recorded during the trial period. For each adverse event, the severity of the event and its relationship to the trial product were recorded.

Statistical methods

With a significance level of 5% and a standard deviation of 10 mU·l⁻¹·h⁻¹, a sample size of 24 subjects ensured that the trial had an 80% chance of detecting a true difference of 9 mU·l⁻¹·h⁻¹ between the two insulin preparations. The endpoints were subjected to analysis of variance (ANOVA), with treatment as a fixed effect and subjects as a random effect. All endpoints, except t_{max} and t_{min}, were log-transformed before analysis. Treatment comparisons were presented by an estimated treatment mean ratio, a P value and a 95% confidence interval for the ratio. The time endpoints were analysed using the Wilcoxon signed rank test, and treatment comparison for these endpoints was presented as an estimated median treatment difference (Hodges-Lehmann estimate) with a P value and a non-parametric 95% confidence interval for the difference. All analyses were made as within-subject comparisons with a significance level of 5%. Statistical analyses were conducted using the Statistical Analysis System (SAS) version 6.09 for UNIX (Statistical Analysis Systems, SAS Institute, Raleigh, NC, USA), and linear models were analysed using PROC MIXED.

Results

Of the 24 subjects that entered, 23 completed the study; one was withdrawn due to non-compliance with the trial protocol (positive drug abuse screen) before the second drug dose.

Pharmacokinetics

The mean serum insulin profiles are presented in Fig. 1. The insulin concentration following BIAsp30 increased faster than after BHI30, as displayed by the AUC(0–90 min), which was approximately 1.8 times larger (Table 1). Following BIAsp30, higher insulin concentrations were reached at an earlier time point, with C_max being 1.5-fold higher than and t_max occurring almost twice as fast with BIAsp30 as BHI30 (Table 1). These differences were statistically significant and support the faster absorption of the soluble fraction of BIAsp30 than.