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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 $U \cdot kg^{-1}$ 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 $mU \cdot l^{-1} \cdot min^{-1}$ [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 $mU \cdot l^{-1}$ [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.

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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 $kg \cdot m^{-2}$ 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 $U \cdot kg^{-1}$ bodyweight) or BHI30 (0.2 $IU \cdot kg^{-1}$ bodyweight). Both insulins were 100 (I)$U \cdot ml^{-1}$ 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, 480, 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 radioimmunoassay using the Pharmacia insulin RIA 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:

$$I_{ex(t)} = I_{tot(t)} - (C_{(t)} I_{(t \le 0)} / C_{(t \le 0)})$$

where $I_{ex(t)}$ is the exogenous insulin concentration at time t post-injection; $I_{tot(t)}$ is the total insulin concentration at time t post-injection; $C_{(t)}$ is the C-peptide concentration at time t; $I_{(t \le 0)}$ is the initial endogenous insulin concentration; and $C_{(t \le 0)}$ 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]:

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

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 $mU \cdot l^{-1} \cdot h^{-1}$, a sample size of 24 subjects ensured that the trial had an 80% chance of detecting a true difference of 9 $mU \cdot l^{-1} \cdot h^{-1}$ 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

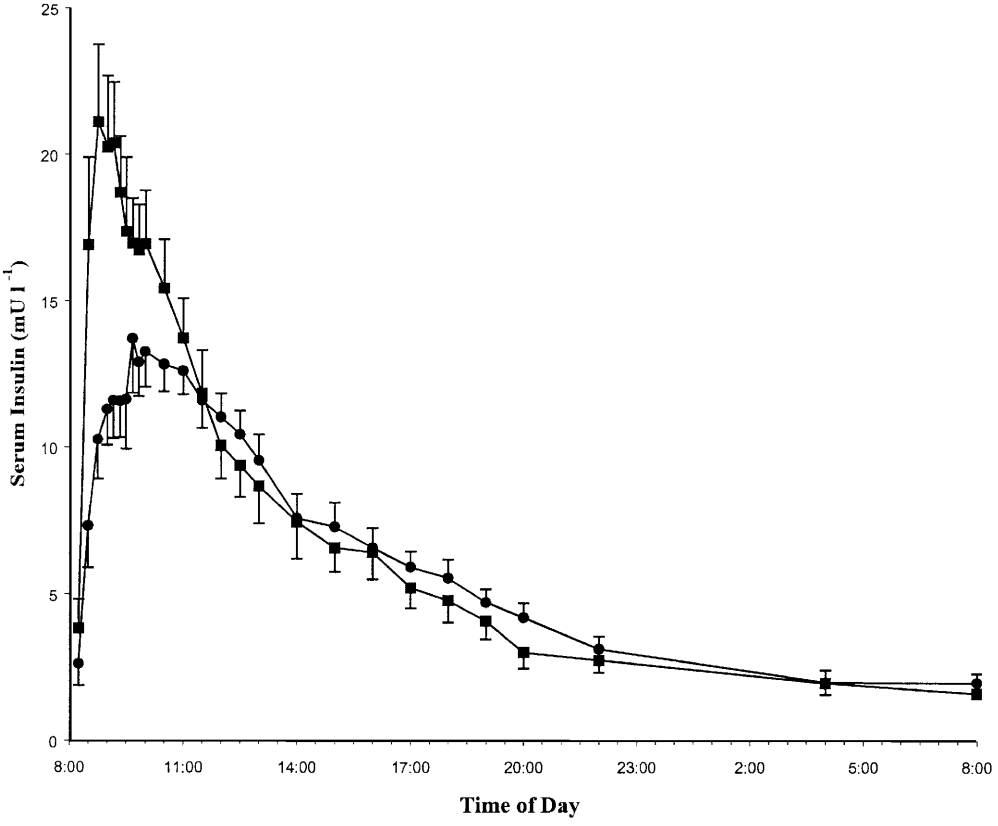

Fig. 1 Mean (±2 SEM) serum insulin profiles in 23 healthy volunteers after a single subcutaneous injection of either biphasic insulin aspart 30 (0.2 $U \cdot kg^{-1}$-bodyweight) or biphasic human insulin 30 (0.2 $IU \cdot kg^{-1}$ bodyweight). *Squares* biphasic insulin aspart 30; *circles* biphasic human insulin 30

that of the soluble fraction of BHI30, as indicated by the primary endpoint. There was no difference between BIAsp30 and BHI30 for $AUC_{(6–24\ h)}$, indicating that there was no difference in absorption between the two protamine-retarded insulins (Table 1). The estimated bioavailability of BIAsp30 relative to BHI30 was 1.04 (95% CI 0.90; 1.20), indicating similar bioavailability of the two insulins. The estimated half-lives were 511 min (384–1057 min) for BIAsp30 and 449 min (363–844 min) for BHI30 [harmonic mean (interquartile range)].

Pharmacodynamics

The mean serum glucose profiles are presented in Fig. 2. The faster absorption of the soluble fraction of BIAsp30 was reflected in the earlier onset of the serum glucose-lowering effect. The pharmacodynamic endpoints, presented in Table 2, show that the $C_{min(0–6\ h)}$ was significantly lower and occurred significantly earlier for BIAsp30 than for BHI30.

Safety

A total of 80 adverse events were reported during this trial, but none of these were serious. There were no differences in relative numbers or drug relation of adverse events between the two treatments. Hypoglycaemic symptoms accounted for the majority of adverse events and were equally distributed between the two study groups.

Table 1 Pharmacokinetic endpoints after injection of biphasic insulin aspart (BIAsp30) or biphasic human insulin (BHI30). The premixed insulins were 30/70 soluble/protamine-retarded. Estimates are means of the ratio BIAsp30/BHI30, except for t_{max}, where the median of the difference BIAsp30-BHI30 is presented. NS not significant; *95%CI* 95% confidence interval; *AUC* area under the insulin concentration curve; C_{max} peak serum concentration; t_{max} time to maximum serum insulin concentration

Pharmacokinetic parameter	BIAsp30 Mean ±SD	BHI30 Mean ±SD	*P* value	Estimated ratio	95% CI
$AUC_{(0–90\ min)}$ (mU l^{-1} min^{-1})	1403 ± 372	752 ± 191	< 0.0001	1.85	1.66, 2.07
C_{max} (mU l^{-1})	23.4 ± 5.3	15.5 ± 3.7	< 0.0001	1.51	1.35, 1.69
t_{max} (min)	60 (45–70)[a]	110 (90–180)[a]	0.0001	−60	−77.5, −42.5
$AUC_{(1.5–6\ h)}$ (mU l^{-1} h^{-1})	53.7 ± 12.2	49.7 ± 7.7	NS	1.07	0.96, 1.19
$AUC_{(6–24\ h)}$ (mU l^{-1} h^{-1})	65.5 ± 21.4	71.3 ± 12.1	NS	0.89	0.76, 1.03
$AUC_{(0–24\ h)}$ (mU l^{-1} h^{-1})	142 ± 31.5	134 ± 15	NS	1.05	0.95, 1.15

[a] Results are presented as the median (interquartile range)

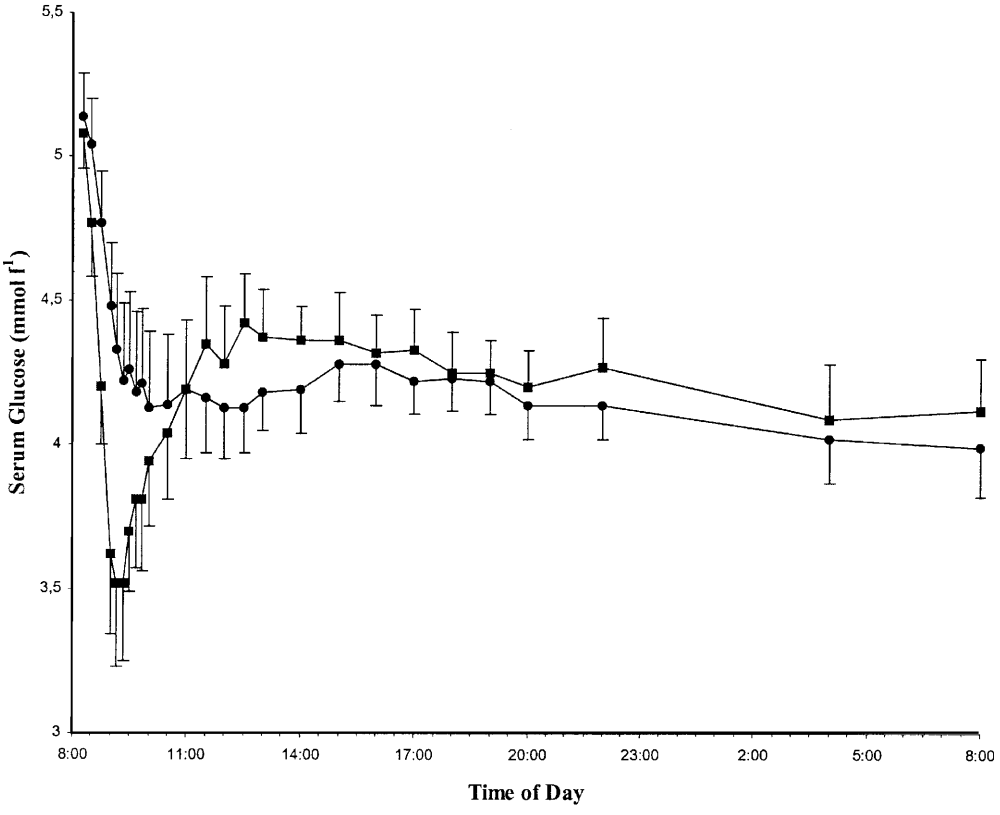

Fig. 2 Mean (±2 SEM) serum glucose profiles in 23 healthy volunteers after a single subcutaneous injection of either biphasic insulin aspart 30 (0.2 $U \cdot kg^{-1}$ bodyweight) or biphasic human insulin 30 (0.2 $IU \cdot kg^{-1}$ bodyweight). *Squares* biphasic insulin aspart 30; *circles* biphasic human insulin 30

Discussion

It is now universally recognised that tight metabolic control is the key to reducing late adverse effects of diabetes, both type-1 [14] and type-2 diabetes [15]. This is best achieved by multiple daily pre-meal injections to cover the meal-related need, and slow-release injections to cover the basal need. However, some patients opt for simpler treatment regimens. Further, many type-2 patients have a residual insulin secretion which, together with one or two injections per day, may provide sufficient blood sugar control.

Soluble insulin aspart has been demonstrated in clinical trials to produce peak insulin concentrations that are more than twice as high as soluble human insulin [9, 10, 11, 16, 17]. Furthermore, t_{max} was half or less than half that of soluble human insulin. Duration of concentration and action were shorter for soluble insulin aspart than for soluble human insulin.

Premixed insulin aspart may provide a ready-made combination of a rapid-acting insulin analogue along with an intermediate-acting insulin, and the present study is the first to characterise the pharmacokinetics of premixed insulin aspart over a period of 24 h. The endpoints were chosen to demonstrate differences in the rapidly absorbed fraction of premixed human insulin [$AUC_{(0–90\ min)}$] and in the more slowly absorbed fraction [$AUC_{(6–24\ h)}$].

The pharmacokinetics of BIAsp30 demonstrated a faster absorption of the rapid-acting component soluble insulin aspart than the soluble short-acting component of BHI30. C_{max} for BIAsp30 was approximately 50% higher than for BHI30 and occurred about 1 h earlier. The difference in peak concentration was slightly less for BIAsp30 in relation to BHI30 than for soluble insulin aspart in relation to soluble human insulin [10]. This is not surprising as NPH-insulin peaks between 2 h and 6 h and contributes to C_{max}. In addition, the difference in time to reach C_{max} for BIAsp30 compared with

Table 2 Serum glucose endpoints after injection of biphasic insulin aspart (BIAsp30) or biphasic human insulin (BHI30). The premixed insulins were 30/70 soluble/protamine-retarded. Estimates are means of the ratio BIAsp30/BHI30, except for t_{min} where the median of the difference BIAsp30-BHI30 is presented. *95%CI* 95% confidence interval; C_{min} minimum glucose concentration; t_{min} time of minimum glucose concentration

Pharmacodynamic parameter	BIAsp30 Mean ±SD	BHI30 Mean ±SD	*P* value	Ratio	95% CI
$C_{min(0–6\ h)}$ ($mmol \cdot l^{-1}$)	3.2 ± 0.5	3.7 ± 0.5	< 0.001	0.86	0.80, 0.93
$t_{min(0–6\ h)}$ (min)	70 (70–80)[a]	180 (100–300)[a]	0.0001	−90	−135, −45

[a] t_{min} results are presented as the median (interquartile range)

soluble insulin aspart (60 min versus 40 min) in healthy subjects [10] could also be due to the NPH-fraction in the pre-mixed formulation.

The rapid absorption of BIAsp30 means that the insulin may be administered immediately before meals instead of 30 min before meals as currently recommended for human insulin and premixed human insulin. The peak concentration following subcutaneous injection of BIAsp30 will mimic the desired endogenous serum insulin profile more closely, and this should result in an improved post-prandial glucose control [2, 3, 4, 17, 18]. Although the present study design is not the most suitable for the evaluation of glucose effects after an insulin injection, it was shown that the hypoglycaemic activity of BIAsp30 occurred earlier and was more pronounced than BHI30. However, the serum glucose responses that occur late after injection should be treated with caution since counter-regulatory responses will be prominent in a study of healthy volunteers.

The intermediate-acting component of BIAsp30 represented by $AUC_{(6–24\ h)}$ was shown to be similar to that of BHI30. Bioavailability of soluble insulin aspart was previously demonstrated to be similar to that of soluble human insulin [10, 19]. Results of the present trial indicate that the bioavailability of BIAsp30 is similar to that of BHI30. Half-lives were also comparable between the two insulin preparations. These findings indicate that BIAsp30 has a duration of concentration long enough to be adequate as a twice daily insulin. In a treatment situation, this would possibly provide improved postprandial glucose control after the breakfast and dinner injections.

In conclusion, the present study demonstrated a faster absorption of the soluble component of BIAsp30 than of BHI30, producing an earlier and more pronounced glucose response, whereas the intermediate-acting component was similar between the two insulins. These results are encouraging and the potential improvement in glucose control should be investigated further in trials including diabetic subjects.

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