Comparative pharmacokinetics and pharmacodynamics of the novel rapid-acting insulin analogue, insulin aspart, in healthy volunteers

Abstract Objective: The pharmacokinetics of a new insulin analogue, insulin aspart, were compared with unmodified human insulin in a double-blind crossover study of 25 fasting healthy men following a single subcutaneous dose.

Methods: Either insulin aspart or human insulin, 0.1 U·kg-body-weight$^{-1}$, was injected subcutaneously and followed by determination of 8-h profiles of serum insulin and plasma glucose concentrations.

Results: The absorption of insulin aspart was, on average, more than twice as fast and reached levels more than twice as high compared with human insulin [t$_{\text{max(ins)}}$ of 52 (23) vs 145 (93) min, $P < 0.0001$; and C$_{\text{max(ins)}}$ of 41 (11) vs 18 (4) mU·l$^{-1}$, $P < 0.0001$; mean with (SD)]. However, total bioavailability did not differ between the insulins, and thus the mean residence time was significantly shorter for insulin aspart [MRT$_{\text{ins}}$ of 149 (26) vs 217 (30) min, $P < 0.0001$]. Plasma glucose (PG) fell more than twice as rapidly [t$_{\text{min(PG)}}$ of 94 (45) vs 226 (120) min, $P < 0.0001$], to a greater extent [C$_{\text{min(PG)}}$ 2.1 (0.6) vs 1.4 (0.4) mmol·l$^{-1}$, $P < 0.0001$], and for a shorter duration with insulin aspart than with human insulin.

Conclusion: With improved subcutaneous absorption characteristics, the insulin aspart concentration–time profile resembles physiological meal-stimulated insulin release more closely than that of unmodified human insulin. This significantly alters the pharmacodynamic response in an advantageous manner in the meal-related treatment of diabetes mellitus.

Key words Insulin aspart · Insulin absorption · Rapid-acting insulin analogue

Introduction

Despite recommendations for the subcutaneous injection of unmodified human insulin some 30 min before a meal, postprandial plasma glucose (PG) levels are not well controlled in patients with diabetes. The circulating insulin level rises comparatively slowly, reaching a peak only after 1.5–2 h and declining only slowly – a situation that does not mimic the normal physiological response to the ingestion of a meal [1, 2].

Self-association of the insulin molecules to form hexamers [3] is thought to be a major reason for the delay in absorption from subcutaneous tissues into the systemic circulation; the rate of absorption is inversely correlated with the degree of self-association [4, 5]. In insulin aspart, proline at position 28 of the B chain of the insulin molecule was replaced by aspartate, discouraging hexamer formation. The resulting analogue has receptor affinity, receptor association and dissociation rates, and in vivo potency similar to human insulin [1, 6–8]. Insulin-like growth factor 1 activity is also similar to human insulin [1, 7]. In animal studies and early clinical studies, insulin aspart was absorbed faster from the subcutaneous injection site than unmodified human insulin [1, 6–9]. A pilot clinical study in healthy humans under euglycaemic glucose clamp conditions showed that insulin aspart had a faster onset of action than human insulin. Moreover, insulin aspart and human insulin appeared to be equipotent in terms of their PG lowering effect [10].

The primary objective of the present study was to compare the serum insulin profile of insulin aspart with that of human insulin in non-diabetic humans to ascertain whether the analogue is absorbed faster after subcutaneous injection and how its duration of action compares with that of unmodified human insulin. The
secondary objective was to compare the efficacies of the two insulins as indicated by the PG profiles.

Subjects and methods

Subjects and study design

Twenty-five subjects were enrolled and 19 completed the study. All 25 were healthy, non-smoking male Caucasians, aged 19–50 years, with a body mass index below 30.0 kg·m⁻², and a fasting PG below 6.0 mmol·l⁻¹. All subjects gave written informed consent. The trial protocol and the consent form were reviewed by the local ethics committee and the trial was carried out in accordance with Good Clinical Practice (GCP) [11].

The trial had a randomized double-blind, crossover design in which each subject acted as his own control. On the first study day, after an overnight fast, each subject received a single dose of 0.1 U·kg-body-weight⁻¹ of either insulin aspart or unmodified human insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark). On the second study day, 1–3 weeks later, subjects received the alternative insulin at the same dose.

Blood samples were collected 10 min before insulin injection, every 15 min until +20 min; then every 10 min until +90 min, every 15 min to +150 min, every 30 min to +240 min and every 60 min to +480 min. The subjects were fasting during the 8-hour sampling period after which they were given a carbohydrate meal, and were subsequently discharged. The subjects returned 1–3 weeks after the second study day for a post-trial examination. The insulin aspart and human insulin cartridges (Penfill, Novo Nordisk A/S) and pen injectors (Novopen II, Novo Nordisk) were identical so as the study design was carried out in accordance with Good Clinical Practice (GCP) [11].

Pharmacokinetic and pharmacodynamic assessments

Venous blood samples were analysed for serum insulin and C-peptide concentrations, and the C-peptide values were used to correct for endogenous insulin using the basal-insulin:C-peptide ratio to obtain a corrected endogenous insulin profile:

\[ I_{\text{ex}(t)} = I_{\text{tot}(t)} - (C(t) \cdot I_{(t \leq 0)}/C_{(t \leq 0)}) \]

where \( I_{\text{ex}(t)} \) is the endogenous insulin concentration at time \( t \) post-injection, \( I_{\text{tot}(t)} \) is the total insulin concentration at time \( t \) post-injection, \( C(t) \) is the C-peptide concentration at time \( t \); \( I_{(t \leq 0)} \) is the initial endogenous insulin concentration; and \( C_{(t \leq 0)} \) is initial C-peptide concentration [12]. This method assumes equivalent plasma clearance of insulin and C-peptide, while in practice that of the latter is 4–6 times longer. As a result the contribution of endogenous insulin will be overestimated when secretion is falling, and underestimated when rising, the degree of error varying with its rate of change. Total area under curve between two steady states will not, however, be affected.

Serum insulin was determined using a standard radioimmunoassay kit from Pharmacia (Uppsala, Sweden). This assay 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:

Insulin aspart \(_{\text{corrected}}\) = \( F \times (1503 \times \text{insulin aspart}_{\text{traction}})/(1138 - \text{insulin aspart}_{\text{traction}}) \)

where \( F \) denotes the dilution factor and \( \text{insulin aspart}_{\text{traction}} \) is in pmol·l⁻¹ as is the diluted assay result.

Endpoints derived from the serum insulin profiles were the mean residence time (MRT\(_{\text{ins}}\)), maximum serum insulin concentration (C\(_{\text{max}(\text{ins})}\)), the time of maximum serum insulin concentration (t\(_{\text{max}(\text{ins})}\)) and the area under the insulin concentration-time curve (AUC\(_{\text{ins}}\)). All endpoints were calculated from the time of insulin administration (\( t = 0 \) min) to the last measured time point (\( t = 480 \) min). The terminal elimination rate constant (\( \lambda_{z(\text{ins})} \)) and the apparent absorption half-life (t\(_{1/2(\text{ins})}\)) for each insulin preparation after subcutaneous administration were estimated by non-compartmental pharmacokinetic analysis using WinNonlin software version 1.1 (Scientific Consulting, Cary, N.C., USA).

MRT\(_{\text{ins}}\) was calculated from:

\[ \text{MRT}_{\text{ins}} = \frac{\text{AUMC}_{\text{ins}}}{\text{AUC}_{\text{ins}}} \]

where AUMC is the area under the statistical moment curve and AUC is the area under the concentration-time curve, calculated by the trapezoidal rule [13]. The apparent terminal t\(_{1/2}\) and terminal elimination rate constant were calculated as the slope of the terminal linear part of the ln(conc) versus time curve. The area under the serum insulin concentration-time curve from time 0 min to infinity (AUC\(_{0-\infty(\text{ins})}\)) was estimated using the equation:

\[ \text{AUC}_{0-\infty(\text{ins})} = \text{AUC}_{0-\infty} + C_{\text{p}} \cdot \lambda_z \]

where \( C_{\text{p}} \) is the estimated concentration at time \( t_\infty \) and \( \lambda_z \) is the estimated terminal rate constant.

The relative bioavailability of insulin aspart (IAsp) versus human insulin (HI) [\( F(\text{AUC}) \)] was derived as the ratio:

\[ F(\text{AUC}) = \frac{\text{AUC}_{0-\infty(\text{IAsp})}}{\text{AUC}_{0-\infty(\text{HI})}} \]

where \( \text{AUC}_{0-\infty} \) is the area under the serum concentration-time curve from time 0 min to infinity.

PG levels were measured at the Bioanalytical Research Corporation (BARC), Gent, Belgium, using the hexokinase method on fluoride plasma. Endpoints derived from the PG profiles were the negative excursions of glucose as assessed as the area below the baseline plasma glucose level and above the glucose concentration-time curve (EXC\(_{\text{PG}}\)), the maximum change in plasma glucose concentration (\( \Delta \text{C}_{\text{min(PG)}} \)), defined as PG₀ – C\(_{\text{min(PG)}} \), and the time (t\(_{\text{min}(\text{PG})}\)) when \( \Delta \text{C}_{\text{min(PG)}} \) first occurs.

Statistical methods

With a significance level of 5%, a sample size of 20 subjects ensured that the trial had an 80% chance of detecting a true relative difference between the insulin preparations. MRT\(_{\text{ins}}\), C\(_{\text{max}(\text{ins})}\), AUC\(_{\text{ins}}\) and EXC\(_{\text{PG}}\) were logarithmically transformed and then analysed by ANOVA with subject as a random effect and treatment condition as a fixed effect. Median differences in t\(_{\text{max}(\text{ins})}\) and t\(_{\text{min}(\text{PG})}\) were compared by the Wilcoxon Signed Rank test using the Hodges-Lehmann approach. All tests were made as within-subject comparisons at the 5% significance level. Statistical analyses were made using SAS for UNIX, version 6.0 (Statistical Analysis Systems, SAS Institute, Raleigh, N.C., USA).

Results

One subject was withdrawn from the trial due to a protocol violation and a further five subjects were excluded as complete profiles were not recorded while taking insulin aspart due to hypoglycaemia. These subjects could therefore not act as their own controls. The maximum serum concentration of insulin aspart (C\(_{\text{max}(\text{ins})}\)) was significantly higher than for human insulin [41 (11) vs 18 (4) mU·l⁻¹, \( P < 0.001 \)], while the time taken to reach this concentration (t\(_{\text{max}(\text{ins})}\)) was significantly shorter [52 (23) vs 145 (93) min, \( P < 0.001 \); Fig. 1]. MRT\(_{\text{ins}}\) was significantly shorter for insulin aspart than for human insulin, indicating a shorter residence time for insulin aspart in subcutaneous tissue (Table 1). The harmonic apparent terminal half-lives, t\(_{1/2(\text{ins})}\), for insulin aspart and human insulin were