**PHARMACOKINETICS OF THE RAPID-ACTING INSULIN ANALOG, INSULIN ASPART, IN RATS, DOGS, AND PIGS, AND PHARMACODYNAMICS OF INSULIN ASPART IN PIGS**

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(Received March 12, 1999; accepted August 12, 1999)

This paper is available online at http://www.dmd.org

**ABSTRACT:**

The objective of this study was to compare the pharmacokinetics and pharmacodynamics of insulin aspart (IA), a rapidly acting insulin analog, with those of human soluble (regular) insulin (HI) in animal models after s.c. and i.v. dosing. Single doses of IA and HI were administered i.v. and s.c. to rats and dogs at three dose levels, and at one dose level to pigs; rats and dogs also underwent repeated s.c. dosing for 1 week. Plasma insulin levels were assessed at predetermined time points after dosing; plasma glucose levels were measured in pigs only. There were no significant pharmacokinetic differences between IA and HI after a single s.c. or i.v. dose in rats or dogs, and no differences were observed after repeated s.c. dosing, implying there was no accumulation. In pigs, there was a strong trend toward more rapid absorption of IA compared with HI after s.c. dosing, whereas there were no differences after i.v. administration. After s.c. dosing in pigs, IA produced significantly lower plasma glucose levels compared with HI during the period 30 to 75 min after dosing (P < .05). In conclusion, IA was more rapidly absorbed than HI after s.c. administration only in the pig; this difference was reflected in earlier and more pronounced effects on plasma glucose levels.

The goal of insulin therapy in patients with type I diabetes mellitus is to mimic the pattern of endogenous insulin secretion seen in healthy individuals, characterized by a relatively constant basal level with sharp peaks after meals (Zinman, 1989). This approach is supported by the wealth of evidence demonstrating that maintenance of plasma glucose as close as possible to physiological levels reduces the risk of late diabetic complications (Wiseman et al., 1985; The Diabetes Control and Complications Trial Research Group, 1993; Reichard et al., 1993; Wang et al., 1993). However, currently available insulin preparations cannot simulate a nondiabetic insulin profile, due partly to the delay in the appearance of insulin in the plasma after s.c. injection (Barnett and Owens, 1997).

Recognition that absorption of soluble (regular) insulin from the s.c. depot is impeded by the formation of hexameric macromolecules in neutral solution (Blundell et al., 1972; Brange et al., 1988) led to the development of insulin aspart (IA), an insulin analog whose molecules repel each other at zinc-free conditions, minimizing association (Brange et al., 1988). Molecular modeling studies of insulin identified the residues important for monomer-monomer interactions, which have no effect on the binding of insulin to its receptor (Baker et al., 1988). IA was created by substituting the amino acid proline with aspartic acid at position 28 on the B chain of insulin, with the aim of developing an insulin analog similar to human soluble (regular) insulin (HI) in all biological respects, but with a faster absorption and thus earlier onset of action. Extensive preclinical testing of IA has confirmed that the chemical and biological properties of insulin have been preserved in terms of potency and characteristics of binding to both the insulin receptor and IGF-1 receptor (Drejer, 1992).

The aim of the present studies was to compare the pharmacokinetics of IA with those of HI after s.c. and i.v. administration. The rat and dog models were chosen as these are the rodent and nonrodent species to be used in the medium and long-term toxicity studies of IA. Pharmacokinetic assessments in rats and dogs were performed after single s.c. and i.v. doses at three dose levels, and after repeated s.c. doses administered over the course of 1 week to assess possible accumulation.

Additional pharmacokinetic studies were conducted in normal pigs after i.v. and s.c. dosing. Pigs are useful models for s.c. dosing studies as their s.c. lipid structure resembles that of humans (Leeson and Leeson, 1976), and the rate of absorption of HI after s.c. administration has been demonstrated to be similar in both pigs and humans (Ribel et al., 1985). However, variability of insulin action may arise not only from pharmacokinetic differences, but also from pharmacodynamic effects (i.e., similar plasma insulin concentrations inducing different metabolic effects). Therefore, the studies in pigs also included analysis of plasma glucose profiles after i.v. and s.c. dosing to allow comparison of the pharmacodynamics of IA and HI.

In this study, doses were selected based on clinical doses, tolerance to hypoglycemia, and known responses to insulin treatment. Generally, when treating type I diabetics, the clinical dose of HI is 1 U/kg b.wt. In dogs, this dose was chosen as the highest s.c. dose, whereas the highest i.v. dose was 0.2 U/kg. In the rats, the selected doses were slightly higher on a weight basis because rats are more tolerable to the insulin-induced hypoglycemia compared with dogs. The highest s.c. dose was 6 U/kg, and the highest i.v. dose was 2 U/kg. In pigs the selected dose was known to result in a reduced plasma glucose concentration, to approximately 2 to 3 mM.
Materials and Methods

All of the studies were performed in compliance with the Organization for Economic Cooperation and Development principles of Good Laboratory Practice.

Animals. Male and female Sprague-Dawley rats (170–209 g) were obtained from the Møllegaard Breeding and Research Center (Lille Skensved, Denmark). The animals were fed a complete pelleted rodent diet (Altromin 1314; Chr. Petersen, Ringsted, Denmark). Food and water were freely available during the study.

Male and female beagle dogs (8.2–13.8 kg) were obtained from Harlan Institute (CPB, Zeist, the Netherlands). The animals were fed a commercially available diet twice daily; tap water was freely available. The animals were exercised outside daily throughout the study.

Female crossbred pigs (LYYD; Land race, Yorkshire and Duroc) weighing 76 to 89 kg were obtained from Lars Holmenlund (Haarlev, Denmark). The animals were fed a commercially available diet twice daily, although animals were fasted before and during the dosing and sampling periods; tap water was freely available. To facilitate drug dosage and blood sampling, pigs were kept in steel racks during the study period.

Test Materials. IA (10 U/ml) and HI (10 U/ml) were used for dosing in all studies except for the s.c. studies in pigs, in which 100-U/ml formulations of both IA and HI were used to obtain injection volumes similar to those used in humans.

Single Dose Studies. The pharmacokinetics of IA and HI were compared after i.v. and s.c. administration to rats (n = 36 at each dose level), dogs (n = 6), and pigs (n = 5). The rats were randomized to the different study groups immediately before dosing. The studies involving dogs and pigs used a crossover design, with doses separated by a washout period of 6 days in each case. Three different doses of IA and HI were administered to rats and dogs. During the course of the study, it became necessary to use rats and dogs in the fed state to prevent threatening attacks of hypoglycemia.

Intravenous dosing. IA or HI was administered to rats as a bolus via the tail vein at doses of 0.5, 1.0, and 2.0 U/kg b.wt. Three heparinized blood samples were taken from each rat by orbital venous plexus puncture under light CO2 anesthesia. Samples were taken at three of the following time points: before dosing, and at 5, 10, 15, 20, 25, 30, 40, and 60 min after dosing. Sampling of individual animals was done at staggered time points, allowing at least a 15-min interval. Plasma was separated and stored at −20°C before analysis.

During the course of the study, each dog received via a foreleg vein individual bolus doses of IA and HI at doses of 0.05, 0.10, and 0.20 U/kg b.wt. Three heparinized blood samples were taken from each rat by orbital venous plexus puncture under light CO2 anesthesia. Samples were taken at three of the following time points: before dosing, and at 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, and 120 min after dosing. Plasma was separated and stored at −20°C. At the start of the study, dogs underwent catheterization of both jugular veins under general anesthesia. Samples were collected at staggered time points, allowing at least a 15-min interval. Plasma was separated and stored at −20°C before analysis.

Subcutaneous dosing. Subcutaneous doses of IA or HI were injected into the necks of rats at doses of 2.0, 4.0, and 6.0 U/kg b.wt. Heparinized blood samples were taken as described above, before dosing, and 15, 30, 45, 60, 90, 120, 180, and 240 min after dosing.

Dogs received s.c. injections of IA or HI in the neck at doses of 0.25, 0.50, and 1.00 U/kg b.wt. Heparinized blood samples were taken before dosing and 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, and 300 min after dosing.

Pigs received IA or HI, approximately 0.125 U/kg b.wt. Each injection was given on one side of the neck using a NovaPen, with the depth adjustment mounted to 5 mm. Blood samples were taken before dosing and 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 240, 300, and 360 min after dosing.

Repeated s.c. Administration (Rats and Dogs). Using the same doses and the same number of animals as in the single-dose studies, IA or HI was administered s.c. twice daily for a period of 7 days, with a single final dose on the eighth day. The doses on each day were separated by approximately 4 h.

Heparinized blood samples were taken on days 1 and 8, as described previously for the single-dose experiments.

Plasma Analysis. Plasma samples were analyzed to determine levels of IA and HI using the Pharmacia Insulin RIA 100 (Uppsala, Sweden). The assay kit uses an insulin-specific antibody raised in guinea pig immunized with monocomponent porcine insulin. The assay was capable of measuring IA and human, porcine, dog, and rabbit insulin with a detection limit of 17.5 pm and a CV of about 8.5% in samples containing from about 80 to 800 pm, which was considered to be in agreement with the manufacturer’s claim for the analysis of HI.

The specificity of the different types of insulin was tested using preparations with insulin concentrations determined by HPLC. The calibration curves were generated using a four-parameter logistic fitting with MultiCalc software from Wallac Oy (Turku, Finland). Porcine and dog insulin were measured with virtually the same specificity (98.4%) compared with HI. This is because the polyclonal insulin antibody used in the Pharmacia insulin RIA 100 binds to a region of the insulin molecule (near the C-terminal end of the B chain) that shows a high degree of homology between different species.

IA and rat insulin did not show a linear relation with the calibration curve, but their response could be described by a Michaelis-Menten model. By using conversion formulae, the measured concentrations could be converted to true concentrations (100% recovery compared with HI). The concentration of IA (z) was found from the measured concentration (y) by the correction formula: z = 1503 + y/(1398 − y). However, the application of this formula would increase the error in z, as y increases. Therefore, samples with y > 600 pm had to be diluted and reanalyzed. The best fit for converting measured concentrations (y) to true concentrations (z) of rat insulin was: z = 452 + y/(286 − y). The endogenous levels of insulin were not taken into account in these calculations. However, these could be ignored in relation to the high levels of HI or IA, which were used in this study.

In the pharmacodynamics study, the plasma glucose concentration was determined in the samples drawn from pigs using a CobasMira analyser (Hoffmann-La Roche, located at Novo Nordisk A/S), using the glucose dehydrogenase method.

Data Analysis. Pharmacokinetics. Data were analyzed by noncompartmental methods (Gibaldi and Perrier, 1982). Pharmacokinetic parameters for IA and HI were compared in the different animal models under different dosing regimens. The parameters included the plasma half-life (t1/2), maximum plasma concentration (Cmax), and time to maximum plasma concentration (tmax). The area under the concentration-time curve (AUC) was calculated according to the trapezoidal rule (Gibaldi and Perrier, 1982) for all single-dose and repeated-dose studies. The extrapolated part of the curve was determined as Cmax/t where Cmax = the last concentration measured and t = slope of the curve for the last phase.

The body clearance (CL) was calculated for single dose i.v. studies as follows: CL = Dose/AUC.

Only parameters for IA and HI generated from the pig studies (n = 5) were compared using a paired t test. The dog studies were carried out using three animals of each sex at each dosing level, which did not allow the use of statistical tests. Only three plasma samples were obtained from each rat. Therefore, the pharmacokinetic data in the rat studies are based on mean concentration-time profiles; S.D. values could not be calculated.

Pharmacodynamics. The effects of IA and HI on relative plasma glucose levels in the pig, and the change from predosing values, were compared separately at each time point using a paired t test.

Results

Only minor differences in pharmacokinetic parameters were observed between IA and HI in the rat and dog studies after either i.v. or s.c. administration. In pigs, similar results were obtained for the two agents after i.v. dosing, but IA exhibited faster s.c. absorption compared with HI. Only minor gender differences were observed during the comparisons of IA or HI; gender effects, therefore, will not be discussed more.

In the pharmacokinetic evaluations, plasma concentrations of IA and HI were not corrected for the baseline level of insulin. This was
because the exact baseline level is difficult to estimate because it may fluctuate during the study, and administration of insulin is expected to suppress endogenous insulin. However, endogenous insulin obviously makes a greater contribution to total plasma insulin levels when the administered dose of insulin is low, but only a small proportional contribution at high dosing levels. Therefore, the pharmacokinetic evaluations of IA and HI were based principally on data obtained after administration of the highest dose used in each study.

**Single Dose Studies. Rats: i.v. dosing.** Both IA and HI were eliminated rapidly after i.v. dosing, with plasma half-lives of 12 and 14 min, respectively, at the highest dose (Table 1). The fast elimination was confirmed by a clearance rate (CL) of 44 ml/min/kg for IA and 58 ml/min/kg for HI. AUC values tended to be higher for IA than for HI. Peak plasma concentrations increased with increasing dose (data not shown).

**Rats: s.c. dosing.** The plasma half-lives of IA and HI after s.c. administration were, respectively, 22 and 23 min (Table 1). The prolonged half-lives and slower elimination after s.c. compared with i.v. dosing may stem from delayed absorption from the s.c. tissue; peak plasma levels of both agents were not reached until 15 min after dosing. Bioavailability of both IA and HI was high after s.c. dosing, reaching 83 and 93%, respectively.

**Dogs: i.v. dosing.** Both IA and HI exhibited rapid elimination, with plasma half-lives of 11 and 12 min, respectively, after the highest dose (Table 2). The rapid elimination was confirmed by rapid clearance rates of 55 to 58 ml/min/kg for IA and 41 to 52 ml/min/kg for HI. AUC values were dose-dependent, indicating linear kinetics for both IA and HI.

**Dogs: s.c. dosing.** In general, only minor differences were observed between IA and HI after s.c. administration; they both exhibited a clear dose-response relationship in peak plasma concentrations and AUC values (Table 2). However, large individual variations in peak plasma concentrations were observed, resulting in large S.D. values for the Cmax values (Table 2). Similarly, the fluctuations in plasma concentration also affected the time to peak plasma levels, leading to large S.D. values in tmax values. Overall, however, IA tended to be absorbed more rapidly from the injection site compared with HI, reaching maximum plasma concentrations at 46 and 60 min, respectively, after the highest dose (1.0 U/kg), although no differences were observed in the maximum plasma concentrations of the two agents. The AUC values indicated a bioavailability of 116 and 93% for IA and HI at a dose of 0.25 U/kg.

**Repeated s.c. Administration. Rats.** Pharmacokinetic parameters for IA and HI after repeated dosing were similar to those described above after a single s.c. dose, although the values for Cmax and AUC were lower than those reported in the single-dose study (data not shown). The reasons for this discrepancy are not clear. Generally, only minor differences were observed between IA and HI. The time to peak plasma concentration was 15 min for all doses of both agents, except the highest dose (6.0 U/kg) of HI, for which tmax was 30 min. Both IA and HI were eliminated rapidly from plasma, with half-lives of 21 to 31 min. At the highest dose level, a tendency toward higher values for Cmax and AUC were observed for HI compared with IA; the reason for these results is unclear.

**Dogs.** No pharmacokinetic differences were observed between the first dose of IA and HI and the results on day 8, after repeated s.c. administration (data not shown). No differences were observed between IA and HI. On day 8, the time to maximum plasma concentration tended to be lower than on day 1 (range 28–48 min compared with 45–90 min on day 1). Values for Cmax showed no consistent differences, although individual values varied substantially for both agents. The apparent differences between days 1 and 8 probably stem from the large individual variations in peak plasma concentrations and absorption rates, resulting in large S.D. values for the Cmax and tmax values. Elimination half-lives were similar on days 1 and 8, ranging from 51 to 97 min on day 1 compared with 41 to 103 min on day 8.

**Pigs.** Pharmacokinetics: i.v. dosing. There were no significant differences between IA and HI after a single 0.025 U/kg i.v. dose in terms of CL (Table 3). Administration of either agent was followed by a similar increase in plasma insulin levels, which declined rapidly to a level comparable with the predosing level (corresponding to the endogenous insulin level for the pig) within 15 to 18 min (Fig. 1). Mean plasma half-lives were 4.5 min for IA and 2.9 min for HI, although there was no difference in plasma clearance (51 ml/min/kg for IA versus 52 ml/min/kg for HI; P = .98).

**Pharmacokinetics: s.c. dosing.** A strong trend was observed toward faster absorption of IA compared with HI after s.c. administration of a single 0.125 U/kg dose, although this difference did not reach statistical significance. IA reached a Cmax of 204 pmol after 73 min, compared with a Cmax of 122 pmol after 99 min for HI. IA was also eliminated more rapidly, exhibiting a half-life of 77 min compared with 121 min for HI, although again this difference did not reach significance (P = .09) (Table 3; Fig. 2).

In two animals, high endogenous insulin levels at the time they were given IA led to much higher values for plasma insulin than were recorded in other pigs after s.c. IA dosing, and in all animals after s.c. HI doses. The impact of this anomaly, and other possible sources of errors mitigating against the demonstration of significant differences between IA and HI after s.c. dosing, are considered in Discussion.

**Pharmacodynamics: i.v. dosing.** Plasma glucose levels exhibited similar changes after i.v. administration of IA or HI (Fig. 1). Although the fall in plasma glucose levels appears slightly greater after dosing...
Period from 30 to 75 min postinjection, glucose levels were significantly greater than baseline at approximately 120 min, before returning toward baseline. During the HI, plasma glucose levels fell in a similar manner over a period of 60 to 90 min after dosing, presumably due to counter-regulation. Returns to a higher plasma glucose level after dosing, presumably due to counter-regulation by endogenous mechanisms.

Pharmacodynamics: s.c. dosing. After s.c. administration of IA and HI, plasma glucose levels fell in a similar manner over a period of approximately 120 min, before returning toward baseline. During the period from 30 to 75 min postinjection, glucose levels were significantly lower after administration of IA compared with HI (P < .05) (Fig. 2). The maximal changes of plasma glucose were 2.1 mM after IA and 1.7 mM after HI. Seventy-five minutes after dosing, the glucose levels fluctuated in both the IA- and HI-treated pigs, presumably due to counter-regulation by endogenous mechanisms.

Discussion
Clinical experience demonstrates that s.c. injection of insulin gives rise to a highly variable metabolic effect, thereby hampering optimal glycemic control (Heinemann et al., 1998). Delayed and unpredictable absorption of standard HI from the s.c. tissue due to the formation of hexameric macromolecules may explain the variable dose response (Blundell et al., 1972; Brange et al., 1988, 1990). IA is a novel insulin analog developed to provide improved and more predictable glycemic control compared with HI by achieving more rapid absorption after s.c. dosing (Brange et al., 1990).

The current study compared the pharmacokinetics of IA and HI after single s.c. and i.v. dosing at three dose levels, and multiple s.c. dosing in Sprague-Dawley rats and beagle dogs. Pharmacokinetic and pharmacodynamic profiles were compared after single s.c. and i.v. doses in crossbred female pigs. IA has been shown to possess similar chemical and biological properties compared with HI in terms of potency and receptor binding (Drejer, 1992). Therefore, few differences between the agents were expected after i.v. dosing, whereas differences in the pharmacokinetic and pharmacodynamic profiles were anticipated after s.c. dosing as a result of more rapid absorption of IA into the systemic circulation.

The single-dose rat and dog studies, however, revealed only minor differences between IA and HI. After i.v. administration, both agents were rapidly eliminated from plasma, with elimination half-lives below 15 min in both species. Much longer half-lives of approximately 60 min were observed after s.c. administration, probably due to the prolonged absorption from the injection site. In dogs there was a trend toward faster absorption of IA from the s.c. depot at the highest dosing level, although there were no differences in the maximum plasma concentrations. The tendency toward higher values for $C_{\text{max}}$ and $AUC$ after s.c. dosing with IA in rats may indicate a more

![Fig. 1](image). Plasma insulin profiles (A) and change in plasma glucose (B) with time after a single i.v. dose of IA or HI (0.025 U/kg) in five crossbred pigs (bars indicate ± S.D.).

The asterisks indicate time points where $P < .05$.
served in smaller steady-state volume of distribution for IA compared with HI.

The rapid absorption rate, but the \( C_{\text{max}} \) may also have been affected by a smaller steady-state volume of distribution for IA compared with HI.

Multiple s.c. dosing of IA and HI in rats and dogs had no apparent effect on pharmacokinetic parameters. The minor differences observed in \( t_{\text{max}} \) in dogs, and in \( C_{\text{max}} \) and AUC in rats have no obvious cause and may be due to the large interindividual variation in these values. Differences between IA and HI were minor, and were generally similar to those observed during the single-dose studies. The lack of differences after repeated dosing indicated that neither IA nor HI accumulates within the body.

As in the rat and dog models, the studies in pigs demonstrated no difference between IA and HI after i.v. dosing, either in terms of pharmacokinetics or the effect on plasma glucose. In contrast, the studies demonstrated a clear trend toward faster absorption of IA compared with HI after s.c. dosing, paralleled by a significantly superior plasma glucose-lowering effect leading to lower plasma glucose from 30 to 75 min after dosing with IA as compared with HI. These results strongly suggest that IA is absorbed more rapidly than HI from the s.c. depot, and that differences between the compounds do not stem from differences in the elimination process.

Taken together, the findings in the three species highlight the importance of selecting an appropriate animal model to assess the pharmacokinetic properties of compounds designed for s.c. administration in humans. Only in the pig were the predicted differences between IA and HI observed in terms of faster absorption and onset of action after s.c. dosing. The probable explanation for these results is that the s.c. tissue of rats and dogs contains much less fat than does equivalent tissue in humans. The lower density of the fat layer allows faster absorption of drugs and fails to differentiate between agents such as IA and HI. The inability of rats and dogs to predict the pharmacokinetics of IA after s.c. dosing has already been noted (Plum et al., 1998). In contrast, the lipid content and structure of the s.c. tissue in pigs is very similar to that in humans, leading to a similar differential pattern of absorption for IA and HI from a s.c. depot. This assertion is supported by previous studies in healthy humans, which have shown that IA reaches maximum plasma concentration faster than soluble (regular) HI after s.c. injection (Heinemann et al., 1998), and that this is accompanied by a faster onset of hypoglycemic action and higher maximal action (Heinemann et al., 1993), resulting in a superior postprandial glucose control (Home et al., 1998).

Despite the close approximation to human s.c. tissue in the pig model, the differences in \( t_{\text{max}} \), \( C_{\text{max}} \), and AUC failed to reach statistical significance. This result may stem from two limitations of the study. First, there was no correction for endogenous insulin in the calculation of the area under the concentration–time curve (AUC\( \text{0–300 min} \)). As noted above, in two pigs receiving IA, high endogenous insulin levels led to much higher values for plasma insulin than were recorded after IA doses in other animals and all HI doses. This is probably responsible for the lack of a significant difference between IA and HI in the calculated AUC\( \text{0–300 min} \) values. Secondly, the assay method used to determine plasma insulin levels (the Pharmacia Human Insulin radioimmunoassay), does not discriminate between IA, HI, and endogenous insulin. Endogenous insulin secretion may therefore mask differences due to IA and HI. Even with the obscuring effect of the pigs’ endogenous insulin, however, a strong tendency toward a difference between IA and HI was observed both for \( C_{\text{max}} \), \( t_{\text{max}} \) and \( t_{1/2} \).

In pigs, which are the preferred model for assessing drug pharmacokinetics after s.c. dosing, peak plasma levels of IA were reached more rapidly than those of HI after s.c. dosing, resulting in a faster onset of hypoglycemic action. The absence of differences between the agents after i.v. dosing confirmed that the differences in the s.c. study were due to faster absorption of IA from the s.c. depot, and not differences in elimination kinetics. No significant differences between IA and HI were observed in rats or dogs after s.c. or i.v. dosing, suggesting that these species are poor models for assessing pharmacokinetic aspects of s.c. dosing in humans.

Acknowledgments. We thank Peter Brinck DVM and Søren Dyring Jacobsen DVM for performing the rat and dog studies at Scantox, Lille Skensved, Denmark; the laboratory assistants Pia Skaarup Larsen and Lisette Gammelgaard Nielsen, Pharmacokinetics Department, Novo Nordisk A/S for performing the pig studies and the plasma glucose analyses; and Declan Walsh and Birgitte Holm, Immunochemistry Department, Novo Nordisk A/S for performing the immense number of radioimmunoassay analyses.

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