Comparative Pharmacokinetic and Pharmacodynamic Studies of Human Insulin and Analogues in Chronic Diabetic Yucatan Minipigs

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Abstract
Pharmacokinetics and pharmacodynamics of insulin analogues were compared with human insulin in streptozotocin-induced chronic diabetic Yucatan minipigs. After overnight fasting, insulin or one of the insulin analogues (0.6 nmol/kg) in acid solutions (pH 3.0) was administered to the minipigs s.c. The plasma insulin concentrations were then measured by radiomunaoassay at predetermined time intervals although blood glucose levels were monitored continuously. The mean (±S.E.) values of ΔCmax (difference between peak and basal plasma insulin levels) were 598 (±21), 528 (±44), 176 (±21), 325 (±60) and 228 (±33) pM, respectively, for analogue AspB9GluB27, AspB9, GluB27, AspB28 and insulin. The differences in ΔCmax values were statistically significant between AspB9GluB27 and insulin (P < .02), and between AspB9 and insulin (P < .01), but not between GluB27 or AspB28 and insulin. Moreover, the mean (±S.E.) values of ΔAUC0−6 (integrated area between plasma insulin concentration curve and basal level) were 1877 (±169), 1987 (±70), 485 (±36), 500 (±32) and 677 (±105) pM × hr, respectively, for AspB9GluB27, AspB9, GluB27, AspB28 and insulin. The differences in ΔAUC0−6 values were statistically significant between AspB9GluB27 and insulin (P < .05) and between AspB9 and insulin (P < .02), but not between GluB27 or AspB28 and insulin. However, there was no significant difference in the values of Δnadir (difference between nadir and basal levels) and ABGC0−12 (integrated area between blood glucose response curve and basal level) between insulin and various analogues. In conclusion, although the insulin analogues are different from human insulin in pharmacokinetics, they exhibit similar biological activity to human insulin in the streptozotocin-induced chronic diabetic minipigs.

Although it is now common practice to treat insulin-dependent diabetic patients with human insulin, normoglycemia has rarely been achieved by subcutaneous administration (Capaldo et al., 1984; Skyler, 1986). Hyperglycemia has often occurred during meals as a result of slow absorption of insulin; and hypoglycemia has often occurred between meals as a result of prolonged duration of absorption. Subcutaneous insulin absorption is influenced by many factors (Brange et al., 1990), among which the association state of human insulin (in hexamer) in pharmaceutical formulation (100 IU/ml ~ 0.6 mM) may be of importance (Brange et al., 1990). A series of human insulin analogues with reduced tendency to self-associate have been developed by recombinant DNA technology (Brange et al., 1987, 1988) and recently marketed (e.g., Humalog) (Howey et al., 1994; Torlone et al., 1994; Trautmann, 1994). These analogues promote rapid absorption, which is better suited to meal-related therapy (Kang et al., 1990; Vora et al., 1988). However, some of insulin analogues (Table 1), such as AspB9GluB27 and AspB9, which have improved absorption properties but less potent than human insulin in receptor-binding affinity (human hepatoma HepG2 cell line) and free-fat cell assay (Drejer et al., 1988; Brange et al., 1988). Nevertheless, these analogues exhibit biological activity similar to or the same as human insulin in mouse blood glucose assay (Drejer et al., 1988; Brange et al., 1988). Analogue AspB9GluB27 was also observed to have full bioactivity in healthy pig assay by euglycemic clamp technique (Ribel et al., 1990). In addition to animal data, AspB9GluB27 and AspB28 were also observed to have similar bioactivities to that of insulin in normal (Kang et al., 1991a, b) and diabetic (Kang et al., 1990, 1991c) human subjects in clinical studies. However, no suitable diseased model has been developed for studying the pharmacokinetic and pharmacodynamic properties of various insulin/analogues formulations before human studies.

Swine's physiological similarity to humans (Panepinto and Phillips, 1986), and the development of effective and gentle animal-handling techniques (Panepinto et al., 1983; Lin and Chien, 1997), have led to increased use of swine as a reliable large-animal model for studying pharmacokinetic and phar-

ABBREVIATIONS: CI, confidence interval; MRT, mean residence time; ABGC, the integrated area between glucose response curve and basal blood glucose; PE, polyethylene; STZ, streptozotocin; TDMAC-heparin, tridodecylmethylammonium chloride-heparin.
TABLE 1
Association behavior and biological characteristics of human insulin and its analogues

<table>
<thead>
<tr>
<th>Human Insulin Analogue/Aggregation State</th>
<th>Association State (Zinc Free) (Osmometry 1 mM, 21°C)</th>
<th>Biological Potency (%)</th>
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<tr>
<td></td>
<td></td>
<td>FFc</td>
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<tr>
<td></td>
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<td>MBc</td>
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<td>RBa</td>
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<tr>
<td></td>
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<td>SSc</td>
</tr>
<tr>
<td>Insulin, 2 Zn²⁺/hexamer</td>
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<tr>
<td>Asp¹⁰⁰Glu⁰²⁷/monomer</td>
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<td>100</td>
</tr>
<tr>
<td>Asp¹⁰⁰/monomer</td>
<td>1.1</td>
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</tr>
<tr>
<td>Asp¹⁰⁰/monomer</td>
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<tr>
<td>Glu⁰²⁷/dimer &amp; tetramer</td>
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<td>26</td>
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<td>87</td>
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</tbody>
</table>

* Data from Dreyer et al. (1988) and Brange et al. (1988).
* Free-cell assay (data from Dreyer et al. (1988) and Brange et al. (1988)).
* Mouse blood glucose assay (data from Dreyer et al. (1988) and Brange et al. (1988)).
* Receptor-binding affinity (human hepatoma cell line) assay (data from Dreyer et al. (1988) and Brange et al. (1988)).

Macromolecular properties of various investigational drugs (Oberle et al., 1994; Kaltenbach et al., 1996; Xing et al., 1998). The swine model has potential for providing a good prediction of clinical performance. In addition, the feasibility of using a continuous blood glucose monitoring system to continuously monitor the glycemic state in the conscious animal has been demonstrated in this laboratory (Lin et al., 1993). The use of the continuous glucose monitoring system has made possible an accurate determination of the nadir value, which often occurs at unpredictable times and lasts only briefly. In this investigation, a streptozotocin-induced chronic diabetic Yucatan minipig model (Marshall, 1979; Wang and Chien, 1996; Stanley et al., 1997) was used and evaluated for studying the pharmacokinetics and pharmacodynamics of four human insulin analogues (Asp¹⁰⁰Glu⁰²⁷, Asp¹⁰⁰, Glu⁰²⁷ and Asp¹⁰⁰⁻¹⁰⁰) for comparison with human insulin in acid solutions.

Methods

Materials. Glucose oxidase membrane, glucose standards and buffer solution used in the Glucose Analyzer (YSI model 27) were purchased from Yellow Springs Instrument (Yellow Springs, OH). Peristaltic pump and tubings (for the pump) were obtained from Cole-Parmer Instrument Co. (Chicago, IL). PE-10 (nonradiopaque polyethylene micro-tubing) was from Clay Adams (Division of Becton Dickinson, Parsippany, NJ). Tridodecylmethylammonium chloride-heparin complex (TDMAC-heparin) was obtained from Polysciences, Inc. (Warrington, PA). STZ, citrate acid monohydrate and sodium phosphate were purchased from Sigma Chemical Co. (St. Louis, MO). Human insulin and insulin analogues were gifts from Novo Research Institute (Bag suaerd, Denmark).

Instrumentation. The continuous blood glucose monitoring system used for this investigation was assembled by connecting the sensor chamber of the glucose analyzer to a peristaltic pump, a temperature used for this investigation was assembled by connecting the sensor chamber of the glucose analyzer to a peristaltic pump, a sensor chamber of the glucose analyzer to a peristaltic pump, and a peristaltic pump and tubings (for the pump) were obtained from Cole-Parmer Instrument Co. (Chicago, IL).

Animal studies. Before injections, the blood glucose level in each test minipig was continuously monitored. After a relatively stable baseline was attained and maintained for a period of at least 30 min, a s.c. dose (0.6 mmol/kg) of human insulin or one of the various analogue solutions was administered at inner-upper region of back leg. Insulin/analogue solutions (0.6 mM) were prepared by dissolving insulin/analogue in normal saline containing phenol (0.2%) and albumin (0.1%), and then adjusted to pH ~3.0 with 1N HCl. The blood glucose levels in the STZ-diabetic minipig were continuously monitored for a period of 12 hr. A recovery period of 1 to 2 wk was allowed between experiments with the same minipig. Each minipig received all insulin/analogue administrations and one placebo, and the sequence of insulin/analogue administrations was randomized. Eighteen experiments were performed, 6 in each minipig, and all were carried out between the 5th and the 6th mo after the initiation of diabetes induction. The mean (±S.E.) body weights of minipigs were 60 (±5) and 62 (±4) kg, respectively, at the time of the first and last experiment.

Insulin radioimmunoassay. During the course of continuous blood glucose monitoring through the first PE-10 tubing, blood sam-
ples were also withdrawn through the second PE-10 tubing at pre-
determined intervals for radioimmunoassay of insulin/analogues. The blood samples were each collected in a chilled microtube and immersed in an ice bath. The blood samples and containers were maintained at 2 to 8°C throughout the entire process of blood collection and handling. The plasma was separated by centrifugation in a refrigerated centrifuge and the plasma was then aspirated and transferred into a microtube and immediately frozen until assayed. Assay of insulin/analogues was performed using Coat-A-Count Insu-
lin kits (Diagnostic Products Co., Los Angeles, CA) with the respective insulin/analogue standards. The standard curves (range from 0–2400 pM) of four human insulin analogues were also evaluated and compared with human insulin.

Pharmacokinetic and pharmacodynamic analysis. For purposes of quantitative comparison of the pharmacokinetics between human insulin and various analogues, the values of C0 (fasting basal plasma insulin level), Cmax (peak concentration observed during the dosing period), tmax (time to Cmax) and MRT after s.c. administration were determined from the plasma insulin (or analogue) concentration-time profiles. Moreover, the quantitative comparison of the pharmacodynamics between human insulin and various analogues, the values of E0 (fasting blood glucose level), nadir (concentration at maximum glycemic reduction observed during the dosing period), t nadir (time to nadir), and E12-hr (blood glucose level at 12-hr after dosing) after s.c. administration were determined from the blood glucose concentration-time profiles.

To minimize the complication of inter-animal variation in basal insulin and glucose levels, the comparison of human insulin and its analogues, normalization of pharmacokinetic and pharmacodynamic parameters is needed. The normalization in this investigation was on the basis that the basal insulin and glucose levels are maintained at a relatively constant state throughout the test period. Therefore, the values of ΔCmax (the difference between Cmax and C0) and ΔAUC (the integrated area between plasma insulin concentration and basal plasma insulin) were calculated by subtraction of the fasting basal insulin levels from the plasma insulin concentration-time profiles. The value of AUC was calculated by trapezoidal rule for a period from 0 to 6 hr. In addition, the values of Δnadir (the difference between nadir and E0) and ABGC were calculated from the blood glucose concentration-time profiles which normalized in percent of the initial fasting basal blood glucose levels. The value of ABGC was calculated by trapezoidal rule for a period from 0 to 12 hr.

Statistical analysis. The Student’s paired t test was used to determine the statistical significance of the difference between pharmacokinetic and pharmacodynamic parameters of each of the various analogues and human insulin.

Results

Chronic diabetic minipig model. A typical set of blood glucose and plasma immunoreactive insulin profiles after a rapid, single i.v. injection of STZ (60 mg/kg) are graphically shown in figure 1. Yucatan minipigs have a baseline fasted glucose level of ≈50 mg/dl [49 ± 2 (mean ± S.E.M., n = 12)] and baseline plasma insulin level of ≈9 μIU/ml [9 ± 1 (mean ± S.E.M., n = 12)]. The results in figure 1 indicate that, after the injection of STZ, hyperglycemia was induced within 1 to 2 hr, and sustained several hours, and then hypoglycemia subsequently appeared. During the period of hypoglycemia, it is critically important to maintain the blood glucose level above 20 mg/dl. The transient fatal hypoglycemia induced by STZ was offset by intravenous administrations of dextrose (25%) solution (10 ml/hr).
The profiles of blood glucose levels and plasma immunoreactive insulin concentrations in the minipigs (n = 3) after diabetes induction (after the two i.v. injections of STZ, with 60 mg/kg each on day 1 and day 8) are shown in figure 2, and indicate that diabetic minipigs have a baseline fasted glucose level higher than 50 mg/dl, and a baseline plasma insulin level lower than 9 μIU/ml (54 pM) compared with that of normal minipigs. It was therefore suggested that this dosing regimen is capable of inducing hyperglycemia in Yucatan miniature pigs, that this hyperglycemia is established immediately after the second dose and that it is maintained for a period of at least 9 mo without any daily insulin supplement (fig. 2).

**Insulin radioimmunoassay.** A linear logit-log relationship exits between the percentage of insulin bound and the concentration of human insulin or its various analogues added into all standard solutions for radioimmunoassay using the Coat-A-Count Insulin kits (fig. 3). The attainment of linearity indicates that the commercially available insulin kits can be used for the measurement and computation of various insulin analogues studied in this investigation. The mean (±S.D.) of sensitivities (95% intercept acquired from logit-log line) calculated from eight repeated RIAs were 27 (±19), 30 (±14), 34 (±26), 26 (±17) and 30 (±19) pM, respectively, for AspB9GluB27, AspB9, GluB27, AspB28 and human insulin. The coefficient variations of inter-assays were 7, 4, 9, 5 and 7%, respectively, for correspondent analogues and insulin.

**Animal studies.** The basal levels normalized plasma immunoreactive insulin/analogues and blood glucose profiles, after the s.c. administration of human insulin or its various analogues (0.6 nmol/kg) in the same group of diabetic minipigs (n = 3) are compared graphically in figure 4A–D. The results indicate that the plasma concentrations of insulin/analogues all increase rapidly and reach their respective concentrations within 1 hr after the s.c. injection. It was then maintained at a relatively steady level for at least another 4 hr, with the exception of AspB9GluB27 (fig. 4A) and AspB28 (fig. 4D), which gradually decline to baseline after reaching peak. However, the hypoglycemic response profiles of all analogues were very similar to that of human insulin: the blood glucose concentration declines gradually, with an onset time of about 30 min (from the baseline level), after s.c. injection, and all reach the maximum level of glycemic reduction (i.e., nadir) within 6 hr. After reaching nadir, the glucose concentrations rise gradually, but the original baseline level is not recovered within the observation period. Moreover, the plasma insulin and blood glucose profiles from the placebo were observed to be maintained at a relatively stable throughout the test period (fig. 4A) that indicates that the normalizations of pharmacokinetic and pharmacodynamic parameters by the basal insulin and glucose levels are possible.

**Pharmacokinetic and pharmacodynamic analysis.** The comparisions of the pharmacokinetics and pharmacodynamics between human insulin and its various analogues, after s.c. administration, were outlined in table 2. The mean (±S.E.) values of ΔCmax were 598 (±21), 528 (±44), 176 (±21), 325 (±60) and 228 (±33) pM for AspB9GluB27, AspB9, GluB27, AspB28 and human insulin, respectively. The differences in ΔCmax values were statistically significant between AspB9GluB27 and human insulin [P = .016, power = 0.95, 95% CI = −576 to −164], and between AspB9 and human insulin (P < 0.01, power > 0.99, 95% CI = −354 to −246), but not between GluB27 (P = .036, power = 0.13, 95% CI = −112 to 216) or AspB28 (P = .133, power = 0.29, 95% CI = −268 to 73) and human insulin. However, there is no difference between the values of time to Cmax between various analogues and human insulin. Moreover, the mean (±S.E.) values of Δnadir were −67 (±9), −74 (±5), −65 (±5), −57 (±15) and −72 (±5)% for AspB9GluB27, AspB9, GluB27, AspB28 and human insulin, respectively. There were no differences in the values of Δnadir and time to nadir between various ana-
logues and human insulin. The mean (±S.E.) values of MRT were 148 (±4), 176 (±12), 165 (±9), 140 (±8) and 171 (±2) min for Asp<sup>9</sup>Glu<sup>27</sup>, Asp<sup>9</sup>, Glu<sup>27</sup>, Asp<sup>28</sup> and human insulin, respectively. The differences in MRT values were statistically significant between Asp<sup>9</sup>Glu<sup>27</sup> (P = 0.048, power = 0.63, 95% CI = 0.6 to 46) and human insulin, and between Asp<sup>28</sup> (P = 0.032, power = 0.78, 95% CI = 7 to 55) and human insulin, but not between Asp<sup>9</sup> (P = 0.700,

Fig. 4. Upper panel, Comparison in the plasma profiles of insulin and analogues in the streptozotocin-induced chronic diabetic Yucatan minipigs (n = 3) after the s.c. administration of human insulin and one of its analogues [Asp<sup>9</sup>Glu<sup>27</sup> (A), Asp<sup>9</sup> (B), Glu<sup>27</sup> (C), and Asp<sup>28</sup> (D)]. Lower panel, Comparison in the reduction profiles of blood glucose (in which the S.E.s are displayed at 1-hr intervals along the course of continuous blood glucose profiles).
TABLE 2
Comparison of pharmacokinetic and pharmacodynamic parameters between insulin and insulin analogues administered subcutaneously in chronic diabetic minipigs (n = 3) *

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Insulin</th>
<th>AspB9/GluB27</th>
<th>AspB9</th>
<th>GluB27</th>
<th>AspB28</th>
</tr>
</thead>
<tbody>
<tr>
<td>C0 (pM)</td>
<td>23 (5)</td>
<td>11 (7)</td>
<td>22 (2)</td>
<td>14 (9)</td>
<td>36 (7)</td>
</tr>
<tr>
<td>Cmax (pM)</td>
<td>242 (27)</td>
<td>609 (27)†</td>
<td>550 (46)†</td>
<td>190 (24)</td>
<td>361 (54)</td>
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<tr>
<td>tmax (min)</td>
<td>100 (40)</td>
<td>80 (42)</td>
<td>147 (52)</td>
<td>93 (44)</td>
<td>80 (40)</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>171 (2)</td>
<td>148 (4)</td>
<td>176 (12)</td>
<td>165 (9)</td>
<td>140 (8)†</td>
</tr>
<tr>
<td>ΔCmax (pM)</td>
<td>228 (33)</td>
<td>598 (21)†</td>
<td>528 (44)†</td>
<td>176 (21)</td>
<td>325 (60)</td>
</tr>
<tr>
<td>ΔAUC0–t (pM × hr)</td>
<td>677 (105)</td>
<td>1877 (169)†</td>
<td>1897 (70)†</td>
<td>485 (36)</td>
<td>500 (32)</td>
</tr>
</tbody>
</table>

Pharmacodynamics

E0 (mg/dL) 134 (24) 164 (11) 114 (17) 117 (10) 146 (41)
Nadir (mg/dL) 37 (6) 54 (16) 31 (10) 41 (6) 69 (37)
tmax (min) 222 (43) 344 (60) 345 (12) 318 (66) 286 (101)
E12a (mg/dL) 73 (12) 108 (28) 55 (11) 65 (7) 105 (28)
Nadir (% E0) −72 (5) −67 (9) −74 (5) −65 (5) −57 (15)
ABGC0–12 (%) 601 (32) 473 (127) 612 (43) 553 (17) 407 (117)

* Data were presented as mean (S.E.).
† C0, fasting basal plasma insulin concentration.
‡ Cmax, maximum concentration observed during the dosing period.
§ The result of paired t test indicated a significant difference (P < .05) vs. human insulin.
∥ tmax, time to reach the maximum concentration (Cmax).
¶ MRT, mean residence time from 0 to 6 hr of period.
*ΔAUC0–t, the difference between Cmax and C0.
** ΔAUC, area between plasma insulin concentration and basal plasma insulin curves calculated by trapezoidal rule from 0 to 6 hr.
· E0, fasting blood glucose level.
· Nadir, concentration at maximum glycemic reduction observed during the dosing period.
·tnadir, time to nadir.
·E12a, blood glucose level at 12-hr after the dosing.
· Δnadir, the difference between nadir and E0.
· ABGC, the area between blood glucose response curve and basal blood glucose calculated by trapezoidal rule from 0 to 12 hr.

Discussion

The transient hyperglycemic state (fig. 1), following the i.v. administration of STZ in minipig, could be attributed to the diabetogenic effect of STZ, whereas the hypoglycemia observed resulted possibly from either liver damage or excessive insulin secretion by the injured Langerham islet. Therefore, it is critically important to offset the transient fatal hypoglycemia induced by STZ by administrations of dextrose to minipig. And, it is not possible without the using of the continuous glucose-monitoring system, which the blood glucose levels can be monitored on a continuous on-line basis.

Although hyperglycemia, after induction of diabetes, was maintained without any administration of antidietic treatment except for the various insulin analogues administered during the study, a lower basal insulin level than that of normal minipig were observed in STZ-induced diabetic minipigs up to 8 mo (fig. 2). The observation of basal endogenous insulin level suggests that the long-term maintenance of chronic diabetic minipigs becomes possible without any daily administration of exogenous insulin. Moreover, severe hyperglycemia can be prevented and then metabolic fluctuations can be minimized by the low basal endogenous insulin. Therefore, the life in these chronic diabetic minipigs can be prolonged, although the successful rate for this diseased model was around 50%. A total of six chronic diabetic minipigs, which had a fasting glucose level in the range of 120 to 200 mg/dl without any hyperglycemic treatment at all time, were involved in the study from its beginning. One of these diabetic minipigs was dropped out from the study, due to the severe of hyperglycemia within the first month. Although the remaining five completed the study, two of them showed some reduction in their diabetes state during the study and so excluded from the final analysis. Therefore, only the data from the three chronically stable diabetic minipigs were reported.

Complete cross-reactivity of antibody with human insulin and its various analogues were observed in figure 3 using the commercially available insulin kits. The antibody binding affinities, calculated from insulin standard curves, in comparison with human insulin are 74% for AspB9GluB27, 78% for GluB27, 69% for AspB28 and 97% for AspB9. The observations suggested that antibody binding affinities of human insulin have not been altered by the replacement of AspB9, but decreased by GluB27 and AspB28.

The finding of a significant difference in the plasma insulin profiles between the analogues and human insulin in diabetic minipig model agrees well with the results that reported in healthy swine (Ribel et al., 1990), healthy human (Kang et al., 1991a, b) and diabetic human (Kang et al., 1990, 1991c). They indicate that plasma insulin concentrations...
have been observed higher for the analogues with low-affinity to the insulin receptor (e.g., AspB9GluB27 and AspB9) than those for human insulin, although plasma insulin levels for the high-affinity analogues (e.g., GluB27 and AspB28) were lower than those for human insulin (fig. 4A–D; table 2). In addition, the blood glucose profiles between the analogues and human insulin in diabetic minipig model have similar results as in healthy swine (Ribel et al., 1990) and suggest that in vivo biological activities between human insulin and analogues with low and high affinity to the insulin receptor are equivalent regardless of the difference in pharmacokinetics.

The pharmacokinetics (ΔCmax, ΔAUC and MRT) and pharmacodynamics (Δnadir and ABGC) of various insulin analogues, after equimolar amount of insulin administered s.c. in chronic diabetic minipigs, in comparison with human insulin are outlined in table 3. Although the values of ΔCmax and ΔAUC in table 3 for AspB9GluB27 and AspB9 were observed 3- to 4-fold higher than that for GluB27 and AspB28, there was no difference in pharmacodynamics. It indicates that the biological potencies for AspB9GluB27 and AspB9 in diabetic minipigs was reached with the results, shown in in vitro receptor-binding affinity (human hepatoma HepG2 cell line) and free-fat cell assay (Drejer et al., 1988; Brange et al., 1988). In addition, the similar in vivo biological activity between these analogues and human insulin was observed in diabetic minipigs, which was the same as reported in mouse blood glucose assay (Drejer et al., 1988; Brange et al., 1988) and in healthy pig assay by euglycemic clamp technique (Ribel et al., 1990). It indicates that the agreement could suggest that the mechanism of these analogues and human insulin in the chronic diabetic minipig could be supported by that in receptor-binding affinity, free-fat cell assay, mouse blood glucose assay and in healthy pig assay with euglycemic clamp technique. Furthermore, it is interesting to observe that there was a direct relationship between MRT and hypoglycemic potency for various analogues in chronic diabetic minipigs. In summary, although the insulin analogues are different from human insulin, the hypoglycemic effect between minipigs and humans needs to be further investigated.

TABLE 3

<table>
<thead>
<tr>
<th>Insulin</th>
<th>AspB9GluB27</th>
<th>AspB9</th>
<th>GluB27</th>
<th>AspB28</th>
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<td>ΔCmax data</td>
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<td>MRT data</td>
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<td>Pharmacodynamics</td>
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<td>ABGC0–12 data</td>
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</table>

have been observed higher for the analogues with low-affinity to the insulin receptor (e.g., AspB9GluB27 and AspB9) than those for human insulin, although plasma insulin levels for the high-affinity analogues (e.g., GluB27 and AspB28) were lower than those for human insulin (fig. 4A–D; table 2). In addition, the blood glucose profiles between the analogues and human insulin in diabetic minipig model have similar results as in healthy swine (Ribel et al., 1990) and suggest that in vivo biological activities between human insulin and analogues with low and high affinity to the insulin receptor are equivalent regardless of the difference in pharmacokinetics.

The pharmacokinetics (ΔCmax, ΔAUC and MRT) and pharmacodynamics (Δnadir and ABGC) of various insulin analogues, after equimolar amount of insulin administered s.c. in chronic diabetic minipigs, in comparison with human insulin are outlined in table 3. Although the values of ΔCmax and ΔAUC in table 3 for AspB9GluB27 and AspB9 were observed 3- to 4-fold higher than that for GluB27 and AspB28, there was no difference in pharmacodynamics. It indicates that the biological potencies for AspB9GluB27 and AspB9 in diabetic minipigs was reached with the results, shown in in vitro receptor-binding affinity (human hepatoma HepG2 cell line) and free-fat cell assay (Drejer et al., 1988; Brange et al., 1988). In addition, the similar in vivo biological activity between these analogues and human insulin was observed in diabetic minipigs, which was the same as reported in mouse blood glucose assay (Drejer et al., 1988; Brange et al., 1988) and in healthy pig assay by euglycemic clamp technique (Ribel et al., 1990). It indicates that the agreement could suggest that the mechanism of these analogues and human insulin in the chronic diabetic minipig could be supported by that in receptor-binding affinity, free-fat cell assay, mouse blood glucose assay and in healthy pig assay with euglycemic clamp technique. Furthermore, it is interesting to observe that there was a direct relationship between MRT and hypoglycemic potency for various analogues in chronic diabetic minipigs. In summary, although the insulin analogues are different from human insulin in pharmacokinetics, they exhibit similar biological activity to human insulin in the streptozotocin-induced chronic diabetic minipigs.

However, the observations of pharmacodynamics are different from that observed clinically in the normal and diabetic human (Kang et al., 1991a, c). Results indicate that the magnitude of the hypoglycemic nadirs is greater for AspB9GluB27 (–2.7 mM) and AspB28 (–2.9 mM) than that for human insulin (–1.9 mM). Moreover, the time of the hypoglycemic nadirs is shorter for AspB9GluB27 (62 min) and AspB28 (65 min) than that for human insulin (201 min). The different observations could be due to either the use of a small number of animal in the study or the difference in the hypoglycemic profiles of insulins between minipigs and humans. In summary, although the pharmacokinetics of AspB9GluB27 and AspB28 in chronic diabetic minipigs have been observed similar to that in healthy and diabetic human subjects, the hypoglycemic effects of these analogues in chronic diabetic minipigs have been shown to be smaller than that in normal and human subjects. Thus, the difference of the hypoglycemic effect between minipigs and humans needs to be further investigated.

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References


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