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Insulin Lispro: In-vivo Potency Determination by Intravenous Administration in Conscious Rabbits

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Abstract

Insulin lispro is a monomeric analogue of human insulin, produced by genetic engineering, and has been reported to have a more rapid absorption following subcutaneous injection than insulin. Since it has been shown to have a similar hypoglycaemic action to insulin in clinical studies and comparable properties in radioimmunoassay, the feasibility of using a bioassay which was designed originally for insulin, to measure insulin lispro potency was evaluated in this investigation.

A random-dose bioassay protocol, in which insulin lispro and two insulin standards were administered intravenously in a random sequence, was used and validated in nine conscious healthy rabbits. The decline in blood-glucose levels, following the intravenous injection of a dose of insulin or its lispro analogue, was monitored by a continuous glucose monitoring system. A glucose response curve was generated, from which various pharmacodynamic parameters were determined. Compared with the insulin standards, the potencies of insulin lispro determined from nadir, basal glucose normalized nadir, glycaemic reduction and ABGC (area of the blood-glucose response curve under baseline) were observed to have mean (95% confidence limits) values of 97·0 (69·5–124·6)%, 106·3 (72·4–140·2)%, 94·9 (51·8–138·0)% and 102·4 (76·3–128·5)%, respectively. In addition, the coefficients of variation for correspondent parameters were 36·9, 41·5, 59·1 and 33·2%, respectively.

The results indicated that the hypoglycaemic potency calculated from the ABGC values was the most accurate (102·4%) with the least coefficient of variation (33·2%). In conclusion, the potency of insulin lispro can be determined accurately from the ABGC values measured by the random-dose bioassay used.

Insulin lispro is a genetically-engineered analogue of human insulin which has been recently approved by the Food and Drug Administration for marketing in the US. Insulin lispro, which differs from human insulin only in the B-chain with the two neighbouring amino acids (i.e. proline and lysine) at positions 28 and 29 inverted in their sequence (Trautmann 1994), has demonstrated a remarkable reduction in tendency for self-association (Ciszak et al 1995; Bakaysa et al 1996). Several clinical studies have shown that the absorption of insulin lispro from a subcutaneous injection site is more rapid than that of regular human insulin (Howey et al 1994; Torlone et al 1994). A fast elimination of insulin lispro from the injection site has also been observed. The outcome is a rapid onset and a short duration of hypoglycaemic activity when insulin lispro is administered subcutaneously, which gives this monomeric analogue an advantage over human insulin, since a better control of meal-induced glycaemic levels is achieved (Howey et al 1995; Pampanelli et al 1995; Garg et al 1996; Heinemann et al 1996; Torlone et al 1996; Anderson et al 1997a, b, c; Burge et al 1997; Kotsanos et al 1997).

The determination of insulin lispro can be achieved by radioimmunoassay (RIA) (Holloway et al 1995; Burge et al 1996), however, RIA data do not provide quantitation of its biological potency. Although the biological activity of insulin lispro has been reported, a survey of the literature indicated that there have been no studies published on the potency determination of insulin lispro. A

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bioassay has been developed in this laboratory for measuring the potency of human insulin using the glycaemic responses following serial intravenous administrations of insulin, in conscious healthy rabbits (Lin & Chien 1995). In view of the similarity in their biological activity with regard to hypoglycaemic effect (Howey et al 1994; Torlone et al 1994; Trautmann 1994) and equivalent RIA displacement curves of insulin standards (Holloway et al 1995) between insulin lispro and human insulin, our bioassay method originally developed for measuring the hypoglycaemic potency of human insulin may be also suitable for insulin lispro. In this report, the results of the bioassay conducted with insulin lispro are presented. The hypoglycaemic potencies obtained from various glycaemic response parameters are compared with those for insulin standards and the implications of the results are discussed.

Materials and Methods

Materials

The YSI Industrial Analyzer (Model 27), glucose oxidase membranes, glucose standards and buffer solutions (for the Industrial Analyzer) were purchased from Yellow Springs Instrument (Yellow Springs, OH). The peristaltic pump and tubing were obtained from Cole-Parmer Instrument Co. (Niles, IL). PE-10 (non-radiopaque polyethylene microtubing) was from Clay Adams (Division of Becton Dickinson/Parsippany, NJ). Sterile water for injection, heparin lock flush solution ($100\,\text{units}\,\text{mL}^{-1}$), and a butterfly intermittent infusion set were supplied by Abbott Hospital, Inc. (North Chicago, IL). Tridodecyl-methylammonium chloride–heparin complex (TDMAC–heparin) was obtained from Polysciences, Inc. (Warrington, PA). Insulin lispro (Humalog, control number: OMK31M) and regular human insulin (Humulin R, control number: OMT76M) were manufactured by Eli Lilly & Co. (Indianapolis, IN) and obtained from a local pharmacy.

Instruments

Set-up. The continuous blood-glucose monitoring system was assembled by connecting together the sensor chamber in the YSI Industrial Analyzer, a peristaltic pump, a specially-designed mixing chamber and a data acquisition station (Lin et al 1993). The system was essentially composed of three stations in series: one for blood sampling and mixing with buffer solution, one for blood-glucose measurement and one for data acquisition. The internal surface of all tubing that could come into direct contact with the blood samples was coated with TDMAC–heparin to create a non-thrombogenic surface and to prevent the formation of blood clots (Grode et al 1969).

Calibration. Before pumping blood samples into the system, the glucose analyser was first calibrated twice with a glucose standard ($200\,\text{mg}\,\text{dL}^{-1}$) and buffer solution (no glucose) to ensure that linearity was attained for glucose concentrations up to $200\,\text{mg}\,\text{dL}^{-1}$. This procedure was repeated for every experiment. The analyser was calibrated routinely with buffer solution ($0\,\text{mg}\,\text{dL}^{-1}$ of glucose) and glucose standards (25, 50, 75, 100, 150 and $200\,\text{mg}\,\text{dL}^{-1}$). If a linear glucose concentration curve could not be attained, the glucose oxidase membrane was replaced. The precision of the glucose monitoring system was evaluated, using six concentrations of glucose standards ($25–200\,\text{mg}\,\text{dL}^{-1}$), and has already been reported (Lin et al 1993). In addition, the results reported previously concluded that linearity is established within the concentration range ($25–200\,\text{mg}\,\text{dL}^{-1}$) of test glucose standards (Lin et al 1993). The stability of the system was also evaluated; the results suggested that the system is very stable throughout the 6-h experiment (Lin et al 1993).

Preparation of animals

Healthy female New Zealand White rabbits (Davidson's Mill Farm, Jamesburg, NJ), weighing 2·5–3·5 kg, were fasted overnight. The following morning, each rabbit was put in a restrainer cage and hair around the outer marginal vein (running along the outer edge of the dorsal surface of the ear) shaved by an electric razor. After shaving, the area (used for cannulation) was first cleaned with an alcohol swab and then lignocaine ointment (5%) was applied to reduce the pain. The marginal ear vein was dilated by local mechanical stimulation. When the vein appeared dilated, a vascular access device with nonthrombogenic PE-10 tubing (10" long) attached was inserted into the vein (the vascular access device was prepared from a butterfly intermittent infusion set by removing the elastic tubing attached to the base of its 21-gauge needle). After gently inserting the PE-10 tubing (through the device) into the vein (to a depth of $\sim 2\,\text{cm}$), the vascular access device was removed leaving the PE-10 tubing inside the marginal vein. During this procedure, pressure was applied to the insertion site with a piece of gauze to stop the bleeding and a

piece of surgical adhesive tape was applied over the insertion site to secure the PE-10 tubing. A heparin lock flush solution ($\sim$0·5 mL) was flushed into the vein, through the PE-10 tubing, to prevent thrombus formation. The procedure was repeated on the other ear of the rabbit. After calibration of the blood-glucose monitoring system (described previously), measurement of blood-glucose levels was then initiated by connecting the nonthrombogenic blood-withdrawing tubing to one of the inserted PE-10 tubings for continuous blood sampling, using a peristaltic pump operating at 10 rev. min^{-1} and having a flow rate of 2–3 mL h^{-1} (tubing i.d. 0·25 mm). Simultaneously, a saline solution of heparin (50 units mL^{-1}) was continuously infused through the other PE-10 tubing, by the same peristaltic pump at a flow rate of 3–5 mL h^{-1} (tubing i.d. 0·35 mm).

Random-dose bioassay

Preparation of insulin standard solution. The insulin standard solution (1·0 int. units mL^{-1}) was prepared by diluting Humulin R with sterile water for injection under aseptic conditions. The insulin standard solutions were given in a volume of 0·1 mL (0·1 int. units per rabbit), representing insulin standard 1, and in a volume of 0·5 mL (0·5 int. units per rabbit), representing insulin standard 2.

Preparation of insulin lispro solution. The insulin lispro solution (1·0 int. units mL^{-1}) was prepared by the same procedure described above except using Humalog, and was given in a volume of 0·3 mL (0·3 int. units per rabbit).

Assay procedure. Before injections, the blood-glucose level in each test rabbit was monitored continuously for the baseline value. After a relatively stable baseline was attained and maintained for a period of at least 10 min, the first intravenous dose (0·1 int. units) of insulin was administered and the blood-glucose profile continuously monitored. The insulin solution was delivered, using a disposable syringe (1·0 mL), into the ear marginal vein through the PE-10 tubing (temporarily disconnected for the process of heparin infusion). Following insulin administration, the tubing was flushed with heparin lock flush solution (1 mL) before reconnection of the PE-10 tubing for continuous infusion of heparin. The blood-glucose levels in the rabbit were monitored continuously for a period of 2 h by the glucose analyser with the glucose concentrations being recorded every second by the on-line computer and graphic recorder, and saved as data on a floppy disk (at 20-s intervals). Two hours after administration of the first dose, the procedure was repeated for administration of the second intravenous dose (0·3 int. units) and the same procedure was followed. Another two hours after administration of the second dose, the procedure was repeated again for administration of the third intravenous dose (0·5 int. units). Two hours later, after administration of the third dose, the experiment was terminated. The sequence of 3 consecutive intravenous administrations (two insulin standards (0·1 and 0·5 int. units) and one insulin lispro (0·3 int. units)) to the rabbit was randomized.

In order to assess the effect of the crossover of dosing period and dosage strength on the blood-glucose response profile, the random-dose bioassay was repeated in an additional eight rabbits. The comparative study was performed using the study protocol wherein three rabbits were randomly assigned to one of three groups.

Data analysis

A method for determination of insulin potency, using several pharmacodynamic parameters attained from the blood-glucose response curve generated from a random-dose bioassay, has been developed (Lin & Chien 1995) and can be applied in this investigation. Therefore, the method was used to calculate the potencies of insulin lispro from several pharmacodynamic parameters, such as nadir, basal glucose normalized nadir, glycaemic reduction and ABGC (area of the blood-glucose response curve under baseline).

Statistical analysis

The Student's paired *t*-test was used to determine the statistical significance of the differences in potency calculated from various pharmacodynamic parameters. The analysis of variance test was used to determine any difference in sequence effect among the groups.

Results

Hypoglycaemic activity of intravenous insulin in a random-dose bioassay

Figure 1 shows a typical set of blood-glucose response profiles following 3 consecutive intravenous injections of insulin solutions (two insulin standards and one insulin sample) in a conscious

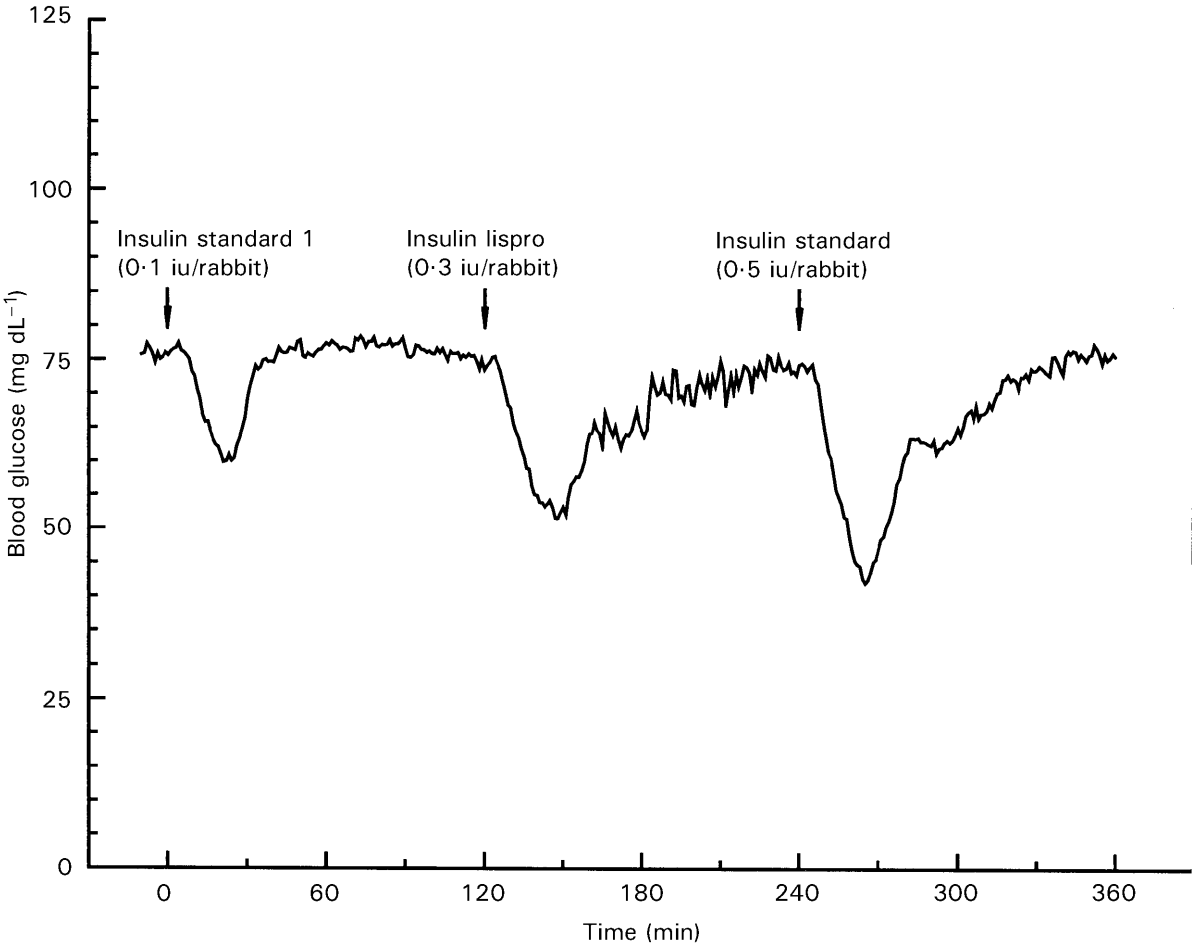

Figure 1. Typical blood-glucose response profiles in a conscious healthy rabbit, measured by the continuous glucose monitoring system, following the consecutive intravenous injections of three insulin doses, using the random-dose bioassay developed previously.

healthy rabbit, using the random-dose bioassay method we had developed previously. The hypoglycaemic response profiles obtained with all three insulin doses were very similar in pattern. Following the intravenous injection of insulin, the blood-glucose concentrations declined rapidly from the baseline level, with an onset time <5 min, and reached the maximum level of reduction (i.e. nadir) within 30 min. After reaching the nadir, the glucose concentrations rose gradually and attained a new baseline level within 90 min. The results in Figure 1 indicate that the depth of the nadir increased as the dosage strength of insulin solution administered increased.

Validation of the random-dose bioassay

The determination of insulin lispro potency was evaluated by repeated random-dose bioassay in nine conscious healthy rabbits and the results are outlined in Table 1. Insulin lispro potency, determined by various pharmacodynamic parameters, varied from one rabbit to the next. The insulin lispro had a mean potency of 106·3% (range 61·9–176·2%) based on the basal glucose normalized nadir or 97·0% (51·7–146·5%) based on the nadir; the coefficient of variation (CV) for the basal glucose normalized nadir is higher than that for the nadir (41·5% vs 36·9%). In addition, the results in Table 1 also suggest that the mean (CV) insulin lispro potency calculated from the glycaemic reduction data (94·9 ± 59·1%; range 49·8–224·5%)

Table 1. Comparison of pharmacodynamic parameters used in determination of insulin lispro potency from repeated random-dose bioassay in conscious healthy rabbits.

Parameters	Potency (%)	CV (%)
Nadir (mg dL^{-1})	97·0 ± 35·9	36·9
Basal glucose normalized nadir (%)	106·3 ± 44·1	41·5
Glycaemic reduction (mg dL^{-1})	94·9 ± 56·1	59·1
$ABGC_{0\rightarrow120}$ (% × min)	102·4 ± 34·0	33·2

Data are means ± s.d. (n = 9). ABGC was defined by the area of the blood-glucose response curve under the baseline.

is less accurate than that determined from nadir (97·0 ± 36·9%). On the other hand, the insulin potency calculated from the ABGC data (102·4 ± 33·2%; range 57·8–146·6%) has the highest accuracy among the pharmacodynamic parameters used (the CV value is 33·2%, which is the lowest CV value for all the pharmacodynamic parameters). However, there is no statistically significant difference ($P > 0·05$) in insulin lispro potency calculated from various pharmacodynamic parameters studied in the investigation.

Sequence effect on random-dose bioassay

The effect of the variation in the sequence of insulin administration on the determination of insulin potency, using this random-dose bioassay, was also investigated in three groups of animals. Statistical analysis of the results suggested that the differences among groups were statistically insignificant ($P > 0·05$) for all the pharmacodynamic parameters studied. The results demonstrate that the determination of insulin potency by this bioassay method is independent of the sequence of insulin injection.

Comparison of insulin lispro potencies calculated from individual and mean data

The insulin lispro potency calculated from individual and mean data obtained from repeated random-dose bioassays were compared. There was no significant difference ($P > 0·05$) between the potency calculated from the mean values obtained by repeated random-dose bioassays and the mean value of the potencies determined from each set of random-dose bioassays. It was concluded that there was no difference in the values of insulin lispro potency calculated from the various pharmacodynamic parameters determined by the mean values or by the individual values of repeated random-dose bioassays.

Discussion

Because insulin lispro was developed to resolve the problems associated with use of regular human insulin (peak of activity reached too late; hypoglycaemic effect possibly lasting too long) by subcutaneous injection (Holleman & Hoekstra 1997), most studies have been performed to compare them via the subcutaneous route, not via intravenous injection. In addition, a major difference between the two types of insulin is the rate of their self-disassociation, which causes differences in absorption rate from the injection site. However, this difference may not exist in intravenous administration. Therefore, comparative biological activity between insulin lispro and regular human insulin need to be evaluated via the intravenous route. Results in Figure 1 indicate that despite differences in insulin dose-strength administered, the hypoglycaemic response profiles after intravenous administration between insulin lispro and regular human insulin are very similar in pattern. Moreover, they reach the nadir almost at the same time. The results of potency calculated from random-dose bioassay in this investigation further confirm that their biological activities are equivalent. The bioequivalence between insulin lispro and regular human insulin in this investigation extends the findings of equivalent displacement between them in RIA reported by Holloway et al (1995).

The potency as calculated from the basal glucose normalized nadir should be more accurate than that calculated from the nadir, since the influence of intrinsic inter-dose variability is minimized during the random-dose bioassay (Lin & Chien 1995). However, it is interesting to note that the nadir normalization did not increase the accuracy in this investigation, due to the smaller inter-dose variation than in the previous study.

The accuracies associated with the pharmacodynamic parameters used in calculating the potency of insulin lispro in the same insulin sample have the following order: ABGC (102·4%) > nadir (97·0%) > glycaemic reduction (94·9%) > basal glucose normalized nadir (106·3%), since the following order is observed for the difference between the determined insulin lispro potency and the theoretical 100% value: ABGC (2·4%) < nadir (3·0%) < glycaemic reduction (5·1%) < basal glucose normalized nadir (6·3%). Because there is no statistical difference among the parameters studied, any one of them could be suitable for the determination of potency. Furthermore, the coefficient of variation for the insulin lispro potency determined using the various parameters have the following order: glycaemic reduction (59·1%) > basal glucose normalized nadir (41·5%) > nadir (36·9%) > ABGC (33·2%). Although ABGC seems to be most accurate parameter, it requires completed glycaemic profiles for the determination of potency and thus it is more complicated than the others. On the other hand, the potency calculated from nadir following random-dose bioassay can be determined easily with acceptable accuracy.

In conclusion, the insulin lispro potency can be calculated accurately from various pharmacodynamic parameters in random-dose bioassay of the insulin lispro with two insulin standards in random sequence, which can be easily completed. The various pharmacodynamic parameters can be attained from the glucose-response curve, which is

generated by a continuous glucose monitoring system following each insulin injection. The use of a continuous glucose monitoring system permits the measurement of blood-glucose levels, in a continuous manner, in the conscious animal. It also makes possible an accurate determination of the nadir value, which often occurs at an unpredictable time and lasts for only a few seconds.

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