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Pharmacokinetic–pharmacodynamic modelling of insulin: comparison of indirect pharmacodynamic response with effect-compartment link models

Senshang Lin and Yie W. Chien

Abstract

The pharmacokinetic and pharmacodynamic modelling of insulin has been reported using a combined pharmacokinetic/pharmacodynamic (PK/PD) model, in which a hypothetical effect compartment is linked to a pharmacokinetic compartment. Review of the literature, however, indicated that the recently developed PK/PD models have consisted of an indirect pharmacodynamic response component, but none of them has been applied to the modelling of insulin. To study the relative relevance of the indirect pharmacodynamic response model and the effect-compartment link model in modelling the pharmacokinetics and pharmacodynamics of insulin, regular human insulin was administered intravenously at a dose of 0.1 IU kg^{-1} to healthy Yucatan minipigs (after an overnight fasting). The plasma concentrations of insulin were measured by radioimmunoassay at predetermined time intervals, while blood glucose levels were monitored continuously using a glucose monitor. Analysis of the plasma insulin and the blood glucose profiles was performed by fitting with various PK/PD models and the results indicated that all of the 12 sets of plasma insulin data (after normalizing by the basal levels) have been adequately fitted to the two-compartment open pharmacokinetic model (a mean$\pm$s.e. correlation coefficient of 0.996$\pm$0.001 was obtained). The mean$\pm$s.e. correlation coefficient, the weighted residuals sum of squares (WRSS), and the Akaike's information criterion (AIC) were found, respectively, to be 0.935$\pm$0.008, 624$\pm$67, and 522$\pm$9 for the inhibitory indirect pharmacodynamic response model and 0.941$\pm$0.010, 547$\pm$63 and 513$\pm$9 for the stimulatory indirect pharmacodynamic response model, as compared with 0.725$\pm$0.041, 2309$\pm$276 and 628$\pm$10 for the effect-compartment link model. Based on these results, one may conclude that the indirect pharmacodynamic response model is a more appropriate approach for modelling the PK/PD of insulin than the effect-compartment link model.

St. John's University, College of Pharmacy and Allied Health Professions, 8000 Utopia Parkway, Jamaica, NY 11439, USA

Senshang Lin

Kaohsiung Medical University, College of Pharmacy, No. 100, Shih-Chuan 1st Road, Kaohsiung 807, Taiwan

Yie W. Chien

Correspondence: S. Lin, St. John's University, College of Pharmacy and Allied Health Professions, 8000 Utopia Parkway, Jamaica, NY 11439, USA. E-mail: linse@stjohns.edu

Introduction

The effect-compartment link model, which assumes the presence of a hypothetical effect compartment and proposes that a drug must first enter this compartment from either the pharmacokinetic central/peripheral compartment before its pharmacological response is exerted, has been reported (Sheiner et al 1979), reviewed extensively (Colburn 1981; Unadkat et al 1986; Verotta & Sheiner 1987; Meibohm & Derendorf 1997) and demonstrated in several drugs (e.g. midazolam (Tuk et al 1998), formoterol (eformoterol) (Derks et al 1997), alprazolam (Lau & Heatherington 1997), glibenclamide (Rydberg et al 1997), ibuprofen and flurbiprofen (Suri et al 1997), as well as insulin (Brown et al 1987)). Recently, four basic

indirect pharmacodynamic response models have been proposed to describe the pharmacodynamic responses to drugs produced by some indirect mechanisms, such as by inhibition or stimulation of the production or dissipation of factors controlling the responses measured experimentally (Dayneka et al 1993; Sharma & Jusko 1996). The applicability of these models has recently been demonstrated to a diverse array of drugs, such as fluocortolone (Lew & Jusko 1993), warfarin, furosemide (frusemide) and terbutaline (Jusko & Ko 1994), tolrestat (van Griensven et al 1995), fenoterol (Bouillon et al 1996), remoxipride (Movin-Osswald & Hammarlund-Udenaes 1995) and alprazolam (Lau & Heatherington 1997), but not yet for insulin.

In view of the physiological similarity of swine to humans (Panepinto & Phillips 1986), and the development of some effective and gentle animal-handling techniques (Panepinto et al 1983; Lin & Chien 1997), swine have been increasingly used as a reliable large-animal model for studying the pharmacokinetic and pharmacodynamic characteristics of 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 blood glucose monitoring system to continuously monitor the glycaemic state in a conscious animal has been demonstrated in this laboratory (Lin et al 1993). Use of the continuous glucose monitoring system has made possible an accurate determination of the nadir value, which is known to occur frequently at an unpredictable time and lasts only briefly. In this investigation, a group of healthy Yucatan minipigs received an intravenous bolus administration of insulin. Thereafter, the plasma insulin and blood glucose profiles were measured and then fitted by the indirect pharmacodynamic response models to evaluate the relevance of these models for simultaneously describing the relationship between the plasma insulin and the blood glucose profiles. In addition, the results were also compared with those obtained from the fitting by effect-compartment link model.

Materials and Methods

Pharmacokinetic/pharmacodynamic animal studies of insulin

Materials

Glucose oxidase membranes, glucose standards and buffer solutions 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. (Vernon Hills, IL). PE-10 (non-radiopaque 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). Human insulin (Novolin R) was from Novo Nordisk Pharmaceuticals Inc. (Princeton, NJ).

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 specially designed mixing chamber, and a data-acquisition station (Lin et al 1993). The system was composed of three stations in sequence: one for blood sampling and mixing with buffer solution, one for blood glucose measurement and one for data acquisition.

Animals

Male Yucatan miniature swine were purchased from Buckshire Corporation (Perkasie, PA). Pigs were housed individually in a pig pen (approximately 3.5 by 7.0 ft) and had free access to fresh water. They were fed a standard, commercially available diet (PMI Feeds Inc., St Louis, MO) formulated for swine, with free access twice daily, and exposed to automated 12-h light cycles. All synchronizers, including the feeding schedule, room temperature (68–70°F) and relative humidity (50%), were fixed. Initially, considerable time was spent hand-feeding and handling each pig to acclimatize it to its surroundings.

Preparation of animals

On the day of experiment, each pig (32–52 kg) was prepared as described elsewhere (Lin & Chien 1997). In brief, following a 16–24-h fast, each pig was comfortably immobilized in an upright position in a sling (Charles River Laboratories, Wilmington, MA) (Panepinto et al 1983). Thereafter, two sections of nonthrombogenic PE-10 tubing, coated on internal surface with TDMAC-heparin complex to create a nonthrombogenic surface of tubing to prevent the formation of blood clots, were cannulated into the veins of both ears (one tube for each ear). The cannulated tubings allowed easy serial/continuous blood sampling during the study.

Animal studies

Following the preparation of pigs, blood samples from each test pig were continuously drawn and pumped to a glucose monitor. This procedure was carried out by

connecting one of the inserted nonthrombogenic PE-10 tubings, described previously, to a peristaltic pump tubing (Tygon, i.d. 0.38 mm) with the pump operating at 10 rev min^{-1} and having a blood-withdrawing rate of $\sim$ 10 mL h^{-1}. After withdrawal, the blood samples were pumped through the glucose monitor and the glucose levels in the blood samples were analysed, instantaneously and continuously, by the glucose monitor and then the results were recorded by a computer at 1-min intervals (Lin et al 1993). After attaining a relatively stable baseline and maintaining for a period of at least 30 min, a single intravenous bolus injection of human insulin (0.1 IU kg^{-1}) was administered. The insulin solution (10 IU mL^{-1}) was prepared by diluting Novolin R (100 IU mL^{-1}) in sterile water for injection under aseptic conditions and delivered via a disposable 1-mL syringe into the ear vein through a PE-10 tubing. Following insulin administration, the tubing was flushed immediately with a heparin lock flush solution ($\sim$ 0.5 mL). The blood glucose levels in the pig were then continuously monitored for a period of 4 h.

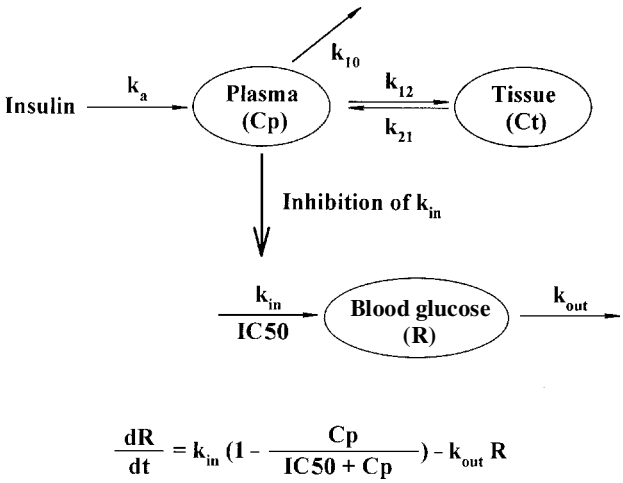
Insulin radioimmunoassay

During the course of continuous blood glucose monitoring through the first PE-10 tubing, serial blood samples ($\sim$ 0.3 mL each) were withdrawn through the second PE-10 tubing at predetermined intervals for radioimmunoassay of insulin. The blood samples were each collected in a chilled microtube and immersed in an ice bath. The blood samples and containers were maintained at 2–8°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 was performed using Coat-A-Count Insulin kits (with a sensitivity of 1.2 μIU mL^{-1}) (Diagnostic Products Co., Los Angeles, CA).

Pharmacokinetic/pharmacodynamic modelling of insulin

Pharmacokinetic model

A general two-compartment pharmacokinetic model has been developed to describe the pharmacokinetics of insulin in the body (Brown et al 1987). This model has the following assumptions: firstly, the basal level of the endogenous insulin remains constant and is insignificant; and secondly, all the rate constants for the kinetic processes, such as k_a, k_{10}, k_{12} and k_{21}, follow first-order kinetics. Based on this pharmacokinetic model, equation 1 has been derived and utilized to describe the plasma concentration profiles of insulin, after intravenous administration, in the central compartment described in Figures 1–3.



$$\frac{dR}{dt} = k_{in}\left(1 - \frac{Cp}{IC50 + Cp}\right) - k_{out}\,R$$

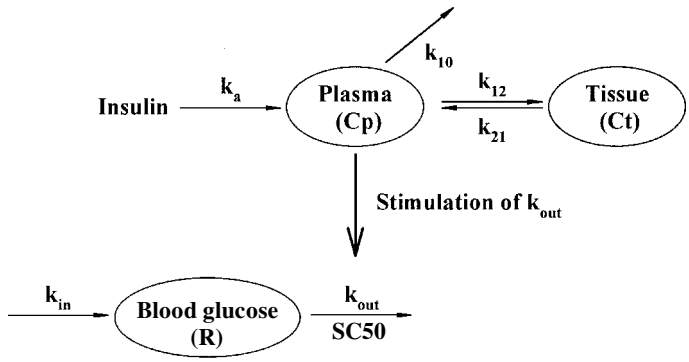
Figure 1 Schematic presentation of a two-compartment open pharmacokinetic model coupled with an inhibitory indirect pharmacodynamic response model. Also shown is the mathematical expression for the rate of insulin's hypoglycaemic effect derived from these models. Cp, plasma insulin concentration; Ct, tissue insulin concentration; t, time after dosing of insulin; k_a, first-order rate constant for insulin absorption; k_{10}, first-order rate constant for insulin elimination; k_{12} and k_{21}, rate constants for the reversible distribution of insulin between plasma and tissue compartments; k_{in}, apparent zero-order rate constant for the production of blood glucose; k_{out}, first-order rate constant for the utilization of blood glucose; IC50, insulin concentration that inhibits the maximal production of blood glucose by 50%; R, hypoglycaemic effect of insulin on blood glucose.

$$Cp = \frac{D}{Vp} \times \left[\frac{(k_{21} - \alpha) \times e^{-\alpha \times t}}{\beta - \alpha} + \frac{(k_{21} - \beta) \times e^{-\beta \times t}}{\alpha - \beta} \right] \quad (1)$$

where Cp is the plasma insulin concentration, D is the insulin dose administered intravenously, Vp is the volume of distribution of insulin in the central compartment, k_{21} is the rate constant for transfer between the central and the tissue compartments, α is the slope value of the distribution phase, β is the slope value of the elimination phase and t is the time following administration. In the case of intravenous administration, the first-order input rate (k_a) does not exist in a practical sense and therefore it is not included in equation 1.

Inhibitory indirect pharmacodynamic response model

The schematic presentation of the two-compartment open pharmacokinetic model in couple with an inhibitory indirect pharmacodynamic response model (Dayneka et al 1993; Model I) is shown in Figure 1 with the corresponding equation. The basic assumptions of this



$$\frac{dR}{dt} = k_{in} - k_{out}\left(1 + \frac{S_{max}\,Cp}{SC50 + Cp}\right) R$$

Figure 2 Schematic presentation of a two-compartment open pharmacokinetic model coupled with a stimulatory indirect pharmacodynamic response model. Also shown is the mathematical expression for the rate of insulin's hypoglycaemic effect derived from these models. Cp, plasma insulin concentration; Ct, tissue insulin concentration; t, time after dosing of insulin; k_a, first-order rate constant for insulin absorption; k_{10}, first-order rate constant for insulin elimination; k_{12} and k_{21}, rate constants for the reversible distribution of insulin between plasma and tissue compartments; k_{in}, apparent zero-order rate constant for the production of blood glucose; k_{out}, first-order rate constant for the utilization of blood glucose; SC50, insulin concentration that stimulates the maximal utilization of blood glucose by 50% ; S_{max}, maximal utilization of blood glucose contributed by insulin; R, hypoglycaemic effect of insulin on blood glucose.

pharmacokinetic/pharmacodynamic (PK/PD) model are that: firstly, the blood glucose level can be expressed by the equilibrium of k_{in} (the apparent zero-order rate constant for the production of blood glucose) and k_{out} (the first-order rate constant for the utilization of blood glucose) at all times; and secondly, the plasma levels of insulin, after an intravenous administration, can be described by a two-compartment open model and the insulin concentration in the plasma compartment has an inhibitory effect on the k_{in} and thus decreases the production of blood glucose. Using the PK/PD model in Figure 1 to establish the pharmacokinetic–pharmacodynamic relationship of insulin administered intravenously, equation 2 can be derived:

$$\frac{dR}{dt} = k_{in} \times \left\{1 - \frac{Cp}{IC50 + Cp}\right\} - k_{out} \times R \qquad (2)$$

where R is the hypoglycaemic effect of insulin on the blood level of glucose, Cp is the plasma concentration of the insulin described previously in the pharmacokinetic model, IC50 is the insulin concentration that could inhibit the maximum production of blood glucose by 50%, k_{in} is the apparent zero-order rate constant for the production of blood glucose and k_{out} is the first-order rate constant for the utilization of blood glucose.

Stimulatory indirect pharmacodynamic response model

The schematic presentation of a two-compartment open pharmacokinetic model in couple with a stimulatory indirect pharmacodynamic response model (Dayneka et al 1993; Model IV) is shown in Figure 2 with the corresponding equation. The basic assumptions of this PK/PD model are similar to the inhibitory indirect pharmacodynamic response model described above, except that the insulin concentration in the plasma compartment has a stimulatory effect on k_{out}, instead of an inhibition of k_{in}, to increase the utilization of blood glucose. Based on the PK/PD model in Figure 2, equation 3 can be derived:

$$\frac{dR}{dt} = k_{in} - k_{out} \times \left\{1 + \frac{S_{max} \times Cp}{SC50 + Cp}\right\} \times R \qquad (3)$$

where R is the hypoglycaemic effect of insulin on blood glucose, Cp is the plasma concentration of the insulin described previously in the pharmacokinetic model, SC50 is the insulin concentration that could stimulate the maximum utilization of blood glucose by 50%, S_{max} is the maximum utilization of blood glucose contributed from the insulin administered intravenously, and k_{in} and k_{out} have the same meaning as described in equation 2.

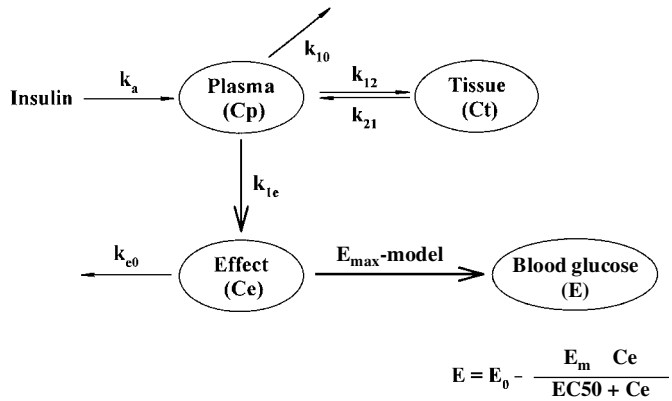


Figure 3 Schematic presentation of a two-compartment open pharmacokinetic model in which the hypothetical effect compartment is linked to the plasma pharmacokinetic compartment. Also shown is the mathematical expression for the rate of insulin's hypoglycaemic effect derived from these models. Cp, plasma insulin concentration; Ct, tissue insulin concentration; t, time after dosing of insulin; k_a, first-order rate constant for insulin absorption; k_{10}, first-order rate constant for insulin elimination; k_{12} and k_{21}, rate constants for the reversible distribution of insulin between plasma and tissue compartments; k_{1e}, rate constant linking the plasma compartment to a hypothetical effect compartment; Ce, insulin concentration in the effect compartment; k_{e0}, first-order rate constant for insulin elimination from the effect compartment; E, hypoglycaemic effect of insulin on blood glucose; E_0, baseline level of blood glucose prior to insulin administration; EC50, insulin concentration that produces 50% of the maximal effect on blood glucose; E_{max}, maximum effect on blood glucose contributed by insulin.

Effect-compartment link model

In this model, a two-compartment open pharmacokinetic model, which relates the plasma concentration of drug to its administered dose, is linked with a model that relates the plasma concentration to the concentration at the effect site, and a pharmacodynamic model that relates the drug concentration at the effect site to the observed effect (Colburn 1981). The schematic presentation of the model is shown in Figure 3, with the corresponding equation. The assumptions of this model are that: firstly, the plasma concentrations of insulin administered can be described by a two-compartment open pharmacokinetic model and the insulin concentrations in the plasma compartment can be linked to a hypothetical effect compartment; secondly, the rate constant linking the plasma compartment to a hypothetical effect compartment follows a first-order kinetics; and thirdly, blood glucose depends on only the insulin concentration in the effect compartment and is time-independent. Based on the PK/PD model in Figure 3, equation 4 can be derived:

$$E = E_0 - \left\{ \frac{E_{max} \times Ce}{EC50 + Ce} \right\} \quad (4)$$

where

$$Ce = \frac{D \times k_{e0}}{Vp} \times \left\{ \frac{(k_{21} - \alpha) \times e^{-\alpha \times t}}{(\beta - \alpha) \times (k_{e0} - \alpha)} + \frac{(k_{21} - \beta) \times e^{-\beta \times t}}{(\alpha - \beta) \times (k_{e0} - \beta)} + \frac{(k_{21} - k_{e0}) \times e^{-k_{e0} \times t}}{(\alpha - k_{e0}) \times (\beta - k_{e0})} \right\} \quad (4A)$$

in which E is the hypoglycaemic effect of insulin on blood glucose, E_0 is the baseline blood glucose level before insulin administration, EC50 is the insulin concentration that produces 50% of the maximum effect on blood glucose, E_{max} is the maximum effect on blood glucose resulting from the insulin administered, Ce is the insulin concentration in the effect compartment, D is the insulin dose administered intravenously, k_{e0} is the first-order rate constant for the elimination of insulin from the effect compartment, and Vp, k_{21}, α, β and t have the same meaning as in equation 1.

Models fitting

Before a model fitting, the plasma levels of insulin were first normalized by subtracting the correspondent basal level (mean of 3 time-point measurements before the administration of insulin) from the actual concentrations measured. The individual set of the normalized plasma insulin data was then fed into the two-compartment pharmacokinetic model described by equation 1, and curve fitting was made, with a weighting factor of -1, using the PCNonlin program (Version 4.2, Statistical Consultant, Inc., A Division of Pharsight Co., Cary, NC). The value of the pharmacokinetic parameters (k_{10}, k_{12}, k_{21}, etc.) required for the PK/PD models (described in equations 2–4) were obtained either as the primary or secondary parameters during the pharmacokinetic model fitting. Subsequently, the values of

pharmacokinetic parameters were fed into the PK/PD models together with the corresponding set of blood glucose data and curve fitting was then made by the PCNonlin program again. Each set of blood glucose data used by the program for the fitting of the various models consisted of a basal level (which was the mean of 30 time-point measurements before the administration of insulin) and 80 data-point measurements (which were the measurements at an interval of every 3 min following the administration of insulin). The mean values and s.e. of the correlation coefficients (R values), the weighted residue sum of squares (WRSS), and the Akaike's information criterion (AIC) (Yamaoka et al 1978) were examined to determine the goodness of each fitting.

Results

Pharmacokinetic modelling of insulin

The mean plasma insulin profiles (normalized by basal levels), in the healthy Yucatan minipigs (n = 12), after a single intravenous bolus administration of insulin (0.1 IU kg^{-1}), were compared with the results fitted by a two-compartment open pharmacokinetic model in Figure 4. The results indicated that all of the 12 sets of plasma insulin data had been adequately fitted by the two-compartment open pharmacokinetic model with the mean (±s.e.) correlation coefficient (r) of 0.996 (±0.001) (Table 1). The pharmacokinetic parameters obtained from the model fitting of the normalized plasma insulin profiles are outlined in Table 1. The mean value of k_{10} (51.64 h^{-1}) appears to be substantially (6-fold) higher than the values of k_{12} and k_{21} (8.453 and 8.646 h^{-1}, respectively). In addition, the mean value of α (61.48 h^{-1}) was also observed to be much (8-fold) higher than that of β (7.26 h^{-1}). The observations indicated that the insulin, after administration, is rapidly eliminated from the systemic circulation. Meanwhile, the administered insulin is distributed at similar rates of transfer between the central and the tissue compartments.

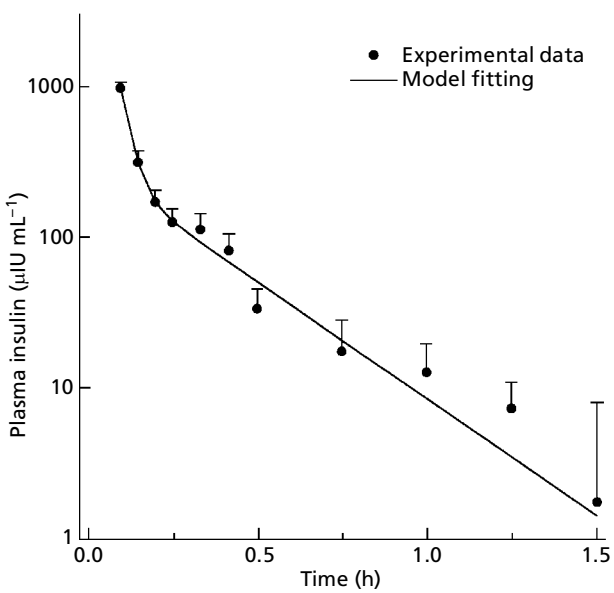


Figure 4 Mean (±s.e.m.) plasma insulin profiles (●), after normalizing by the baseline levels, following a single intravenous bolus administration of insulin (0.1 IU kg^{-1}) to healthy Yucatan minipigs (n = 12). The result fitted by a two-compartment open pharmacokinetic model is also shown (—).

Table 1 Pharmacokinetic parameters obtained from the model fitting, by a two-compartment open pharmacokinetic model, of the normalized plasma insulin profiles following its intravenous administration in healthy minipigs.

Parameters	Value
Dose (IU/pig)	4.01±0.2
k_{12} (h^{-1})	8.453±1.473
k_{21} (h^{-1})	8.646±0.953
k_{10} (h^{-1})	51.64±7.79
α (h^{-1})	61.48±9.03
β (h^{-1})	7.26±0.85
R	0.996±0.001
Sample size	12

Values are presented as means±s.e. k_{12}, rate constant for insulin transport from the plasma to the tissue compartments; k_{21}, rate constant for insulin transport from the tissue to the plasma compartments; k_{10}, first-order rate constant for insulin elimination from the plasma compartment; α, the slope value of the distribution phase; β, the slope value of the elimination phase; R, correlation coefficient among the observed concentrations and the predicted values.

Pharmacokinetic/pharmacodynamic modeling of insulin

The mean measured reduction profile of blood glucose following a single intravenous administration of insulin (0.1 IU kg^{-1}) to the healthy Yucatan minipigs (n = 12) was fitted by various PK/PD models in Figure 5. The hypoglycaemic profile in Figure 5 demonstrates that following the intravenous injection of insulin, a typical profile of insulin-induced hypoglycaemia is attained: the blood concentrations of glucose decline rapidly from the baseline level and reach the maximum level of reduction at around 30 min. After reaching the maxi-

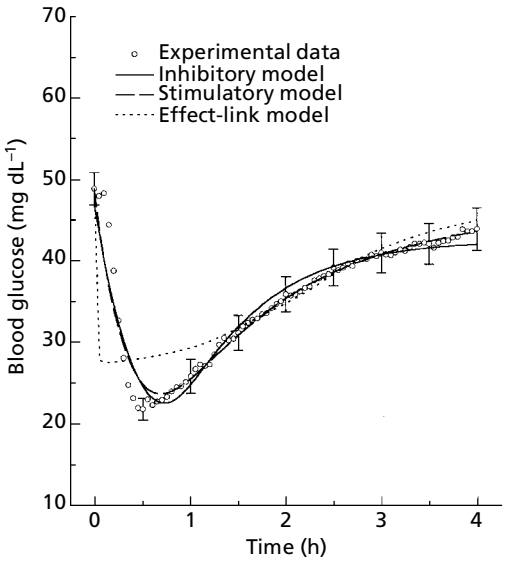


Figure 5 Mean (±s.e.m.) blood glucose profiles (○), after a single intravenous bolus administration of insulin (0.1 IU kg^{-1}) to healthy Yucatan minipigs (n = 12). The resultant fittings by various PK/PD models are also shown for comparison: solid line for fitting by the inhibitory indirect pharmacodynamic response model; dashed line for the fitting by the stimulatory indirect pharmacodynamic response model; and dotted line for the fitting by the effect-compartment link model. The standard errors are displayed at half-hour-intervals along the course of continuous blood glucose profiles.

mum reduction, the blood glucose concentrations rise gradually and then return to the original baseline level.

The pharmacodynamic parameters obtained from fitting of the blood glucose profile by the various PK/PD models are listed in Table 2 with the values of pharmacokinetic parameters. The mean (±s.e.) values of the correlation coefficient, the weighted residuals sum of squares (WRSS) and the Akaike's information criterion (AIC) determined were, respectively, 0.935±0.008, 624±67 and 522±9 for the inhibitory indirect pharmacodynamic response model, 0.941±0.010, 547±63 and 513±9 for the stimulatory indirect pharmacodynamic response model, and 0.725±0.041, 2309±276 and 628±10 for the effect-compartment link model. This suggests that the indirect pharmacodynamic response model is more relevant for the PK/PD modelling of insulin than the effect-compartment link model.

Discussion

By using the continuous blood glucose monitoring system, the maximum glucose response produced by insulin was detected and accurately determined in conscious swine, even though it occurred at an unpredictable moment and lasted for only a few minutes. Therefore,

Table 2 Comparison of pharmacodynamic parameters obtained from model fitting by the various PK/PD models following the intravenous administration of insulin to healthy minipigs.

Parameters	Indirect pharmacodynamic response		Effect-compartment link	
	Inhibition	Stimulation	Without time-lag[a]	With time-lag
k_{in} (mg $dL^{-1}h^{-1}$)	67.6±6.9	49.6±8.2	—	—
k_{out} (h^{-1})	1.62±0.16	1.14±0.21	—	—
IC50/SC50 (μIU mL^{-1})	23±17	13±8	—	—
S_{max} (mg/dL)	—	4.9±2.2	—	—
k_{e0} (h^{-1})	—	—	1.71±0.48	1.70±0.55
EC50 (μIU mL^{-1})	—	—	49±26	98±20
E_{max} (mg dL^{-1})	—	—	26.2±2.4	31.4±1.6
t_{lag} (h^{-1})	—	—	—	0.34±0.05
R	0.935±0.008	0.941±0.010	0.725±0.041	0.820±0.031
WRSS	624±67	547±63	2309±276	1854±359
AIC	522±9	513±9	628±10	597±19
Sample size	12	12	10	12

Values are presented as means±s.e. [a]Two of the 12 sets of data could not be fitted by the PK/PD model. k_{in}, apparent zero-order rate constant for the production of blood glucose; k_{out}, first-order rate for the utilization of blood glucose; IC50, the insulin concentration that inhibited 50% of the maximum blood glucose production; SC50, the insulin concentration that produced 50% of the maximum blood glucose utilization; S_{max}, maximum utilization of blood glucose attributed to the administered dose of insulin; k_{e0}, first-order rate constant for insulin elimination from the effect compartment; EC50, insulin concentration that produced 50% of the maximum effect on blood glucose; E_{max}, maximum effect on blood glucose attributed to the insulin dose administered; t_{lag}, lag-time or time delay; R, correlation coefficient between the observed concentrations and the predicted values; WRSS, weighted residual sum of squares; AIC, Akaike's information criterion.

the underestimation of maximum response, which generally occurs in the intermittent sampling method, can be avoided. The more accurate empirical determination of maximum response will permit a more faultless estimation of PK/PD parameters during the PK/PD modelling analysis. Despite the difference in the animal models (dog vs minipig) and in the disease state (alloxan-induced diabetes vs healthy) as reported in the literature (Brown et al 1987), agreement was observed in insulin pharmacokinetics which is better described by a two-compartment open model. The values of the model parameters may differ, in theory, depending upon the type of animals and disease states, not the model used. For example, in the case of the two-compartment open model for insulin pharmacokinetics, the attainment of a higher elimination rate (k_{10}) value than one measured previously in the same patient may indicate development of a rapid metabolism of insulin. The clinical benefits of the extrapolation of the various model parameters obtained from the model fitting have been well described elsewhere (Brown et al 1987).

It was surprising to observe a poor fitting of the blood glucose profiles, which have been reported in the literature to have a better correlation (Brown et al 1987), in this investigation when using the effect-compartment link model. This disagreement could result from the difference in the pharmacodynamic (i.e., glucose) profiles used: the fraction of maximum glucose response, which has involved some mathematical manipulation, was used in the previous study, while glucose level, which was measured directly by the instrument, without any mathematical treatment, was utilized in this investigation.

Artificial pancreas with a closed-loop system has been considered to be a better therapy for the treatment of insulin-dependent diabetes mellitus. The advantage of the closed-loop system is that it consists of a glucose sensor, which is capable of measuring the concentrations of glucose in the body, and a PK/PD model to analyse the glucose levels measured. The results are fed back to adjust the dose of insulin to be administered without any delay. To achieve this goal, we intend to develop a PK/PD model for utilization of the glucose data measured directly from a glucose sensor, and therefore avoid the need for use of the maximum glucose response to calculate the fraction of maximum glucose response.

In addition, the sites for glucose measurements, such as capillary, venous, arterial and subcutaneous tissue, also need to be evaluated during development of the PK/PD model, since differences in the glucose concentration measurements have been associated with the sites of sampling.

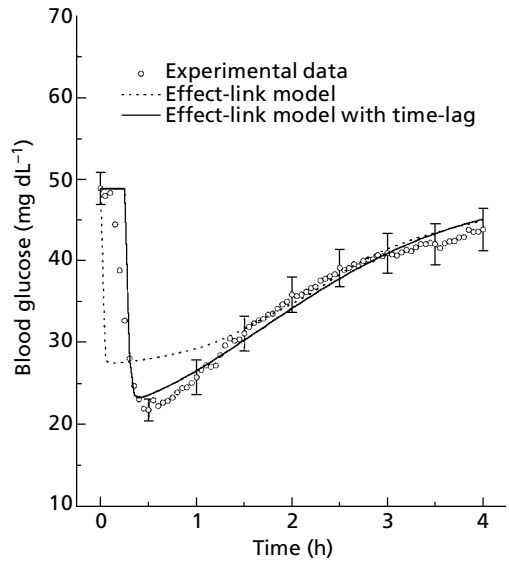


Figure 6 Mean (±s.e.m.) blood glucose profiles (○), after a single bolus intravenous administration of insulin (0.1 IU kg^{-1}) to healthy Yucatan minipigs (n = 12). The resultant fittings by the effect-compartment link models with (—) and without (- - -) time-lag are also shown for comparison. The standard errors are displayed at half-hour-intervals along the course of continuous blood glucose profiles.

One of the major objectives for this investigation is to compare the indirect pharmacodynamic response model with the effect-compartment link model for PK/PD modelling of insulin. Based on the resultant correlation coefficient, WRSS, and AIC data listed in Table 2, the relationship between the plasma insulin and the blood glucose profiles has been better fitted by the indirect pharmacodynamic response models than by the effect-compartment link model. The results suggested that the hypoglycaemic effect of insulin on blood glucose could be better described by the indirect response mechanism than by the direct response mechanism. This finding of indirect response of insulin on the reduction of glucose agrees well with the results obtained from the euglycaemic clamp studies which concluded that insulin acts slowly in-vivo although it acts rapidly under in-vitro situations (Doberne et al 1981).

For the indirect response mechanism underlying the insulin-induced hypoglycaemic effect, the effect-compartment link model might be more appropriate if it takes the time-lag into consideration. To evaluate this hypothesis, the plasma insulin and the blood glucose profiles were further evaluated using the effect-compartment link model with time-lag taken into account (Figure 6). Results in Table 2 indicate that the values of correlation coefficient, WRSS, and AIC suggest a better

fitting has been attained by the effect-compartment link model with time-lag than without it. This observation supports the involvement of an indirect pharmacodynamic response concept in the insulin-induced hypoglycaemic effect. Based on the resultant correlation coefficient, WRSS, and AIC data (Table 2), the effect-compartment link model, however, still could not demonstrate its superiority, even with time-lag taken into consideration, over the indirect pharmacodynamic response model.

While the mechanism involved in insulin's action in reducing blood glucose is a complex one and has not been fully explored at a cellular level, a single gateway hypothesis has been proposed (Bergman 1997). This hypothesis could be utilized to support the results obtained from the PK/PD modelling of insulin in this investigation. The single gateway hypothesis suggests that following its administration, insulin is first transported slowly across the transendothelial barrier, binds to its receptors on the membrane of muscle and adipose tissue, and then produces the translation and activation of insulin-dependent glucose transporters, such as GLUT4 (Verhey et al 1995). Blood glucose, after entering the cells, is utilized as the energy source or stored as glycogen. And more importantly, during this glucose utilization process, a signal is triggered instantly and sent immediately to the liver to inhibit the output of hepatic glucose. Therefore, the effect of insulin on the control of glucose output from the liver is proposed to be an indirect rather than a direct response.

Based on the results obtained from the indirect pharmacodynamic response in this investigation, the insulin concentration required to trigger the glucose utilization responses has a lower value (13 vs 23 μIU mL^{-1}) than that needed to inhibit the output of hepatic glucose. In other words, the insulin in the peripheral circulation has a faster effect on glucose utilization than on the inhibition of hepatic glucose output. This observation agrees with the single gateway hypothesis and may suggest that the inhibitory indirect response model may be an appropriate PK/PD model to described the pharmacokinetic and pharmacodynamic relationship of insulin.

Despite the attainment of a better fitting of insulin-induced hypoglycaemic profiles by the indirect pharmacodynamic response models than the effect-compartment link model, none of these models developed is based on the physiological action of insulin which has effects on the inhibition of hepatic glucose output in the liver as well as the stimulation of glucose in the peripheral tissues, like muscle and adipose tissue. As a result, an appropriate physiologically-based PK/PD model needs to be developed. Currently, a model which consists of both inhibitory and stimulatory indirect pharmacodynamic responses is under evaluation in this laboratory and the results will be reported in the future.

Conclusions

The pharmacokinetics of insulin administered intravenously could be described by a two-compartment open model. The hypoglycaemic response to insulin could be predicted more accurately by using the indirect response models than the effect-compartment link model. However, none of these indirect response models studied is based on the physiological actions of insulin. Appropriate physiologically-based PK/PD models, which may consist of both inhibitory and stimulatory indirect pharmacodynamic responses, need to be developed.

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