An Approach to Predict the Ductus-Arteriosus Dilating Effect Induced by Lipo-Prostaglandin E₁ in Newborn Rats Lacking Plasma Concentration-Time Data by the Pharmacological Response Kinetic Model

Keita YAMAUCHI, Shoji YASUNAGA, Hiromu KAWASAKI, and Yuji KUROSAKI*

Department of Clinical Pharmaceutical Science, Graduate School of Natural Science and Technology, Okayama University; 1–1–1 Tsushima-naka, Okayama 700–8530, Japan. Received July 29, 2002; accepted October 22, 2002

The usefulness of kinetic analysis of pharmacological response data was discussed in investigating the ductus arteriosus dilating effect (DADE) of lipo-PGE₁ (a lipid emulsion preparation of prostaglandin E₁ for injection) preparations. Lipo-PGE₁ was administered intravenously via the umbilical vein by a bolus injection or an infusion in newborn rats 60 min after the delivery. The DADE data were expressed as the inner diameter ratio between the ductus arteriosus and the main pulmonary artery, and were analyzed by a pharmacological response kinetic (PRK) model consisting of an $E_{\text{max}}$ model and a simple pharmacokinetic model as the pharmacodynamic- and the pharmacokinetic-component, respectively. The former component includes the release process of free-PGE₁ from the lipid phase of lipo-PGE₁, followed by distribution to the effect compartment. The $E_{\text{max}}$ value was estimated by the DADE observed 10 min after the bolus administration of each dose, and the value was fixed in the PRK analysis. The regression curves given by simultaneous non-linear least squares regression analysis were satisfactorily fitted to the observed DADE data at all doses. Prediction of the DADE of lipo-PGE₁, in an infusion study was satisfactorily done using the estimated parameters in the i.v.-study. These findings indicate that PRK modeling based on the intensities of the observed pharmacological response-time data is a meaningful tool in some targeting-type drugs, for which pharmacokinetic analysis itself is meaningless or acquisition of pharmacokinetic data is technically impossible, in predicting the time courses of the drug’s pharmacological response in different dosage regimens.

Key words lipo-prostaglandin E₁; ductus-arteriosus; pharmacokinetic/pharmacodynamic model; pharmacological response kinetic model; newborn rat; targeting-type drug

Prostaglandin E₁ (PGE₁) and Prostaglandin E₂ selectively dilate ductus arteriosus in many mammal neonates.¹ A cyclodextrin preparation of PGE₁ (PGE₁-CD)² and a lipid microsphere preparation of PGE₁ (lipo-PGE₁)³,⁴ have both been widely used in emergency treatment in neonatal patients with ductus dependent congenital heart diseases (D-CHDs). However, a fatal adverse drug reaction, apnea, may occur when PGE₁ is overdosed.⁵ Lipo-PGE₁ is a passive targeting-type drug delivery system (DDS) of PGE₁, in which PGE₁ is dissolved in the lipid emulsion of soybean oil.⁶ Momma reported that the ductus-dilating intensity of lipo-PGE₁ was approximately ten times higher than that of PGE₁-CD by the clinical monitoring of arterial blood pO₂ in D-CHD patients.³ Thus, lipo-PGE₁ therapy allows for a reduction of the total dose of PGE₁. Further, the ductus-dilating effect after the discontinuation of lipo-PGE₁ administration lasts much longer than that of PGE₁-CD.³,⁷,⁸ However, its kinetics have not been clarified from the biopharmaceutical viewpoint. This makes it difficult to present evidence-based precise lipo-PGE₁ therapy in D-CHD patients.

Pharmacokinetic analysis based on blood or plasma concentration profiles of drugs has been utilized for optimization of the dosage regimen. Pharmacokinetic/pharmacodynamic (PK/PD) modeling, which is an integrated mathematics model of a pharmacokinetic (concentration-time) and a pharmacodynamic (effect-concentration) event, is a useful tool for predicting the time course of pharmacological response.⁹ Ordinary PK/PD modeling studies require validation of both the pharmacokinetic and pharmacodynamic aspects.¹⁰ However, it is difficult to obtain both the pharmacokinetic and pharmacodynamic data in, for example, the following cases: as a highly potent drug is administered in quite a low dose, it is difficult to determine plasma concentration of the drug; blood or plasma concentration has no meaning in the targeting-type DDS drugs because systemic drug level does not reflect its local pharmacological response. The ductus-dilating effect of PGE₁ has been reported to be extremely potent.¹¹ The clinical dose of lipo-PGE₁ (5 ng/kg/min), a targeting-type DDS, is too low to determine the plasma concentration profiles during the treatment. Thus, in the case of lipo-PGE₁ therapy in D-CHD patients, the acquisition of systemic pharmacokinetic data is technically impossible, and the pharmacokinetic analysis itself is meaningless. In such a case, validation of the model by systemic pharmacokinetic data is impossible, and the only way we can validate the model will be validation by the pharmacological response-time data.

In this study, we have assessed changes in the diameter of ductus arteriosus after the bolus i.v. administration of lipo-PGE₁ in a multi-dose study to define a suitable pharmacological response kinetic model (PRK model) and related kinetic parameters. Also, we have investigated whether the model could predict the time course of the ductus-dilating effect under the constant infusion of lipo-PGE₁. The utility of the new modeling concept, in which the model lacks PK validation, has been discussed.

MATERIALS AND METHODS

Chemicals Lipo-PGE₁ preparations, in which PGE₁ was dissolved in lipid microspheres (PGE₁ content: 5 or 20 µg/ml), were kindly supplied by Taisho Pharmaceutical Co., Ltd. (Tokyo, Japan). All other chemicals were of reagent grade.

Animals Female Wistar rats on days 8—14 of pregnancy
were obtained from Shimizu Laboratory Supplies (Kyoto, Japan). On day 21 of pregnancy, the animals were sacrificed by cervical dislocation, and all the fetuses, together with the placentas, were delivered quickly by Caesarean section. The delivered newborn rats were placed on a thermo regulated platform at 38 °C during the experiment.

Administration of Lipo-PGE

In the bolus i.v. administration study, lipo-PGE, was diluted to final concentrations of 1.0, 2.5, 4.0 and 20 μg/ml with isotonic saline. Diluted preparations were injected (10 μl/kg) with a microsyringe via the umbilical vein of neonatal rats 60 min after the Caesarean section. For the experiments of constant infusion, a diluted lipo-PGE, (10 μg/ml) was prepared with isotonic saline, and the preparation was constantly infused through the cannula inserted in the umbilical vein at a rate of 2.5 μg/kg/min for 120 min.

Microscopic Measurement of Inner Diameters of Ductus Arteriosus and Pulmonary Artery

The ductus-dilating effect induced by lipo-PGE, determined by a previously published method. The newborn rats administered lipo-PGE, were fixed by a rapid whole-body freezing technique using hexane cooled by dry ice. The thorax of the frozen newborn was trimmed and mounted on a tissue holder to obtain a sectioning surface perpendicular to ductus arteriosus. The pulmonary artery and ductus arteriosus was sectioned from the start of the pulmonary artery to the end of the ductus with a cryostat, and mounted on the slide glass. The sections were stained with hematoxylin and eosin. The inner diameter of the pulmonary artery and ductus arteriosus was measured with a micrometer under a binocular microscope. Since the pulmonary artery did not change by administration of PGE, the ductus-dilating effect was expressed by the DA/PA ratio, where the diameters of ductus arteriosus (DA) and pulmonary artery (PA) were obtained by the minimum diameter of ductus arteriosus and by the diameter measured in the middle section of pulmonary artery, respectively.

Pharmacodynamic Modeling

The relationship between the dose of lipo-PGE, and the ductus-dilating effect data was determined by the following equation:

\[ E = \frac{E_{\text{max}} \cdot D}{ED_{50} + D} + E_0 \]  

(1)

where \( D \) is the dose of lipo-PGE; \( E_{\text{max}} \) and \( E_0 \) represent the peak of the ductus-dilating effect observed in each dose after bolus injection of lipo-PGE; the intrinsic maximal DA/PA level in which lipo-PGE, can archive and the base line effect of saline, respectively; \( ED_{50} \) represents the dose of lipo-PGE, evoking a one-half maximal effect. The \( E_{\text{max}} \) value was fixed when the PK/PD parameter were estimated. The parameters were estimated by a nonlinear least squares program, MULTI.

Pharmacokinetic and Pharmacodynamic Modeling

We hypothesized that the basic kinetic model of PGE, is simple. The PK/PD model for the ductus-dilating effect induced by lipo-PGE, consists of the central compartment (plasma) and lipid phase (lipid microspheres), and the effect compartment (Fig. 1).

In the bolus i.v. injection of lipo-PGE, the time courses of the amount in each compartment are expressed by the following differential equations:

\[ \frac{dA_1}{dt} = -k_1 \cdot A_1 \cdot A_2(0) = x \cdot D \]  

(2)

\[ \frac{dA_2}{dt} = k_1 \cdot A_1 - K \cdot A_2 \cdot A_4(0) = (1-x)\cdot D \]  

(3)

\[ \frac{dA_4}{dt} = k_{1e} \cdot A_1 - K \cdot A_2 \cdot A_4(0) = 0 \]  

(4)

where \( t \) represents the time after administration; \( A_1, A_4 \) and \( A_4 \) represent the amounts of PGE, in the lipid phase, the central compartment and the effect compartment, respectively; \( A_2(0), A_4(0) \) and \( A_4(0) \) represent the initial amount of \( A_1, A_4 \) and \( A_4 \), respectively; \( k_1, k_{1e}, k_1, k_{1e} \) and \( K \) are the first-order rate constants. \( K \) represents the sum of \( k_1 \) and \( k_{1e} \). Our previous study suggested that the retention rate of PGE, in lipid emulsion changes with dilution ratio and/or the type of aqueous infusion. Therefore, lipo-PGE, preparations required a correction by the drug distribution ratio, “x”. The rates of PGE, retention in lipid emulsion were 0.855, 0.689, and 0.468, respectively, when diluted 2, 5 and 10 times with physiological saline.

In the constant infusion study, the time courses of the amount in each compartment are expressed by the following differential equations:

\[ \frac{dA_1}{dt} = I \cdot x - k_1 \cdot A_1 \cdot A_2(0) = 0 \]  

(5)

\[ \frac{dA_2}{dt} = I \cdot (1-x) + k_1 \cdot A_1 - K \cdot A_2 \cdot A_4(0) = 0 \]  

(6)

\[ \frac{dA_4}{dt} = k_{1e} \cdot A_1 - k_{1e} \cdot A_2 \cdot A_4(0) = 0 \]  

(7)

where \( I \) represents an infusion rate.

The ductus-dilating effect is directly related to the amount of drug in the effect compartment. Therefore, the time course of the ductus-dilating effect is based on the following equation:

\[ E(t) = \frac{E_{\text{max}} \cdot A_4}{EA_{50} + A_4} + E(t)_{\text{cont}} \]  

(8)

where \( EA_{50} \) represents an amount of drug in the effect compartment evoking a one-half maximal effect, and \( E(t)_{\text{cont}} \) is the time course of the base line, which is defined as the following monoexponential function for convenience:

\[ E(t)_{\text{cont}} = a \cdot e^{-t} \]  

(9)
where $a$ and $b$ are the constants.

The parameters were estimated by the simultaneous fitting of the observed pharmacological data in the bolus injection study to the theoretical kinetic equation derived as its integrated form using a nonlinear least-squares program, MULTI, by the Simplex method. In this estimation, $(E_i)^{0.017}$ was adopted as the weight where $E_i$ represents the observed ductus-dilating effect of the $i$-th point. The area under the pharmacological response curve ($AUPRC$) of the observed ductus-dilating effect and simulation curves were obtained by the trapezoidal rule.

RESULTS

**Bolus i.v. Administration** Immediately after delivery, the ductus arteriosus of newborn rats was fully dilated. The inner diameter ratio of DA to PA determined was 0.748 ± 0.036 (mean ± S.E.) in this study. Thereafter, the ductus arteriosus constricted rapidly. The DA/PA ratios decreased to 0.097 ± 0.017 within 60 min after the delivery (Fig. 2). However, the ratio increased, i.e., the ductus arteriosus dilated again after the bolus i.v. of lipo-PGE$_1$ preparations. At all doses, the DA/PA ratio reached its maximum at 10 min after the injection of lipo-PGE$_1$. Then the DA/PA ratio decreased gradually. The ductus arteriosus closed completely 180 min after the injection.

As the relationship between the dose of lipo-PGE$_1$ and the maximal dilating effect observed 10 min after the injection showed a typical nonlinear profile (Fig. 3), an $E_{\text{max}}$ model expressed by Eq. 1 was adapted to describe the relationship. The model-dependent parameters, $E_{\text{max}}$ and ED$_{50}$, were estimated to be 0.500 and 1.01 $\mu$g/kg, respectively. However, the latter parameter, ED$_{50}$, was uncertain, because the degree of the maximal was almost the same, even at the lowest dose of lipo-PGE$_1$ in this study. Thus, the $E_{\text{max}}$ value estimated for intravenously administered lipo-PGE$_1$ preparations in ductus arteriosus dilating action was used as the only parameter fixed in the following PRK analysis.

The PRK model shown in Fig. 1 was designed to express the entire range of DA/PA ratio profiles after bolus i.v. injection of lipo-PGE$_1$ preparations (Fig. 4). The DA/PA ratio data obtained in 4 different doses were simultaneously fitted to the theoretical kinetic equation (Eq. 8) by using a non-linear least squares regression program ‘MULTI'. The estimated parameters for the ductus-dilating action of lipo-PGE$_1$ and the resultant regression curves are shown in the Table 1 and Fig. 4, respectively. Thus, this constructed PRK model seemed to satisfactorily express the ductus-dilating action of intravenously administered lipo-PGE$_1$ at any dose. As we do not have information on concentration-time profiles of lipo-PGE$_1$ in this study, validation of the PK-model component in the PRK model is impossible. However, the PRK modeling has effectively expressed the time courses of the pharmacological effect after the i.v. bolus injection of lipo-PGE$_1$ in rats.

$AUPRC$ is a parameter representing the pharmacological availability of drugs. The $AUPRC$ values of the observed ductus-dilating effects in newborn rats were calculated and plotted against the dose of lipo-PGE$_1$ (Fig. 5). Lipo-PGE$_1$ increased $AUPRC$ dose-dependently. The $AUPRC$ values obtained from the simulation curves based on the PRK modeling also well represented those values from the observed data. This suggests that the PRK model constructed in this study is useful in estimating the bioavailability of the pharmacological effect of intravenously administered lipo-PGE$_1$.

**Infusion Study** In the infusion study, the lipo-PGE$_1$ (10 $\mu$g/ml) preparation was constantly infused into the umbilical vein at a rate of 2.5 $\mu$g/kg/min for 120 min. The DA/PA ratio increased to a maximal level of 0.630 at 60 min after the start of the infusion, and was maintained at a steady state level until the infusion was stopped (Fig. 6). A striking pharmacological response profile was recognized after termination of the infusion. The ductus-dilating effect continued to remain at the steady state level for about 30 min after stopping the infusion. Then, the DA/PA ratio decreased gradually, and the ductus arteriosus was closed completely by 180 min after discontinuation of the infusion of lipo-PGE$_1$. A simulation curve predicted by the PRK model incorporated with the estimated parameters in the bolus i.v. administration study was extremely well fitted to the observed data obtained in the infusion study. Thus, a satisfactory prediction of the time course of the ductus-dilating effect of lipo-PGE$_1$ after constant infusion was achieved by the PRK modeling, although the model lacked validation by the pharmacokinetic data.
DISCUSSION

PGE₁ is an effective agent in treating peripheral vascular disorders such as Buerger's disease and connective tissue disease. However, its biological half-life is quite short because of its rapid metabolic elimination in the circulation, especially in the lung. A lipid microsphere preparation consisting of soybean oil and lecithin has been marketed as an intravenously injected nutritional supplement. Lipo-PGE₁, in which PGE₁ was dissolved in lipid microspheres, has succeeded in increasing the clinical efficiency of PGE₁. Approximately 90% of PGE₁ exists in the lipid phase in lipo-PGE₁. As PGE₁ molecules in the lipid microspheres can effectively avoid metabolic elimination in the lung, their biological half-life is much more prolonged. As a result, the total dose of PGE₁ in lipo-PGE₁-therapy in the neonatal D-CHD can be reduced compared with the pharmacotherapy by PGE₁-CD. On the other hand, clinical reports also clarified that the duration of the effect of lipo-PGE₁, especially the duration after the discontinuation, was longer than that of
PGE₁-CD. These factors make it difficult to predict the profiles of its clinical effect, which will be necessary for making precise dosage regimens for lipo-PGE₁-therapy.

We have investigated the time courses of the ductus-dilating effect following the i.v. bolus injection or constant infusion of lipo-PGE₁ into neonatal rats. From the time-course data in the bolus injection study, we estimated kinetic parameters representing the PRK model (Fig. 1). Then, we validated the model by predicting the ductus-dilating profile in neonatal rats receiving a constant infusion of lipo-PGE₁. As shown in Fig. 6, the prediction was satisfactory.

In our previous study, PGE₁ in lipo-PGE₁ was not distributed solely in the lipid microspheres but also in the aqueous phase. Therefore, we assumed that the PGE₁ distributed in the aqueous phase was immediately distributed into the plasma compartment. In contrast, PGE₁ in lipid microspheres was released into the plasma compartment by a first order rate constant when lipo-PGE₁ was injected into newborn rats. However, the observed peak of the ductus-dilating effect appeared at 10 min in each dose of lipo-PGE₁ after i.v. bolus administration. We assumed the presence of anti-clockwise hysteresis on the relationship between the plasma concentration of PGE₁ and the ductus-dilating effect.

Generally, there are several reasons for the delay in the pharmacological effect against plasma drug concentration. The major reason for this delay is that the effect site compartment is considered to be different from the central compartment. The pharmacological effect evoked by active metabolites is another possibility for the delay. The PRK model designed in this study includes an effect compartment. Clyman et al. reported that 13,14-dihydro-prostaglandin E₁, one of the metabolites of PGE₁, has ductus-dilating activity similar to PGE₁. The contribution of the active metabolite in estimating the EA₅₀ value (Table 1) expressing the amount of drug in the effect compartment evoking a one-half maximal effect is unclear in this study. Leonhardt et al. reported that plasma concentrations of PGE₁ and its metabolite are almost the same. So, it has been thought that PGE₁ and its active metabolite play the same role in the ductus-dilating effect.

The effect compartment model proposed by Sheiner et al. is one of the general and practical models for correcting the anti-clockwise hysteresis in the PK/PD relationship. In the present study, we introduced the concept of the effect compartment model to the PRK model. Pharmacodynamic data sets obtained in different PGE₁ doses were fitted simultaneously to the PRK model. Cawello et al. reported that the plasma elimination of PGE₁ exhibited two phases, that is, their half-lives were 0.2 min and 8.2 min for the α-phase and the β-phase, respectively, in human subjects. However, by using the estimated parameters, the plasma concentration of PGE₁ in the newborn rats was predicted to decline mono-exponentially, and the biological half-life was estimated to be 3 min. Thus, we suggest that the approximate plasma elimination kinetics of PGE₁ can be simply presented by a 1-compartment model in this PRK modeling.

The intravenous infusion of lipo-PGE₁ is the most common clinical treatment to dilate ductus arteriosus in D-CHD. The ductus-dilating effect reached a plateau by 60 min after the start of lipo-PGE₁ infusion. The DA/PA values at the steady states ranged from 0.316 to 0.630, owing to the infusion rate of lipo-PGE₁. The steady state ductus-dilating effect lasted for approximately 30 min after the discontinuation of the infusion. This ductus-dilating effect disappeared 180 min after the discontinuation. Chino et al. reported that the ductus-dilating effect of PGE₁-CD decreased rapidly after discontinuation of the infusion, whereas that of lipo-PGE₁ remained over 30 min. Therefore, this long-lasting action in ductus-dilating effect is distinctive in lipo-PGE₁. We simulated the kinetics of the ductus-dilating effect in the infusion study by using the parameters estimated in i.v. bolus injection studies. The simulation curves satisfactorily represented the observed pharmacological-effect profiles. This result suggested that our newly constructed PRK model is suitable for simulating the ductus-dilating effect of lipo-PGE₁. Among three transfer rate constants, i.e., kᵣ, K and kₑ, estimated in this PRK modeling, kₑ showed the smallest value (Table 1). Thus, the elimination process of PGE₁ from the effect compartment is probably the rate-determining step in the long-lasting lipo-PGE₁ action after discontinuation of the infusion.

The PK/PD modeling approaches usually require both the plasma concentration data and pharmacological response data to discuss the validation of its rationalities in prediction. However, in some cases, for example, in the case of a high potency drug being administered at quite a low dose, or with administration of a targeting-type DDS drug, it is technically difficult to obtain blood or plasma concentration in the former case, and plasma concentration does not reflect its local pharmacological response in the latter case. PGE₁ has a very high vaso-dilating effect, and lipo-PGE₁ showed a long-lasting ductus-dilating action compared with PGE₁-CD. The PK/PD modeling based on the observed pharmacological-response is considered to be a meaningful approach in some drugs, in which pharmacokinetic analysis is otherwise incapable of or unreliable in predicting the time-course of the pharmacological response in different dosage regimens. We intend to clarify the targeting function of lipo-PGE₁ to the ductus arteriosus in ductus-dilating action by constructing an extended PRK model in the next step.
**APPENDIX**

The integrated solutions for the lipo-PGE$_1$ in the PRK model are described as follows.

(a) **I.V. Bolus Injection Study**

\[
A_t = k_{le} \left\{ \frac{A_0}{(K - k_e)(k_e - k_i)} e^{-k_i \cdot t} + \frac{A_0}{(k_i - K)(k_i - k_e)} e^{-k_e \cdot t} + \frac{A_0}{(k_i - k_e)(k_e - k_i)} e^{-k_i \cdot t} \right\}
\]

where $f(t)$ means

\[
f(t) = \frac{A_0}{k_{le}}
\]

Ductus-dilating effect is expressed as follows:

\[
E(t) = \frac{E_{max} \cdot f(t)}{E_A_{50}/k_{le} + f(t)} + E(t)_{cont}
\]

(b) **Constant Infusion Study**

\[
A_t = k_{le} \left\{ \frac{I}{K - k_e} + \frac{I \cdot x}{(K - k_e)(k_e - k_i)} e^{-k_i \cdot t} - \frac{I \cdot x \cdot k_i + I \cdot (1 - x) \cdot (k_i - K)}{K \cdot (k_e - K)(k_i - k_e)} e^{-k_i \cdot t} - \frac{I \cdot x \cdot k_i + I \cdot (1 - x) \cdot (k_i - K)}{k_i \cdot (k_i - k_e)(K - k_e)} e^{-k_i \cdot t} \right\}
\]

Ductus-dilating effect is expressed as follows:

\[
E(t) = \frac{E_{max} \cdot g(t)}{E_A_{50}/k_{le} + g(t)} + E(t)_{cont}
\]

where $g(t)$ means

\[
g(t) = \frac{A_0}{k_{le}}
\]