Comparison of Simulated and in-Vivo Plasma Levels of Cilastatin Following Intravenous in-Line Drug Administration

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The primary objective of this work was to establish a method to simulate the plasma levels of cilastatin, a model drug, following an intravenous in-line delivery scheme. In-vivo data in dogs obtained from this work were used to demonstrate the validity of the proposed approach. The in-line drug delivery system consists of a drug containing device which is placed between a large volume parenteral and a patient. Numerous advantages have been identified for this automatic in-line reconstitution delivery system. The numerical convolution integral algorithm was used in this work to perform plasma profile simulation. The results indicated that the simulated cilastatin plasma profile following in-line delivery closely agreed with the in-vivo data.

KEY WORDS: intravenous drug delivery; in-line administration; zero-order infusion; bolus injection; numerical convolution integral; cilastatin.

INTRODUCTION

An in-line delivery system for intravenous (I.V.) drug administration consists of a drug containing device which is placed between a large volume parenteral (LVP) and a patient (1). A schematic representation of the device is shown in Figure 1. The system administers drug intravenously via automatic in-line reconstitution as the large volume parenteral solution flows through the system at a controlled flow rate. This in-line system has various advantages over the conventional infusion systems. The system eliminates the secondary infusion set and the mini-bag used in a typical I.V. piggyback drug delivery. Since the reconstitution of the powder or liquid drug in the device is performed automatically as the parenteral solution (also termed as the diluent) flows through the system, the in-line drug delivery is deemed labor-saving. The device is also convenient to use and the potential for human error in preparing the medication using conventional I.V. admixture practices may be reduced. In addition, the system eliminates drug waste because the device is activated immediately prior to I.V. administration. Finally, unstable drugs can be formulated in solid dosage forms and be placed in the device with improved shelf-life. All these advantages are intended to reduce the costs associated with I.V. drug therapy.

The release kinetics of drug from the in-line system in general depends on a number of factors (1), including 1) the dissolution rate of a solid drug, and 2) the dispersion effect, which indicates the residence time distribution of the drug molecules in the system. The dissolution rate is a function of drug solubility (2). Whereas the dispersion effect is a function of the diluent flow rate, the volume of the fluid in the system, and the geometric configurations of the system (3). Due to these factors, the resulting plasma profile may therefore be different from those obtained using currently accepted I.V. administration practices; for example, zero-order (constant rate) drug delivery. The method of numerical convolution integral algorithm was therefore proposed to simulate the plasma concentrations following this in-line drug therapy.

The primary objective of this work was to establish a method for the simulation of plasma levels following I.V. in-line drug administration. The validity of this method was verified by the in-vivo data in dogs. The approaches to achieve this objective include: 1) obtaining the pharmacokinetic parameters of a model drug via bolus injection and 2) using these parameters to simulate drug plasma profiles and compare these profiles with the in-vivo data following zero-order and in-line administrations in dogs.

EXPERIMENTAL

Animal Preparation

Six male mongrel dogs weighing 20 to 35 kg were used in this study. The dogs were ordered and maintained in the laboratory services animal care facility under the direction of staff veterinarians.

Selection of a Model Drug

The commercially available cilastatin for the I.V. administration purpose is contended in Primaxin®, which is a sterile powder manufactured by Merck Sharp & Dohome consisting of imipenem and cilastatin in 1:1 ratio. Cilastatin is provided in the form of cilastatin sodium (4) which is very soluble in water (>2 g/ml), and its delivery kinetics in the in-line system can be regarded as dispersion control, as discussed in the INTRODUCTION section. Imipenem (5) is provided as a free acid form with relatively poor aqueous solubility (10 mg/ml), and its delivery kinetics is a function of dissolution and dispersion, yielding a slower delivery rate than cilastatin sodium. The delivery and simulation studies in this work focus on cilastatin sodium only.

Drug Administration

The cilastatin, in the form of cilastatin sodium, was administered with imipenem to six male mongrel dogs. A bolus injection (10 mg Primaxin®/kg, an average dose of approximately 137 mg of cilastatin), reconstituted with normal saline, was administered over 3-4 minutes to the dogs. For

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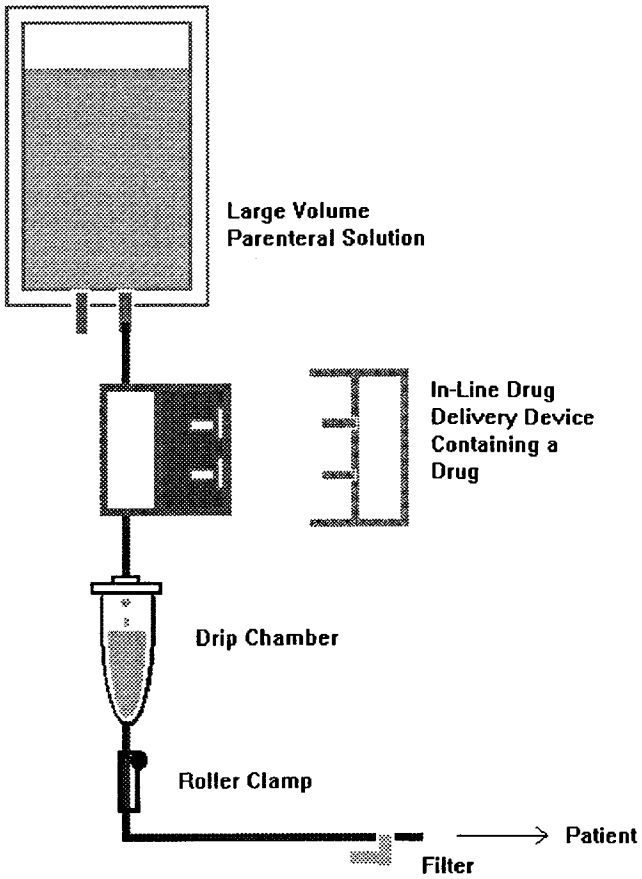

Fig. 1. In-line drug administration.

in-line delivery and zero-order infusion, normal saline was also used as the diluent to deliver the drugs. A 0.22 micron downstream filter was used during the in-line infusion. A dose of 250 mg cilastatin was given via zero-order or in-line infusion. For the zero-order infusion, the 250 mg dose (5 mg cilastatin per ml concentration) was infused at a constant flow rate of 2 ml/min for 25 min. Whereas a 250 mg dose was delivered via the in-line system at 2 ml/min for 60 min.

In-Vivo Experimental Design

Six mongrel dogs were equally and randomly divided between two groups (Blocks 1 and 2; see Table I) in accordance with the Latin Square design (6). Since it was difficult to perform administrations for 6 dogs on the same day due to limited resources, the drugs were administered in two consecutive days to these dogs. Blocks 1 and 2 represent two consecutive treatment days. For example, on day one dog number 5 (Block 1) was administered the drug under Treatment Order I (see Table I). After the treatment day, the dogs were allowed six days to clear the residual drug before proceeding to Treatment Order II, and so on for Treatment Order III. Block 2 dogs were treated in an identical fashion beginning one day following that of the Block 1 animals.

Blood samples from each dog were collected prior to treatment. Following the administration of Primaxin®, blood samples from each dog were taken at predetermined time periods for determination of plasma cilastatin concentration. Plasma was promptly separated in a refrigerated centrifuge

Table I. Latin Square Design*

Block*	Dog number	Treatment order[a]		
		Day 1	*Day 7*	*Day 14*
1	5	I	II	III
	2	II	III	I
	4	III	I	II
		Day 2	*Day 8*	*Day 15*
2	3	I	II	III
	6	II	III	I
	1	III	I	II

*: Blocks 1 and 2 repesent two consecutive treatment days.
[a] The treatments were: I; I.V. bolus. II; zero-order infusion. III; in-line infusion.

(2°C to 5°C), stabilized with the addition of an equal volume of a 1:1 mixture of 1 M morpholineethanesulfonate buffer (pH 6.0) and ethylene glycol, followed by storing at −70°C to −80°C until assay.

The cilastatin in plasma was assayed by a high performance liquid chromatography (HPLC). A Waters HPLC system equipped with an Alltech Adsorbosphere C8 column (15 cm X 0.46 cm; 5 micron particles) was used. A filtered mobile phase containing 40 ml of methanol, 100 ml of 0.1 M MOPS buffer, and 860 ml deionized water was prepared prior to assays. The 0.1 M MOPS buffer contained 20.9 g of 3-[N-morpholino]propane-sulfonic acid in 1000 ml of deionized water at pH 7.0. The mobile phase flow rate was set at 4.0 ml/min and the temperature of the HPLC column was equilibrated at 50°C. The injection volume was 10 microliters and the detector wavelength was set at 245 nm.

SIMULATION OF PLASMA DRUG LEVELS

In-Line Cilastatin Delivery Profile

The in-vitro delivery profile of 250 mg cilastatin via the in-line system was experimentally determined by collecting the effluent and assaying the cilastatin concentration using a conductance method (7). The results are presented in Figure 2. The vial content of cilastatin was calculated by averaging the values of the last four data points in the plateau region of the delivery profile. This vial content was denoted as 100% of cilastatin delivered. Based on this vial content value, each cilastatin delivery data point in the same profile was expressed as percent delivered.

The mathematical expression of an in-line drug delivery profile was previously described using a Weibull function (3). The mathematical expression is

$$\%\text{Delivery} = 100(1\text{-}e^{-F_2(t-T_{lag})^{F_1}}) \quad (1)$$

where the lag time, T_{Lag}, is the time required to analytically detect drug following the activation of the in-line device, and t is the time after activation. The accumulated mass delivered at any time, M_t, can be expressed in terms of %Delivery, as

$$M_t(t) = \frac{X_0\ \%\text{Delivery}}{100} \quad (2)$$

where X_0 is the dose of cilastatin.

The delivery rate profile is obtained by taking the first derivative of Equation 2 with respect to t, as given by

$$\text{Rate}(t) = X_0 F_1 F_2 (t - T_{Lag})^{(F_1-1)} e^{-F_2(t-T_{Lag})^{F_1}} \quad (3)$$

A non-linear least squares data fitting program (8,9) was used to compute the parameters, F_1, F_2, and T_{Lag}. The experimental and fitted in-line delivery profiles for cilastatin sodium are presented in Figure 2. As can be seen in Figure 2, the delivery profile of the in-line infusion for a given flow rate is not linear over time. The in-line drug administration rate, as indicated in Equation 3, is therefore not constant.

Pharmacokinetic Model

The distribution and elimination of cilastatin in plasma follow a two-compartment open model (10), as depicted in Figure 3 (11). The plasma level of cilastatin in the central compartment can be expressed by

$$C_p(t) = \frac{X_0}{V_p}\left\{\frac{K_{21} - \alpha}{\beta - \alpha} e^{-\alpha t} + \frac{K_{21} - \beta}{\alpha - \beta} e^{-\beta t}\right\} \quad (4)$$

where α and β are the hybrid first order rate constants for the distribution phase and elimination phase, respectively. Other symbols in Equation 4 are defined as follows:

X_0: The cilastatin (in form of cilastatin sodium) dose administered.
V_p: The apparent volume of distribution in the central compartment.
K_{21}: First order rate constant.

Based on the in-vivo biexponential decline (see Equation 4) of cilastatin concentrations in plasma following the bolus injection (see Figure 4), the pharmacokinetic parameters were computed using the curve fitting software SigmaPlot® (Jandel Scientific, California).

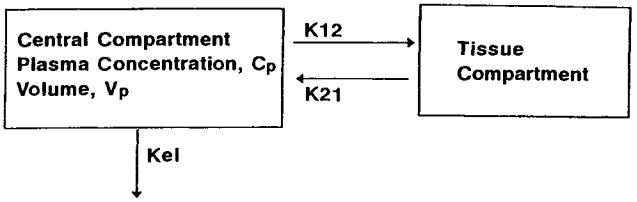

Fig. 3. Two-compartment pharmacokinetic model. The descriptions of K_{12}, K_{21}, and K_{el} are shown in Equation 4 and Table II.

Plasma Cilastatin Levels of Various Administration Schemes Computed by Numerical Convolution Integral Algorithm

For any drug administration scheme, the plasma drug levels can be simulated using the following numerical convolution integral algorithm, provided that the pharmacokinetics is linear (12):

$$\text{Con}(t) = \int_0^t \text{Rate}(t') E(t - t') dt' \quad (5)$$

where t' is a dummy variable used for convolution integration. The term E(t), which is equal to $C_p(t)/X_0$, is the normalized plasma cilastatin concentration profile following the bolus injection as described earlier. It is noted that the integration of E(t) from time zero to infinity gives a value of one, indicating that E(t) is a probability density function.

For in-line drug administration, Equation 3 describes the drug input rate, Rate(t), which is time dependent. Equation 6, however, is applicable to all I.V. drug administration practices with constant input rate. For zero-order infusion or bolus injection, the term Rate(t) can be simplified as

$$\text{Rate}(t) = X_0/(T_0) \quad (6)$$

where T_0 is the total infusion or injection time. In order to perform a fair comparison for the simulated plasma responses using bolus injection, zero-order infusion, and in-line delivery, the lag time T_{Lag}, in the in-line, in-vitro delivery profile (as indicated in Equations 3) was excluded in the computation. For a given value of t, the integration of Equation 5 gives rise to the concentration of cilastatin in the

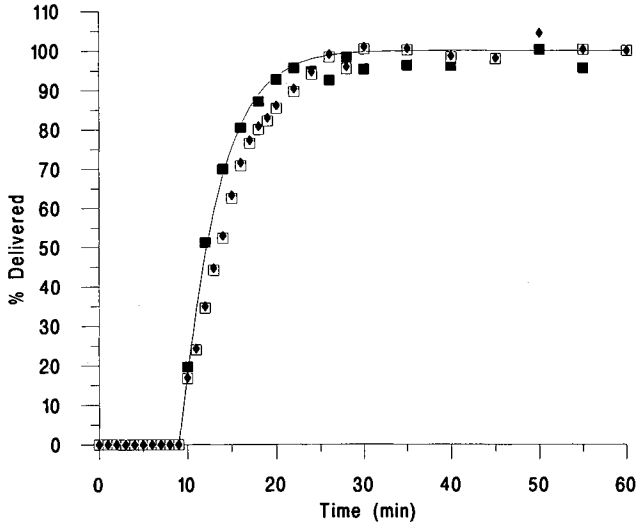

Fig. 2. Experimental [Run 1 (■); Run 2 (□); Run 3 (♦)] and simulated (—) in-line delivery profiles for cilastatin sodium.

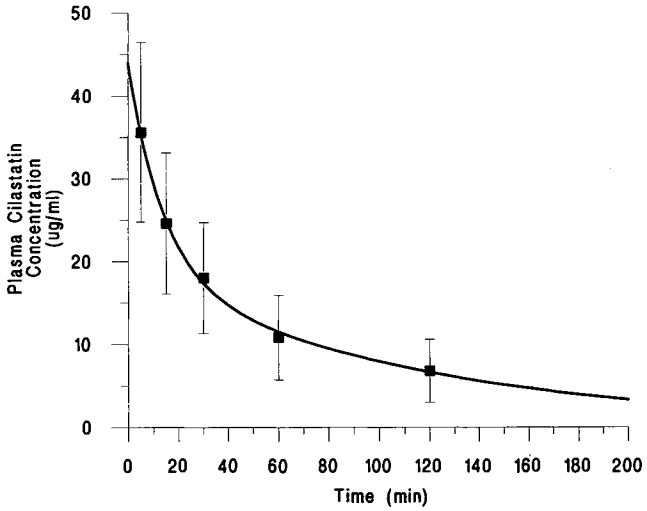

Fig. 4. Experimental (■) and simulated (—) plasma profiles of cilastatin sodium following bolus injection.

Table II. Pharmacokinetic Parameters (Two Compartment Pharmacokinetic Model) for Cilastatin in Dogs

Parameters	
V_p	3.11×10^3 ml
K_{12}	0.02735 min^{-1}
K_{21}	0.03617 min^{-1}
K_{el}	0.01738 in^{-1}
α:	0.07219 min^{-1}
β:	0.008710 min^{-1}

V_p: Volume of distribution in the central compartment.
α: First order rate constant for the distribution phase.
β: First order rate constant for the elimination phase.
K_{21}: First order rate constant.
K_{el}: First order elimination rate constant ($K_{el} = \alpha\beta/K_{21}$) (11).
K_{12}: First order rate constant ($K_{12} = \alpha + \beta - K_{el} - K_{21}$) (11).

plasma. The numerical integration of Equation 5 was performed by a FORTRAN program developed in this work.

RESULTS

In-Vivo Experiments

Using the in-vivo plasma cilastatin elimination profile following a bolus injection (see Figure 4), the parameters, α, β, K_{21}, and V_p (see Equation 4) in dogs were determined using SigmaPlot®, as described earlier. The fitted profile is indicated by the solid curve in Figure 4 and the resulting pharmacokinetic parameters are presented in Table II. The V_p, K_{21}, α, and β values were used for the simulation of plasma cilastatin levels under various administration schemes.

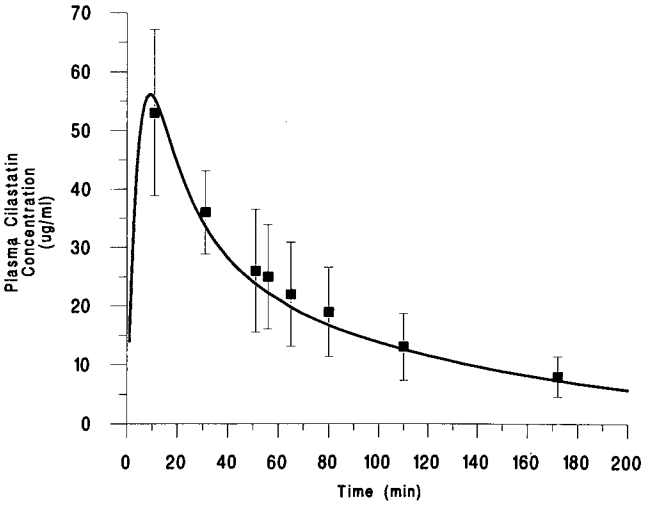

Fig. 5. Experimental (■) and simulated (—) plasma profiles of cilastatin sodium following in-line infusion.

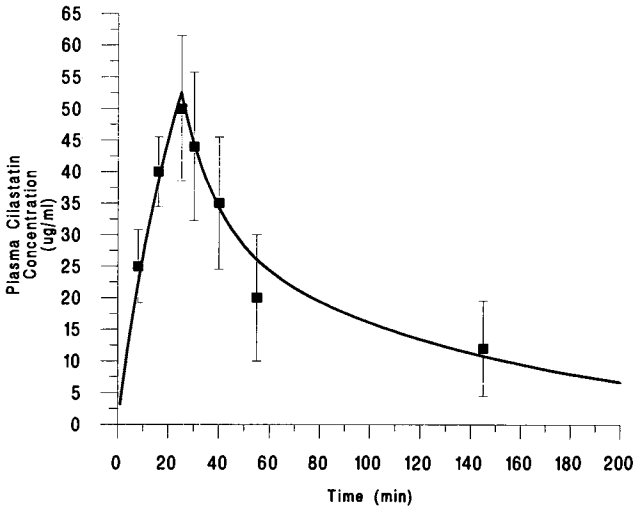

Fig. 6. Experimental (■) and simulated (—) plasma profiles of cilastatin sodium following zero-order infusion.

Simulation Using Convolution Integral

The S-shaped percent delivery versus time data for an in-vitro profile, as depicted in Figure 2, was conveniently fitted by the Weibull function (see Equation 1) using the nonlinear least squares algorithm (8,9). The F_1, F_2, and T_{Lag} values were determined to be 1.1034, 0.1951, and 8.9994 min, respectively. Once the parameters F_1 and F_2 had been determined, the plasma cilastatin levels were calculated by using the numerical convolution integral algorithm. As can be seen in Figure 5, the simulated plasma cilastatin concentration profile following in-line administration closely agrees with the in-vivo results. In addition, the in-vivo plasma levels following zero-order infusion (see Figure 6) were also successfully simulated using the same approach.

Table III shows the simulated plasma peak concentrations (C_{max}), the time values to reach C_{max} (T_{max}), and area under curve values (AUC) for bolus, zero-order, and in-line administrations in dogs. As can be seen, the C_{max} and AUC of cilastatin following in-line administration are comparable to the zero-order infusion.

Table III. Comparison of the Simulated T_{max}, C_{max}, and AUC Values Following in-Line Cilastatin Administration with the Corresponding Values Simulated Using Currently Accepted I.V. Injection/Infusion Methods

	Administration dose (mg)	T_{max} (min)	C_{max} (μg/ml)	AUC (μg · min/ml)
Injection (3–4 min)	137	4	41	2524
Infusion (2 ml/min for 25 min)	250	25	53	4542
In-Line (2 ml/min for 60 min)	250	9	56	4341

CONCLUSION

The simulated plasma levels of cilastatin following bolus, zero-order, and in-line administrations strongly agree with the in-vivo data obtained from mongrel dogs using the same schemes of administrations. This study therefore demonstrates the validity of the numerical convolution integral algorithm proposed in this work for simulating plasma drug concentrations following I.V. in-line drug administration. Furthermore, the C_{max} and AUC values for in-line and zero-order infusions were comparable. Thus, the clinical responses of drug delivery using the in-line system is anticipated to be similar to those resulting from zero-order drug infusion. The FORTRAN source codes for computing the convolution integral is available upon request from W. Kuu.

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