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An *In Vitro* Model for the Study of Antibacterial Dosage Regimen Design

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Abstract □ A model was developed that is capable of simulating antibacterial agent concentration *versus* time profiles commonly observed following intravenous and intramuscular bolus injections, intravenous infusions, and oral doses, administered as single or multiple doses. The model consisted of two physical compartments separated by a membrane of a commercial hemodialyzer. The 1.08 m^2 membrane surface area allowed rapid transmembrane passage of drugs and other small molecules, while membrane pore size prevented bacterial passage. These characteristics allowed bacteria in one of the two compartments of the model to be exposed to time-variant drug concentrations without affecting the number or concentration of bacteria. The model was used to study the effects of a multiple intravenous bolus dosage regimen of ampicillin on *Escherichia coli* ATCC 12407.

Keyphrases □ Penicillin—*in vitro* model for the study of antibacterial dosage regimen design □ Models—*in vitro*, study of antibacterial dosage regimen design □ Antibacterials—*in vitro* model, study of dosage regimen design

The antibacterial agent concentration profiles to which bacteria are exposed *in vivo* vary with the method of drug administration. Continuous intravenous infusion yields constant plasma and tissue drug concentrations once steady state is achieved, while the short elimination half-life of most antimicrobial agents results in rapid decreases in plasma and interstitial fluid drug concentrations (1) following bolus intravenous, intramuscular, or oral doses.

Considerable progress has been made during the past 15 years in determining the mechanism of action of β-lactam antibiotics (2, 3). However, the relative therapeutic effectiveness of intermittent and continuous dosage regimens for these compounds is uncertain (4, 5). Attempted correlations of therapeutic effectiveness with various pharmacokinetic parameters, such as maximum plasma concentration (6), the time period during which drug levels exceed the minimum inhibitory concentration (7), area under the plasma level curve (8), intensity factor (9), and the degree of serum protein binding (10), have been difficult due to the many interactions between the drug, bacteria, infection site, and host. Information ob-

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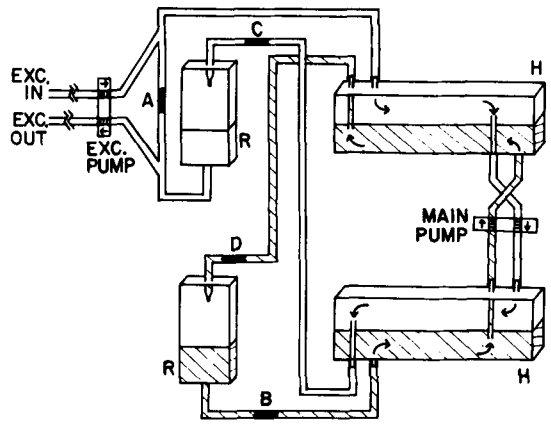

Figure 1—*Schematic diagram of model showing sampling ports (A,B,C,D), hemodialysis units (H), reservoirs (R), tubing, main pump, and exchange pump. Arrows indicate direction of broth flow.*

tained from *in vitro* studies of bactericidal kinetics at constant drug concentrations is not directly applicable to the situation *in vivo* where drug concentrations may undergo rapid changes. The importance of phenomena such as the postantibiotic effect in multiple dose antibiotic regimens has also been difficult to assess in animal models and clinical situations due to uncontrollable factors (11).

The present report describes the theory, characterization, and testing of an *in vitro* model which can be used to monitor the antibacterial effect of simulated plasma drug concentration profiles from constant intravenous infusions or from single or multiple intravenous bolus, intramuscular, or oral doses.

EXPERIMENTAL

Drugs—Solutions of anhydrous ampicillin powder[1] in Mueller-Hinton broth[2] were prepared immediately prior to use. Drug concentration was monitored by the inclusion of a trace amount of [^{14}C]penicillin G potassium[3] (16.22 mCi/mmole) in a broth solution of the unlabeled drug. Scintillation counts were obtained on 0.1-ml broth samples in 4.0 ml of a scintillation solution[4].

Bacteria—An overnight culture of *Escherichia coli*, ATCC 12407[5], in Mueller-Hinton broth was serially diluted in broth to ~10^2 colony-forming units/ml (cfu/ml) and allowed to grow to 10^8 cfu/ml. A 1.0-ml aliquot of this solution was used to inoculate the model. Bacterial growth was monitored in 16 × 125-mm glass tubes using optical density measurements obtained spectrophotometrically at 500 nm[6]. Optical density was linearly related to bacterial concentrations between 6×10^5 and 4×10^8 cfu/ml. The minimum inhibitory concentration for ampicillin against the test organism was obtained by the broth dilution method (12) with an initial inoculum size of 5×10^4 cfu/ml and incubation at 37° for 18 hr.

Viable Cell Assay—Viable cell counts were obtained by performing six consecutive serial dilutions of a 0.1-ml broth sample in 0.9 ml of 0.9% sodium chloride solution at 10°. Nutrient agar[2] plates were divided into eight sectors and streaked with duplicative 10-μl aliquots of each dilution. Visual inspection of the number of colonies formed in each sector after incubation at 37° for 12 hr gave the number of colony-forming units per milliliter in the original sample. Colony recounts following an additional 12 hr of incubation failed to show an increase in colony numbers.

Model Description—The model consisted of a closed loop of 0.95-mm i.d. tubing[7] on each side of the membrane of a hemodialyzer[8] (Fig. 1).

[1] Wyeth Laboratories, Philadelphia, Pa.
[2] Difco Laboratories, Detroit, Mich.
[3] Radiochemical Centre, Amersham, England.
[4] Aquasol, New England Nuclear, Boston, Mass.
[5] American Type Culture Collection, Rockville, Md.
[6] Spectronic 88, Bausch and Lomb, Rochester, N.Y.
[7] Tygon tubing.
[8] Gambro-Lundia Minor hemodialyzer, Gambro-Lundia, Lund, Sweden.

Table I—Kinetic Data for Ampicillin in the Experiment [a]

Dose	Time, Above MIC[b], hr	Time, Below MIC, hr	$t_{1/2}$, min	V_{TOT}, ml	C_0[c] MIC	AUC[d], μg min/ml, Above MIC	AUC[d], μg min/ml, $t^{0 \to 6\ hr}$
1	2.6	3.4	75	823	4.12	167	389
2	2.6	3.4	78	824	4.11	172	399
3	2.8	3.2	83	857	3.95	171	407

[a] Data presented in Fig. 5. The three ampicillin doses were administered at 6-hr intervals. [b] Minimum inhibitory concentration. [c] Ratio of drug concentration immediately after injection to the minimum inhibitory concentration for *E. coli*, ATCC 12407. [d] Area under ampicillin concentration *versus* time curve that is above the minimum inhibitory concentration and the area under the total curve during the 6 hr following each dose (minimum inhibitory concentration = 0.9 μg/ml).

Each dialyzer contained a 17-μm thick membrane[9] of 0.54-m^2 surface area. Two dialyzers were connected in series in order to increase membrane surface area and to decrease ultrafiltration of fluid across the membrane. The tubing was gas sterilized, and the model was assembled aseptically and filled with Mueller-Hinton broth. The broth was synchronously pumped through both tubing loops using a dual channel peristaltic pump[10] (main pump) in opposing flows past the membrane. The total volume of broth in the system was ~800 ml (Table I). A 1-liter glass infusion reservoir on each side of the system permitted pressure equalization and allowed the broth volume on each side of the model to be monitored. Access into the closed system was by syringe through injection sleeves from the arterial infusion sets[11]. A broth exchange loop, EXC. IN and EXC. OUT in Fig. 1, was connected to a tubing loop on one side of the model to allow aseptic introduction of fresh broth and removal of a mixture of broth and drug using a dual channel peristaltic pump[10] (exchange pump). The entire system was maintained at 37° in a 0.9-m^3 incubator[12].

Model Theory—The side of the model containing the broth exchange loop was designated the drug compartment; the other side was designated the bacterial compartment. Bacteria in the bacterial compartment could not penetrate the dialysis membrane and, thus, could not enter the drug compartment. Drug introduced into either compartment could rapidly equilibrate across the membrane due to the large membrane surface area, and could be removed from the system through the broth exchange loop on the drug compartment side of the model.

Simulated intravenous infusion concentration profiles in the bacterial compartment were obtained by injecting drug as a bolus into the drug compartment with the broth exchange loop closed (Fig. 2A). Drug concentration in the bacterial compartment C_{Ba} at time t is described as:

$$C_{Ba} = \frac{D}{V_{TOT}}[1 - e^{(k_{12}+k_{21})t}] \qquad \text{(Eq. 1)}$$

where D is the administered dose, V_{TOT} is the total volume of broth in the system, and k_{12} and k_{21} are first-order rate constants for drug movement across the dialysis membrane. Once drug equilibration in the model is achieved, the steady-state drug concentration is given by:

$$C_{Ba}^{ss} = D/V_{TOT} \qquad \text{(Eq. 2)}$$

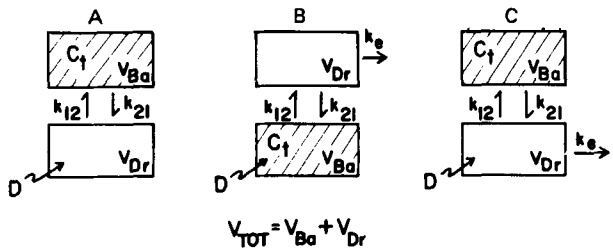

Figure 2—*Schematic representations of drug kinetics in the model during (A) intravenous infusion, (B) intravenous bolus injection, and (C) intramuscular or oral dosage simulations.* V_{Dr} *and* V_{Ba} *are the drug and bacterial compartment volumes, respectively.* C_t *is the concentration of drug in the volume* V_{Ba} *at time t, described in the text as* C_{Ba}. *Other symbols are described in text.*

[9] Cuprophan membrane.
[10] Model 1210, Harvard Apparatus, Millis, Mass.
[11] Travenol Laboratories, Deerfield, Ill.
[12] National Appliance, Hollywood, Fla.

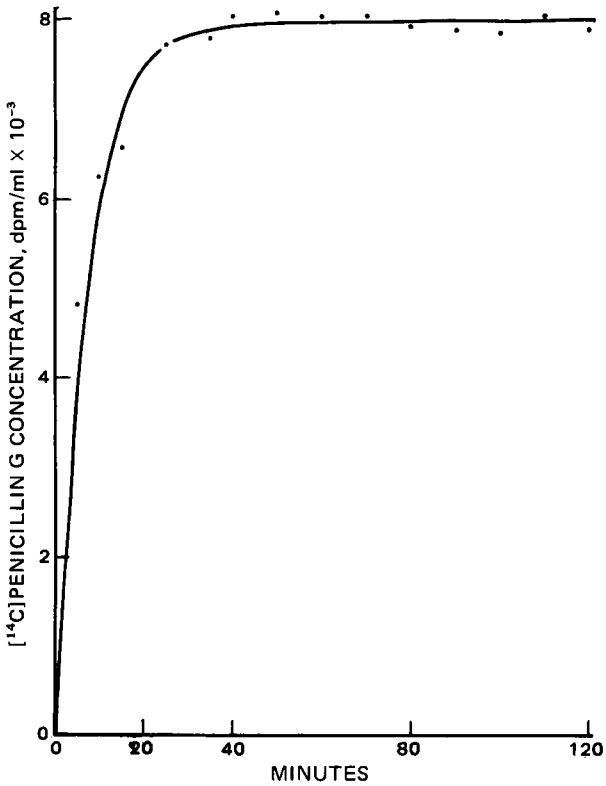

Figure 3—*Intravenous infusion simulation in the model showing experimentally determined drug concentration* versus *time data points.*

The desired C^{ss} value in the bacterial compartment of the model was obtained by selecting the proper dose for the total system volume according to Eq. 2. The time taken for drug levels in the bacterial compartment to reach C^{ss}_{Ba} is a function of the rate of drug passage across the membrane.

Intravenous bolus simulations were achieved by bolus drug injection into the bacterial compartment with the broth exchange loop functioning. The exchange pump speed controlled the rate of drug removal from the model (Fig. 2B). The bacterial compartment drug concentration is described by:

$$C_{Ba} = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{(Eq. 3)}$$

where

$$A = \frac{D(k_{21} + k_e - \alpha)}{V_{Ba}(\beta - \alpha)} \quad \text{(Eq. 4)}$$

$$B = \frac{D(k_{21} + k_e - \beta)}{V_{Ba}(\alpha - \beta)} \quad \text{(Eq. 5)}$$

and

$$\begin{pmatrix}\alpha\\ \beta\end{pmatrix} = \frac{1}{2}\left[(k_{12} + k_{21} + k_e) \pm \sqrt{(k_{12} + k_{21} + k_e)^2 - 4k_{21}k_e}\right] \quad \text{(Eq. 6)}$$

As drug removal by the broth exchange loop was a first-order process, and passage of drug across the membrane was fast compared to drug elimination, a plot of the decrease in the logarithm of the drug concentration with time was linear.

Oral and intramuscular dosage simulations were obtained by injecting a drug bolus into the drug compartment with the broth exchange loop functioning. The membrane passage controlled the rate at which drug concentration increased in the bacterial compartment, while the rate of drug elimination was controlled by the exchange pump rate (Fig. 2C). Equation 7 describes the bacterial compartment drug concentration profile:

$$C_{Ba} = \frac{Dk_{12}}{V_{Ba}(\alpha - \beta)}\left[e^{-\beta t} - e^{-\alpha t}\right] \quad \text{(Eq. 7)}$$

The elimination rates for the intravenous bolus, intramuscular, and oral dose simulations are controlled by the exchange pump speed according to:

$$t_{1/2} = \frac{0.693V_{TOT}}{Q} \quad \text{(Eq. 8)}$$

where Q is the broth exchange loop flow rate.

Use of the Model to Examine the Antibacterial Effect of Ampicillin—The antibacterial effect of a multiple intravenous bolus dosage regimen of ampicillin on *E. coli*, ATCC 12407, was examined. A 1-ml inoculum of 1×10^8 cfu/ml of *E. coli*, which was in logarithmic growth phase, was injected into the bacterial compartment. Logarithmic bacterial growth in the model was monitored by means of optical density. When the bacterial concentration reached 5×10^6 cfu/ml, the first of three 3-mg ampicillin doses was administered as a 1-ml bolus into the bacterial compartment with the broth exchange loop functioning. Similar doses of ampicillin were given at 6 and 12 hr after the initial dose. Drug and viable bacteria concentrations were monitored every 0.5 hr for 18 hr following the first ampicillin dose. The serial dilutions in the bacterial assay served to dilute ampicillin in samples to noninterfering levels.

RESULTS

The kinetics of penicillin G disposition in the model were examined by injecting 0.5 ml of a mixture of 11.2 μg/ml of [^{14}C]penicillin G potassium and 962 μg/ml of unlabeled penicillin G potassium into the system. With the main pump flow rate set at 120 ml/min, circulation time for drug in the model was found to be <3 min. A 0.5-min drug equilibration half-time across the membrane was calculated from an intravenous infusion dose. Observed C^{ss} values under these conditions indicated that the drug did not bind to a significant degree to the dialysis membrane.

Drug concentration profiles in the bacterial compartment during intravenous infusion and oral dosage simulations are shown in Figs. 3 and 4. Multiple bolus intravenous injection simulations are shown in Fig. 5. The residual plot for the absorption phase of the oral simulation was linear, indicating that the approach to steady-state drug concentration in the bacterial compartment can be represented by a first-order process. The absorption and elimination half-lives for the oral dose simulations were ~5 and 53 min, respectively.

The possible effect of changes in the exchange pump flow rate on

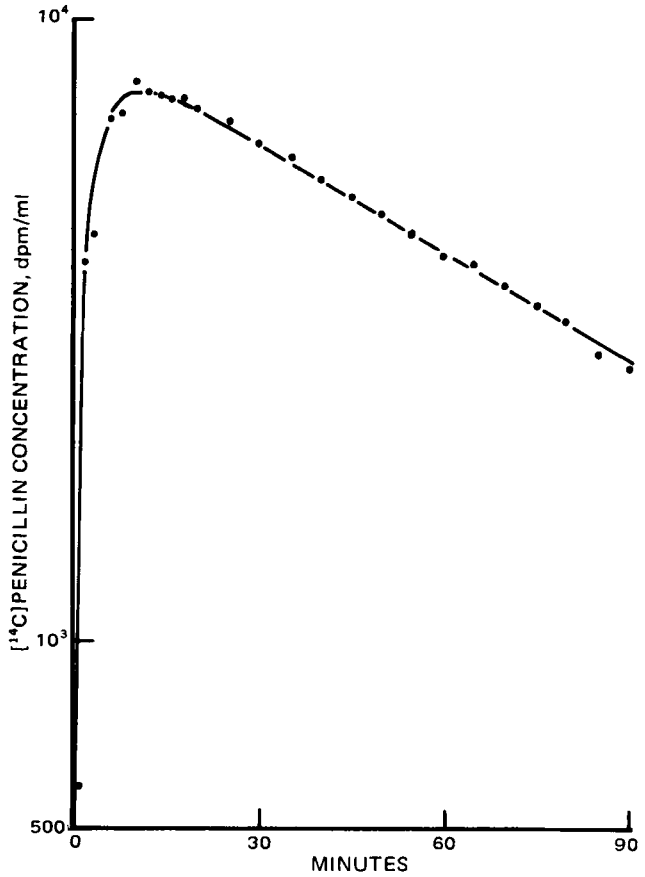

Figure 4—*Intramuscular injection or oral dosage simulation in the model showing experimentally determined bacterial compartment drug concentration* versus *time data points.*

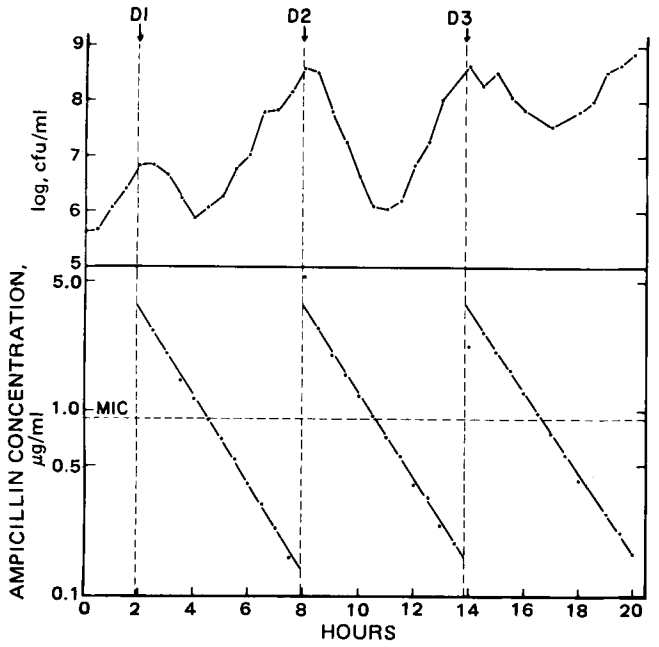

Figure 5—*Plots of the logarithm of the number of colony-forming units per milliliter (cfu/ml) and the log of the drug concentration* versus *time following three (D1,D2,D3) simulated intravenous bolus injections of ampicillin against* E. coli, *ATCC 12407. The minimum inhibitory concentration (0.9 μg/ml) is indicated as a horizontal dotted line.*

bacterial growth in the absence of drug was determined by varying the pump flow rates to yield simulated drug half-lives of infinity (exchange loop closed), 75, and 37 min. The tests were performed on different days using different bacterial inocula. The slopes ($\pm SD$), obtained by linear regression, for the logarithmic bacterial growth curves were 0.72 (±0.05) for $t_{1/2} = \infty$, 0.75 (± 0.02) for $t_{1/2} = 75$ min, and 0.78 (±0.01) for $t_{1/2} = 37$ min. These values were not significantly different from each other and show that bacterial growth rates were unaffected by broth exchange rates within the range of values studied.

The results of the repeated dose intravenous bolus simulation, using ampicillin against *E. coli*, ATCC 12407, are shown in Fig. 5. Pertinent data are presented in Table I. Bacterial kill occurred following each dose of antibiotic, and regrowth resumed shortly after antibiotic levels had fallen below the minimum inhibitory concentration. The time that elapsed between dosing and observed bactericidal effect tended to decrease slightly, but the maximum kill rate and the absolute amount of bacterial kill appeared also to differ with succeeding doses.

DISCUSSION

Antimicrobial therapy is generally administered on an empirical basis and optimization of effect through accurate dosage regimen design has not been accomplished in most cases.

It is difficult to establish accurate relationships between drug kinetics and antibacterial effects *in vivo* because of the contribution of many, often uncontrollable, factors such as tissue penetration, development of bacterial resistance, variable binding of drug to plasma proteins, and interactions of drugs with other substances. Failure to generate useful data in clinical situations, or by the use of animal models, has given rise to an increasing interest in the development of *in vitro* systems which may simulate in an idealistic manner the *in vivo*, clinical situation. While it is recognized that such systems exclude many of the variables that exist *in vivo*, they are nonetheless justified in that they attempt to establish relationships between changing drug concentrations and antibacterial effect in a controlled situation.

Some *in vitro* studies have examined the kinetics of antimicrobial effects using constant drug concentrations (13, 14). However, the results obtained in these studies do not apply to many clinical situations in which drug levels are constantly changing.

Other models have been designed to simulate varying drug levels with respect to time (15–19). In most of these systems, the addition of fresh broth in order to change the concentration of antibiotic results in simultaneous dilution of the organism (15, 16, 18, 19). In some models there is less than optimal control of the drug concentration *versus* time profiles.

The described model has advantages over previous systems in that the addition of fresh growth medium in order to dilute the concentration of antibiotic does not dilute the bacteria, and also the closed, continuously recycling system provides considerable flexibility for the simulation of a variety of single and repeated dosage regimens.

The rapid rate of drug equilibration across the dialyzer membranes observed with the present system can be altered by varying the membrane surface area. This would have the effect of altering the drug absorption rate in oral or intramuscular dose simulations and changing the time for steady-state drug levels to be achieved in the bacterial compartment during infusion simulations.

Tracer quantities of [^{14}C]penicillin G were used in this study to monitor both penicillin G and ampicillin levels, characterize the model, and provide preliminary data on dosage simulation. It is recognized that circulating total radioactivity does not necessarily represent microbiologically active drug, and the preliminary concentration effect relationships described in Fig. 5 must be interpreted in this light. However, the good agreement between drug concentrations relative to the minimum inhibitory concentration and the antibacterial effect serves to illustrate the usefulness of the model for this type of study. To characterize accurately the concentration–effect relationship of any chemically or bacteriologically unstable compound, it will be necessary to measure the concentration of active drug.

Various improvements to the described model are possible and are being investigated. For example, with appropriate engineering, the overall size of the system and the membrane surface area may be reduced to permit greater ease in handling without loss of model flexibility. However, the present model is suitable to study drug antibacterial activity under a variety of dosing conditions. The data in Fig. 5 were generated with the bacteria in logarithmic growth at the time of drug administration. With appropriate adjustment of experimental conditions, drug activity can be determined against bacteria in either logarithmic or stationary growth phases.

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