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Pirbenicillin: Pharmacokinetic Parameters in Mice

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The rapid intravenous administration to mice of pirbenicillin, carbenicillin, and ampicillin produced biexponential blood concentration-time curves when assessed by frequent blood samplings at short intervals. The pharmacokinetic behavior of pirbenicillin and the other penicillins was analyzed by the two-compartment open model. This is thought to be the first study giving detailed pharmacokinetic values of penicillins in mice. Some significant differences were noted between the pharmacokinetic values of pirbenicillin, ampicillin, and carbenicillin. These values suggest that the interchange of pirbenicillin between the central and peripheral body compartments of the mouse was slower than that of either carbenicillin or ampicillin and indicated that a greater fraction of the pirbenicillin than the ampicillin dose reached the peripheral compartment.

Pirbenicillin (former designation, CP-33,994), a new broad-spectrum, semisynthetic penicillin, has demonstrated two to four times greater activity than carbenicillin against several experimental infections in mice (12a). In vivo effectiveness of an antibiotic results from the complex interdependence of antibacterial activity at the site(s) of infection and pharmacokinetic processes. In addition to drug concentration in blood and urine, the processes that characterize and regulate the movement of a drug through the body are represented by its biological half-life, apparent volume of distribution, various distribution and elimination rate constants, and serum-tissue drug ratios. Usually these detailed pharmacokinetic parameters are determined in the late stages of antibiotic evaluation by studies in large animals or in man. However, as mice are routinely used in the early stages of antibiotic evaluation, it was of interest to determine these properties of pirbenicillin in this species.

MATERIALS AND METHODS

Pirbenicillin (sodium salt), ampicillin (trihydrate), and carbenicillin (disodium salt) were obtained from members of Chemical Research, Pfizer, Inc. Dosage forms were prepared as solutions in physiological saline.

Mice averaging 25 g were purchased from Blue Spruce Farms, Altamont, N. Y. After a single intravenous injection of 10 mg of drug per kg, whole blood samples were obtained from the orbital sinus of mice by using heparinized hemocrit tubes (14). Each mouse had an identification mark to insure bleeding in the same sequence at sampling times. A second group of 10 mice were maintained in a plastic metabolism cage to insure collection of feces-free urine. These mice were used only in the urine excretion study. Urine was collected in a tube packed in ice.

Antibiotic in mouse whole blood and urine was assayed using filter-paper disks (6.5 mm) moistened with 0.025 ml of sample placed on seed agar (Baltimore Biological Laboratories) in large plates. Two or three disks were prepared from each mouse at each time sample. Standard curves, as well as necessary sample dilution, were prepared in the respective fluid from normal mice. *Micrococcus luteus*, ATCC 9341, was used as the assay organism for pirbenicillin and ampicillin. The Ellsworth strain of *Pseudomonas aeruginosa*, NCTC 10490, was used to assay carbenicillin.

The mouse data at each time interval represent the mean value of samples from 15 to 20 individual mice, which were used during three to four individual experiments. Mean values and the standard error of the mean were calculated on a programmable desk-type calculator (Monroe 1860). In some instances, the significance of differences between mean values of the pharmacokinetic parameters of the antibiotics was determined by the t-test.

RESULTS

The calculated pharmacokinetic values presented are independent of dose; values obtained with dosages of 5 mg of pirbenicillin and ampicillin per kg and 20 mg of carbenicillin per kg agreed well with those presented for the 10-mg/kg dosage.

Semilog plots of mean whole blood concentrations of pirbenicillin, carbenicillin, and ampicillin after a 10-mg/kg intravenous injection versus time are presented in Table 1 and Fig. 1A, B, and C. The plots consist of two segments: (i) the first segment, or "α-phase" (distribution phase), which results primarily from rapid distribution of the drug between blood and the extravascular tissue compartment; and (ii) a

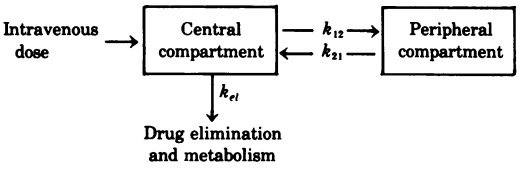
TABLE 1. *Pirbenicillin, carbenicillin, and ampicillin in mice: mean whole blood concentrations in mice (10 mg/kg, intravenous)*

Sample time (min)	Mean concn (μg/ml) with SEM[a]		
	Pirbenicillin	Carbenicillin	Ampicillin
1	18.20 ± 0.86 (18.20)[b]	16.6 ± 1.78 (17.7)[b]	18.70 ± 0.97 (20.4)[b]
3	9.40 ± 0.56 (9.51)	7.40 ± 0.54 (6.9)	10.60 ± 0.43 (10.15)
5	6.00 ± 0.36 (5.90)	4.67 ± 0.28 (4.70)	7.98 ± 0.34 (7.72)
10	3.65 ± 0.25 (3.37)	3.37 ± 0.17 (3.62)	6.36 ± 0.25 (6.24)
15	2.75 ± 0.21 (2.74)		5.20 ± 0.20 (5.38)
20		2.47 ± 0.04 (2.20)	
30	1.59 ± 0.25 (1.74)	1.82 ± 0.05 (1.84)	3.27 ± 0.28 (3.47)
45		1.37 ± 0.03 (1.11)	
60	0.64 ± 0.17 (0.72)	0.78 ± 0.01 (0.67)	1.58 ± 0.23 (1.45)
90	0.28 ± 0.09 (0.30)		0.58 ± 0.09 (0.60)
120	0.14 ± 0.05 (0.12)		0.26 ± 0.05 (0.25)

[a] SEM, Standard error of the mean.

[b] The numbers within parentheses were calculated using the biexponential equation of $C_p = Ae^{-\alpha t} + Be^{-\beta t}$. We thank J. Taylor of Pfizer, Inc. for these computer simulations.

straight segment, or "β-phase," the slope of which is determined by the rates of distribution, excretion, and/or metabolism of the drug. The decline in whole blood concentrations of pirbenicillin and the other penicillins occurs in a biexponential manner that can be mathematically fitted to a two-compartment open model (13):



Solution of the differential equations resulting from this model yield the biexponential equation, $C_p = Ae^{-\alpha t} + Be^{-\beta t}$. C_p is the drug concentration in the blood at time t; A and B are the intercepts on the ordinate axis. At zero time, $C_p = A_0 + B_0$ (2, 7). The first-order distribution rate constants k_{12} and k_{21} control the movement of drug between the two compartments; k_{el} is the sum of the first-order constants relevant to metabolism and excretion. These various parameters were determined by plotting the data on semilogarithmic paper and subsequent regression analysis by the method of least squares. A linear regression, constructed from the β-phase, was extrapolated to the ordinate. The importance of drug elimination that occurs in the very early stages after intravenous administration was considered in that the extrapolated β-phase values were subtracted from the observed α-phase values to give the residual lines shown in Fig. 1A, B, and C (7, 10). A_0 and α are the intercept and rate constant, respectively, of the residual α-phase line; B_0 and β are the intercept and rate constant, respectively, of the β-phase line. Biological half-life of each segment of the distribution-elimination curve is related to the slope of the segment and was calculated as $T1/2 = 0.693/\text{rate constant}$ (7). Values of A_0, B_0, α, and β for pirbenicillin, carbenicillin, and ampicillin are presented in Table 2. Also presented is the area under the concentration curve ($AUC_{0\to\infty}$) expressed as μg·ml/min as determined by the trapezoidal rule (11). That the blood concentration-time curves are well described by the use of the above-mentioned values in the biexponential equation is shown by the similarity of the calculated data points to observed points, which are presented in Table 1.

The individual rate constants in the two-compartment model were calculated following the detailed mathematical procedures described by Gillette (7). The first-order rate constant, k_{21}, for the passage of the drug from the peripheral to the central compartment was calculated as $k_{21} = (A_0 \cdot \beta + B_0 \cdot \alpha)/(A_0 + B_0)$; the first-order rate constant, k_{el}, of the elimination of drug from the central compartment was calculated as $k_{el} = \alpha \cdot \beta / k_{21}$; the first-order rate constant, k_{12}, for the passage of the drug from the central compartment to the peripheral compartment was calculated as

$$k_{12} = (\alpha + \beta) - (k_{21} + k_{el})$$

A summary of these individual rate constants for pirbenicillin and the other penicillins is presented in Table 3.

Each antibiotic left the vascular system rapidly and penetrated the tissue compartment (Table 2). However, based on the half-life values of the α-phase (distribution), pirbenicillin did distribute more slowly than did either carbenicillin or ampicillin: 1.90 min compared

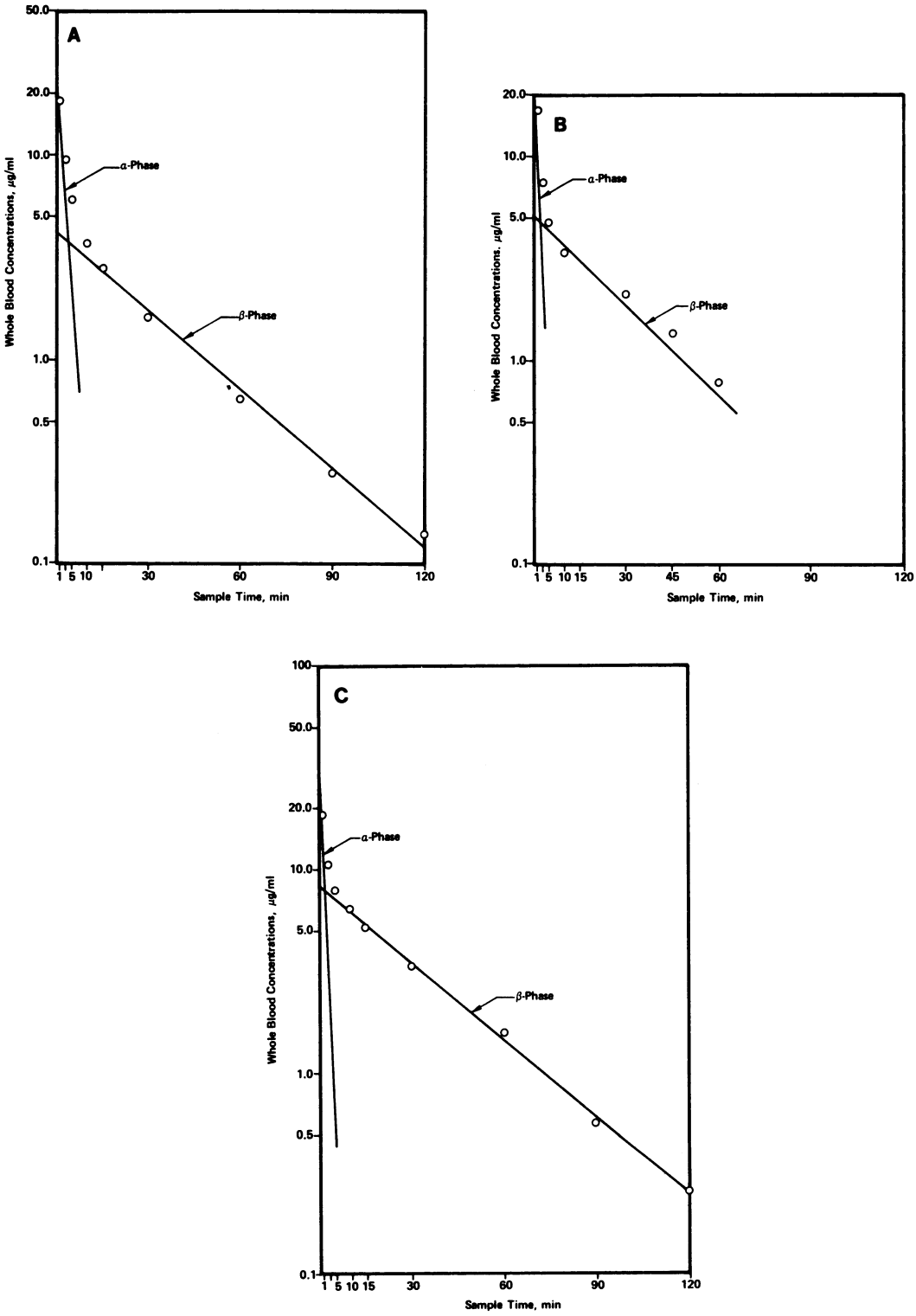


FIG. 1. *(A) Pirbenicillin; (B) carbenicillin; (C) ampicillin. Concentrations in mouse whole blood after a 10-mg/kg intravenous dosage. Constructed α- and β-phase lines.*

TABLE 2. *Pirbenicillin, carbenicillin, and ampicillin in mice: intravenous administration (10 mg/kg)—pharmacokinetic parameters of α- and β-phases*

Antibiotics	Pharmacokinetic parameters of:						AUC[a] curve (μg·ml/min) $0 \rightarrow \infty$
	α-Phase			β-Phase			
	A_0 (μg/ml)	α[b]	Half-life (min)	B_0 (μg/ml)	β[b]	Half-life (min)	
Pirbenicillin	19.50 ± 2.49[c]	−0.389 ± 0.031	1.90 ± 0.17	4.33 ± 0.31	−0.032 ± 0.002	22.20 ± 1.68	178.89 ± 20.23
Carbenicillin	28.50 ± 3.49	−0.817 ± 0.072	0.93 ± 0.11	5.04 ± 0.29	−0.033 ± 0.002	21.27 ± 0.88	153.15 ± 4.90
Ampicillin	20.50 ± 3.38	−0.633 ± 0.059	1.18 ± 0.11	8.50 ± 0.25	−0.031 ± 0.001	23.20 ± 0.40	296.82 ± 23.44

[a] AUC, Areas under concentration.
[b] Rate constant of decline.
[c] Standard error of the mean.

TABLE 3. *Pirbenicillin, carbenicillin, and ampicillin in mice: intravenous administration (10 mg/kg)—elimination rate constants*

Antibiotics (10 mg/kg, i.v.)	Elimination rate constants		
	k_{12} (blood → periphery)	k_{21} (min^{-1}) (periphery → blood)	k_{el} (min^{-1}) (elimination from blood)
Pirbenicillin	0.197 ± 0.026[a]	0.095 ± 0.004	0.129 ± 0.007
Carbenicillin	0.516 ± 0.061	0.156 ± 0.011	0.177 ± 0.017
Ampicillin	0.359 ± 0.051	0.213 ± 0.016	0.092 ± 0.008

[a] Standard error of the mean.

with 0.93 and 1.18 min. The difference between pirbenicillin and carbenicillin was significant (t-test) at $P = 0.005$; the difference between pirbenicillin and ampicillin, $P = 0.001$.

Relatively short half-lives in the distribution phase are seen also in larger animals; for example, for ampicillin the distribution half-lives are 4.8 min in beagles and 15.6 min in man (3, 10). Similarly, k_{12}, the rate constant describing the distribution of pirbenicillin from the blood to the peripheral compartment, was only one-third that of carbenicillin (t-test: $P = >0.001$) and one-half that of ampicillin (t-test: $P = 0.02$) (Table 3). Riegelman et al. have pointed out that the rate of uptake of a compound into tissues is controlled by several factors, including the rate of blood flow through the tissue, the mass or volume of the tissue, and the partition characteristics of the compound between plasma and the tissues (13). As the first two factors are common to all the antibiotics in these mouse studies, the partition characteristics may account for the observed differences in the distribution phase. Other studies have shown that in a butanol-water system, pirbenicillin is more polar than ampicillin but is less polar than carbenicillin (R. Rosati, Pfizer, Inc., unpublished data). Thus one might expect the k_{12} value of pirbenicillin to be between that of ampicillin and carbenicillin. Because it is substantially less than the k_{12} value for both ampicillin and carbenicillin, some other unknown factor may play an important role.

Relevant to the elimination phase, pirbenicillin appears more similar to carbenicillin than to ampicillin. The extrapolated value of B_0 (Table 2) in the elimination phase of pirbenicillin is similar to that of carbenicillin and only one-half that of ampicillin (t-test: $P = <0.001$). However, β, the rate constant of the decline in the second phase of pirbenicillin, is very similar to that of ampicillin and carbenicillin. All three penicillins have similar half-life values in the elimination phase (Table 2). The rate constant, k_{21}, describing the movement of drug from periphery to blood is about two times smaller for pirbenicillin than for carbenicillin (t-test: $P = <0.001$) and ampicillin (t-test: $P = 0.01$) (Table 3). The hybrid rate constant, k_{el}, for both pirbenicillin and ampicillin is similar and smaller than that for carbenicillin.

The whole blood-peripheral distribution of pirbenicillin and the other penicillins was estimated by calculating the fraction of the administered dose in the extravascular peripheral and central compartments using average data for all mice and the analysis described by Rattie and Ravin (12):

$$A_{\text{tissue}} = \frac{k_{12}}{\alpha - \beta}\left(e^{-\beta t} - e^{-\alpha t}\right) \text{ and}$$

$$A_{\substack{\text{central}\\\text{compartment}}} = \left[\left(\frac{\alpha - k_{21}}{\alpha - \beta}\right)e^{-\alpha t} + \left(\frac{k_{21} - \beta}{\alpha - \beta}\right)e^{-\beta t}\right]$$

Figure 2A presents the percentage of pirbenicillin dose estimated in the peripheral compartment and in the central compartment. Similar plots for carbenicillin and ampicillin are presented in Fig. 2B and C. The amount of pirbenicillin in the tissue compartment peaked at about 45% of the administered dose at 5 min, after which time the percentage of dose in this compartment was always greater than in the central compartment. The peak carbenicillin

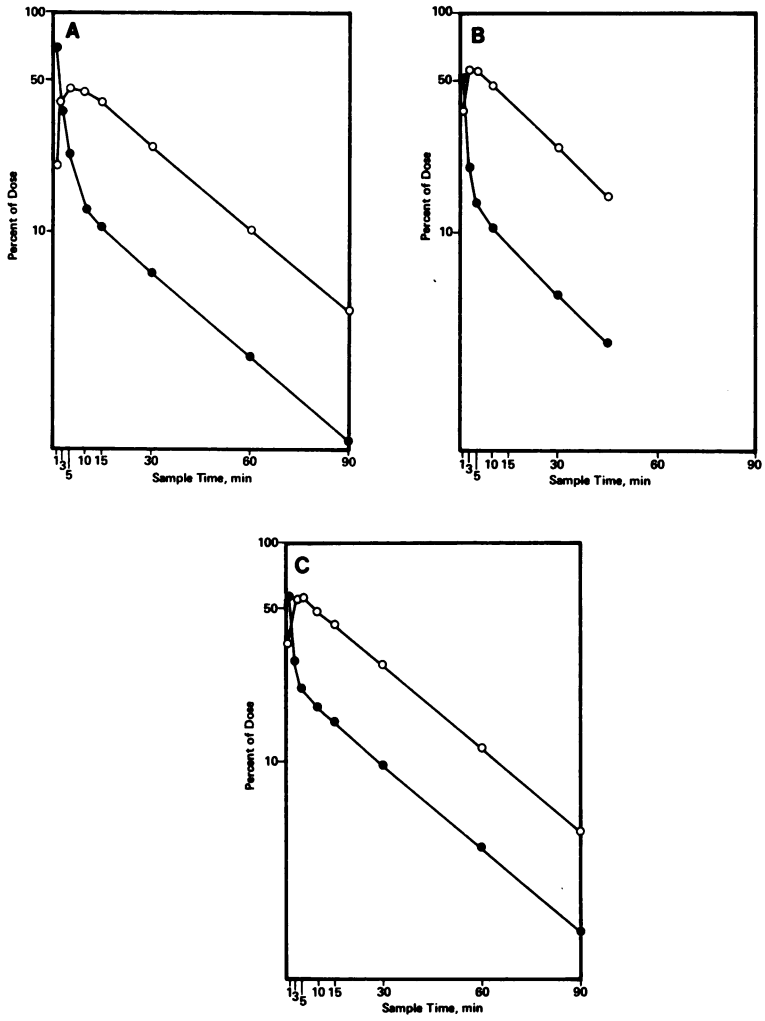


FIG. 2. *(A) Pirbenicillin; (B) carbenicillin; (C) ampicillin. Percentage of intravenous dose in mice (10 mg/kg) in peripheral (●) and central compartments (○).*

and ampicillin percentage of dose in the tissue compartment was about 55% after 3 to 5 min postinjection. As was true with pirbenicillin, subsequent decreases in both tissue and blood compartments were parallel. Again, the percentage of the dose in the tissue compartment after peaking was always greater than in the blood compartment. This has been observed in man with both penicillins and cephalosporins (5, 12).

The kinetic volume of distribution of the entire body was calculated from the parameters of the two-compartment model as follows:

$$V_{d(k)} = \frac{\text{dose } (T1/2 \text{ of } \beta\text{-phase})}{A_{a(0)} \cdot (T1/2 \text{ of } \alpha\text{-phase}) + B_{a(0)} \cdot (T1/2 \text{ of } \beta\text{-phase})} \quad (7)$$

Values for V_p were calculated by dividing the dose by C_p^0, the whole blood concentration at zero time ($A_0 + B_0$). Values for V_t were calculated by the relationship:

$$V_t = V_p \cdot \frac{k_{12}}{k_{21}} \quad (8)$$

In addition, the fraction V_t/V_p is equivalent to the ratio of the amount of drug in the extravascular tissue compartment to the amount of drug in the central compartment. These values are presented in Table 4.

The kinetic volume of distribution of ampicillin approximated the volume of the mouse; however, those of pirbenicillin and carbenicillin were greater than the mouse volume. The

$V_{d(k)}$ value of pirbenicillin was significantly greater than those of carbenicillin (t-test: $P = 0.05$) and ampicillin ($P = 0.001$). The apparent volume of the central compartment of pirbenicillin also was significantly greater than that of carbenicillin (t-test: $P = 0.025$) and, in addition, the V_t value of pirbenicillin was greater than that of ampicillin (t-test: $P = 0.001$) (Table 4).

Volumes of distribution values in mice and rabbits for antibiotics in addition to penicillins, i.e., tetracyclines, streptomycin, and macrolides, have been found greater than body volume (9, 15; A.R.E., unpublished data). Localization in a body tissue or fluid has been suggested as a possible explanation (1, 9). Also, serum binding is an important determinant in drug distribution and activity. It is generally agreed that only free drug can distribute into tissues because drug-protein complexes cannot penetrate cell membranes (6). However, as indicated in Table 5, serum binding to mouse serum proteins by pirbenicillin, carbenicillin, and ampicillin were nearly equivalent. It is likely, therefore, that the contribution of serum binding to the observed differences in distribution volumes was minimal.

Total clearance rates (TCR) were evaluated as $TCR = k_{e1} \cdot V_p$ (12). The TCR value for pirbenicillin was similar to that of carbenicillin. Both were greater than that for ampicillin (t-test: $P = 0.001$) (Table 6). It should be recalled that this is a measure of elimination by all processes and is always greater than elimination as a result of renal function only. Renal clearance rates (RCR) were calculated by using the fraction (f) of the dose recovered in the urine multiplied by TCR: $RCR = TCR \cdot f$ (12).

These data are presented in Table 6. The RCR of pirbenicillin and ampicillin were quite similar; both were higher than that of carbenicillin. Percentage of urinary recovery of the administered dose during a 24-h period for pirbenicillin, carbenicillin, and ampicillin, respectively, amounted to 43.2 ± 0.85%, 38.4 ± 0.25%, and 69.8 ± 1.9%.

DISCUSSION

The rapid intravenous administration of pirbenicillin, carbenicillin, and ampicillin to mice produced biexponential blood concentration-time curves when assessed by frequent blood samplings at short intervals (Table 1; Fig. 1A, B, and C). The two-compartment open model and the graphical technique for measuring the various parameters were adequate to interpret the pharmacokinetic behavior of pirbenicillin and the other penicillins studied in mice. The present study is thought to be the first giving detailed pharamacokinetic values for penicillins, or any other antibiotic, in mice. The biexponential curve is typical of penicillins, other antibiotics, and other therapeutic agents in man and larger animals (3–5).

The pharmacokinetic values of pirbenicillin, in a number of instances, were significantly different from those of carbenicillin and ampicillin, e.g., volume of distribution values, k_{12}, k_{21}, and α-phase half-life. Generally, the pharmacokinetic values suggest that the interchange between the central and peripheral compartments for pirbenicillin is slower than that of the other penicillins. The V_t/V_p ratio (fraction of the dose in the peripheral compartment) suggests that a greater fraction of the pirbenicillin dose than the ampicillin dose reaches the peripheral compartment; the values of both these antibiotics are smaller than that for carbenicillin.

Urinary recovery of pirbenicillin (43.2%) was intermediate between ampicillin (69.8%) and carbenicillin (38.4%). The difference between TCR and RCR suggests either considerable non-renal elimination or urinary excretion

TABLE 5. *Pirbenicillin, carbenicillin, and ampicillin serum binding[a] in mouse serum*

Penicillins	Mouse serum
Pirbenicillin	24.2% ± 1.2[b]
Carbenicillin	22.6% ± 1.9
Ampicillin	23.2% ± 0.0

[a] As determined by an ultrafiltration procedure.
[b] Standard error of the mean.

TABLE 4. *Pirbenicillin, carbenicillin, and ampicillin in mice: intravenous administration (10 mg/kg)—volume distribution values*

Antibiotics	Apparent vol of:			V_t/V_p ratio
	Distribution V_{dk} (ml)	Central compartment V_p (ml)	Extravascular compartment V_t (ml)	
Pirbenicillin	46.93 ± 2.47[a]	12.60 ± 1.36	34.33 ± 2.07	3.03 ± 0.39
Carbenicillin	39.49 ± 2.30	8.20 ± 1.21	31.29 ± 2.02	4.39 ± 0.53
Ampicillin	27.25 ± 0.77	9.63 ± 0.89	17.62 ± 1.04	2.10 ± 0.37

[a] Standard error of the mean.

TABLE 6. *Pirbenicillin, carbenicillin, and ampicillin in mice: intravenous administration (10 mg/kg)-TCR and RCR rates*

Antibiotics	TCR[a]	RCR[a]
Pirbenicillin	1.60 ± 0.16[b]	0.60
Carbenicillin	1.43 ± 0.07	0.49
Ampicillin	0.83 ± 0.05	0.56

[a] TCR, Total clearance rate; RCR, renal clearance rate (milliliters per minute).

[b] Standard error of the mean.

of a biologically less active material for pirbenicillin, carbenicillin, and, to a lesser extent, ampicillin in the mouse.

Pharmacokinetic parameters represent important processes that characterize and regulate the movement of a drug through the body. However, overall assessment of efficacy of antibiotic against infections involve the above plus the further complexity and interplay or relative bacteriostatic and/or bactericidal activity at the site(s) of infection. In this regard, pirbenicillin has been shown previously to have greater antibacterial activity in vitro than carbenicillin, and this potency advantage was also observed in murine protection models with *Pseudomonas, Serratia*, and *Escherichia coli* (12a).

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