height. Since the nitrate ion absorbs at this wavelength, an injection of isopilocarpine nitrate solution gave an extra peak at ~40–50 min after injection, presumably due to ammonium nitrate elution. This peak can be avoided, if desired, by first passing the isopilocarpine nitrate sample through an ion-exchange column to replace the nitrate ion with a UV inactive ion.

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Effect of Dosing Volume on Intramuscular Absorption Rate of Aminoglycosides

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Received July 30, 1980, from the *Department of Drug Metabolism and Pharmacokinetics, Bristol Laboratories, Syracuse, NY 13201.* Accepted for publication September 29, 1980.

Abstract □ The Loo–Riegelman method was applied to serum amikacin level data after intravenous and intramuscular administration. Intramuscular amikacin absorption can be described by first-order kinetics, but the absorption rate constant decreased from 1.95 hr^{-1} at a 125-mg dose to 1.00 hr^{-1} at a 750-mg dose. This rate change apparently is a physical phenomenon due to differing dosing volumes at different doses and attendant changes in the surface area to volume ratio at the injection site. Amikacin absorption rates on intramuscular injection can be maximized by giving several smaller injections rather than a single larger injection. This phenomenon should be generally observed with aminoglycoside antibiotics and could be partly responsible for reported variations in absorption rate and the poor predictability of serum concentrations.

Keyphrases □ Aminoglycosides—effect of dosing volume on intramuscular absorption rate □ Absorption, intramuscular—aminoglycosides, dosing volume effects □ Amikacin—intramuscular absorption rates, dosing volume effects

In a review of the clinical pharmacokinetics of aminoglycoside antibiotics, Pechere and Dugal (1) referred to reports of wide intramuscular absorption rate variations within and between studies and antibiotics. They commented: "In view of the fact that rates of absorption vary widely, nomograms based on equations which make use of a universal absorption rate constant should be critically examined. This, incidentally, may be partly responsible for the poor predictability of serum aminoglycoside concentrations noted by some authors." According to Greenblatt and Koch-Weser (2), nonlinear intramuscular absorption may be fairly common because local and systemic factors that influence the absorption rate rarely remain constant while absorption is taking place.

In this study, available intravenous and intramuscular amikacin data were used to estimate intramuscular absorption kinetics of an aminoglycoside antibiotic. Since the data were derived from separate studies, mean values were used for the pharmacokinetic parameters and intramuscular serum levels. A crossover intravenous–intramuscular amikacin study at 125- and 500-mg doses was reported, but no estimates of the intramuscular absorption kinetics were made (3).

EXPERIMENTAL

The data analyzed were drawn from three Phase I amikacin clinical investigations (Studies 1, 2, and 7). The subjects in all three studies were normal, healthy adult males ranging from 21 to 40 years and from 60 to 90 kg.

In Study 7, 7.5 mg/kg was administered intravenously as 5-, 30-, and 60-min infusions. Six subjects received both the 30- and 60-min infusions, according to a balanced crossover design, into an antecubital vein using an infusion pump[1]. Six other subjects received the 5-min infusion as a push injection into an antecubital vein.

In Studies 1 and 2, amikacin was administered into the right or left superior lateral gluteal quadrant. In Study 1, 12 subjects each received 250- and 500-mg doses; some results were previously published (4). In Study 2, three subjects each received 125-, 250-, 500-, or 750-mg doses.

Blood samples were collected for serum preparation, and serum samples were analyzed for amikacin content using a standard cup plate bioassay (5) with *Bacillus subtilis* (ATCC 6633) as the bioassay organism. The minimum quantitative sensitivity of the assay was 0.06 μg/ml. Typically, an analysis of variance of the regression slope of the standard response line (zone diameter *versus* log concentration) yielded $F(1, 3) = 240$.

Mean postinfusion intravenous data were fitted to the biexponential equation for an open, two-compartment model of drug distribution with central compartment elimination only:

$$C = A' \exp(-\alpha t') + B' \exp(-\beta t') \qquad \text{(Eq. 1)}$$

[1] Model 940, Harvard Instrument Co., South Natick, Mass.

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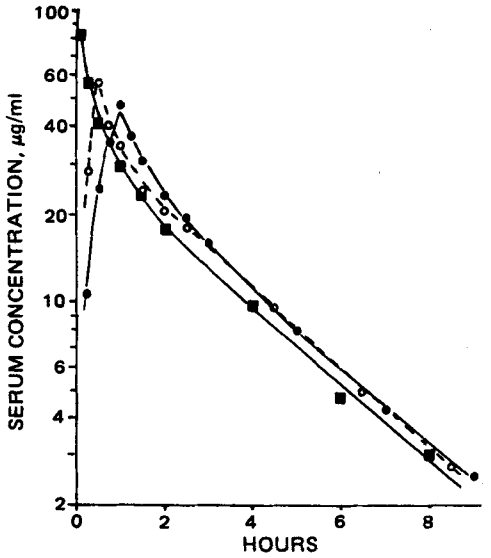

Figure 1—*Mean serum amikacin concentrations after intravenous infusion (Study 7). Key: ●, 60-min infusion; ○, 30-min infusion; and ■, 5-min infusion.*

where C is the observed serum concentration at time $t' = t - T$, t is time from the start of the infusion, and T is the duration of the infusion. The fitting was performed by nonlinear regression analysis using the digital computer program NONLIN (6), which employs Hartley's modification of the Gauss–Newton method (7). Goodness of fit was measured using the sum of squares of the deviations of the fitted concentrations ($\hat{C}$) from the observed concentrations:

$$S = \sum(C - \hat{C})^2 \qquad \text{(Eq. 2)}$$

and a coefficient of determination:

$$R^2 = \frac{\sum C^2 - \frac{(\sum C)^2}{n} - S}{\sum C^2 - \frac{(\sum C)^2}{n}} \qquad \text{(Eq. 3)}$$

The coefficients A' and B' were corrected to their equivalent values for intravenous bolus injection, A and B, using (8):

$$A = A'\alpha T/[1 - \exp(-\alpha T)] \qquad \text{(Eq. 4a)}$$

$$B = B'\beta T/[1 - \exp(-\beta T)] \qquad \text{(Eq. 4b)}$$

Given A, α, B, and β, the pharmacokinetic parameters could be calculated according to standard methods (9).

The intramuscular absorption profiles, as percent of dose unabsorbed *versus* time, for amikacin were determined using the mean intramuscular serum data and the mean intravenous pharmacokinetic parameters according to the Loo–Riegelman method (10). The equation defining peripheral compartment concentrations was used without the Taylor expansion simplification. All cumulative areas under the mean intramuscular drug concentration *versus* time curves were determined using the trapezoidal rule. The values representing percent of dose unabsorbed *versus* time were analyzed by suitable transformation and least-squares linear regression analysis to determine kinetic order, lag time, and absorption rate constants.

Table I—Pharmacokinetic Parameters from Mean Intravenous Data

Parameter	60-min Infusion	30-min Infusion	5-min Infusion	Mean (*SD*)
A^a, μg/ml	37.4	39.8	46.0	41.1 (4.4)
α, hr^{-1}	1.39	2.78	1.85	2.01 (0.71)
B^a, μg/ml	28.4	35.5	31.9	31.9 (3.6)
β, hr^{-1}	0.290	0.313	0.301	0.301 (0.012)
K_{21}, hr^{-1}	0.766	1.48	0.934	1.06 (0.37)
K_{12}, hr^{-1}	0.388	1.02	0.595	0.669 (0.325)
K_{el}, hr^{-1}	0.527	0.589	0.619	0.578 (0.047)
V_1, liters/kg	0.114	0.100	0.096	0.103 (0.009)
$\int_0^\infty C_{dt}$, μg hr/ml	125	128	131	128 (3)
R^2	0.9958	0.9982	0.9837	
S	7.7574	4.3811	91.1586	

[a] Values of A and B after correction to bolus administration case.

Table II—Results of Linear Regression Analysis on Natural Logarithm of Percent of Dose Remaining to be Absorbed *versus* Time

Study	Dose, mg	K_a, hr^{-1}	$t_{1/2}$, min	t_0, min
1	250	1.38	30	3
	500	1.08	38	3
2	125	1.95	21	3
	250	1.36	31	8
	500	1.00	42	4
	750	1.05	40	4

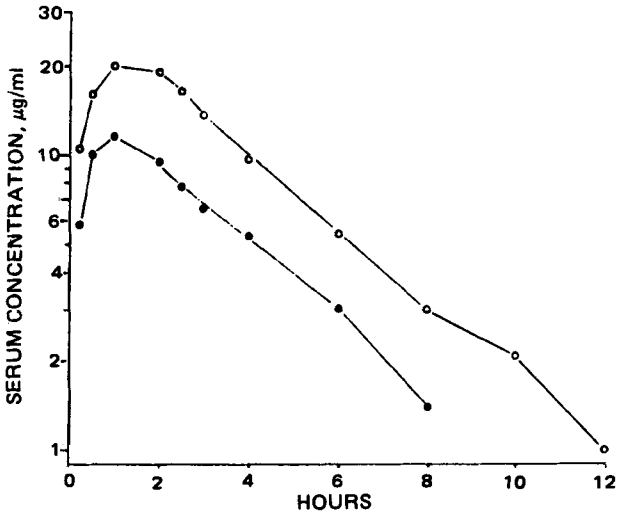

Figure 2—*Mean serum amikacin concentrations after intramuscular injection (Study 1). Key: ●, 250-mg dose; and ○, 500-mg dose.*

RESULTS AND DISCUSSION

Figure 1 is a semilogarithmic plot of the mean intravenous data. The results of the pharmacokinetic analyses of these data are listed in Table I. The reason for the poorer fit to the 5-min infusion data probably was the difficulty in timing a push injection exactly. The overall mean values of K_{21}, K_{12}, and K_{el} of 1.06, 0.669, and 0.578 hr^{-1}, respectively (Table I), were used in the Loo–Riegelman analysis.

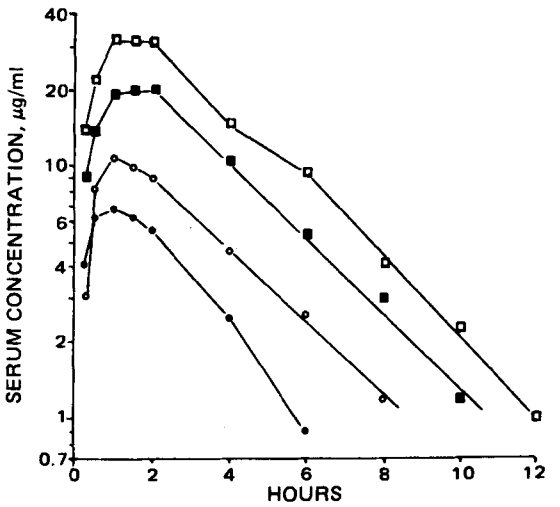

Figure 3—*Mean serum amikacin concentrations after intramuscular injection (Study 2). Key: ●, 125-mg dose; ○, 250-mg dose; ■, 500-mg dose; and □, 750-mg dose.*

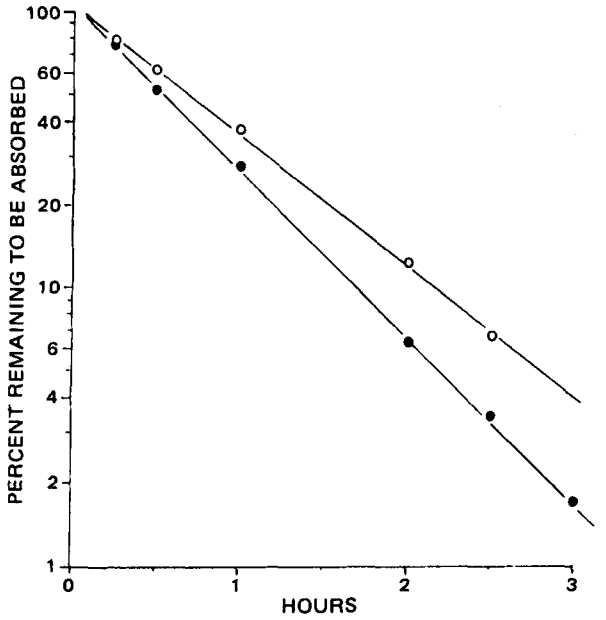

Figure 4—*Absorption profiles from Study 1 data. Key:* ●, *250-mg dose; and* ○, *500-mg dose.*

The mean intramuscular serum levels from Studies 1 and 2 are plotted semilogarithmically in Figs. 2 and 3. The calculated intramuscular absorption profiles were straight lines when plotted semilogarithmically (Figs. 4 and 5), indicating that intramuscular amikacin absorption could be a single first-order process. In all cases, the correlation coefficients from least-squares linear regression analysis of the natural logarithm of the percent remaining to be absorbed *versus* time were ≥0.9998. The absorption rate constants (K_a), absorption half-times ($t_{1/2} = \ln 2/K_a$), and absorption lag times (t_0) are listed in Table II. The overall mean $t_{1/2} \pm SD$ was 34 ± 8 min.

The values for K_a, 1–2 hr^{-1}, were sufficiently close to the values for α (Table II), 1.4–2.8 hr^{-1}, so that, on intramuscular administration, K_a and α would effectively disappear as separate coefficients, producing a compound rate constant that could be misinterpreted as an absorption rate constant.

The value of K_a decreased as the dose increased. There was a statistically significant linear regression of ln K_a on ln D (D is dose in milligrams):

$$\ln K_a = 2.388 - 0.370 \ln D \qquad \text{(Eq. 5)}$$

$$df = 4 \quad r = 0.957 \quad p < 0.01$$

which transformed to (Fig. 6):

$$K_a = 10.9D^{-0.370} \qquad \text{(Eq. 6)}$$

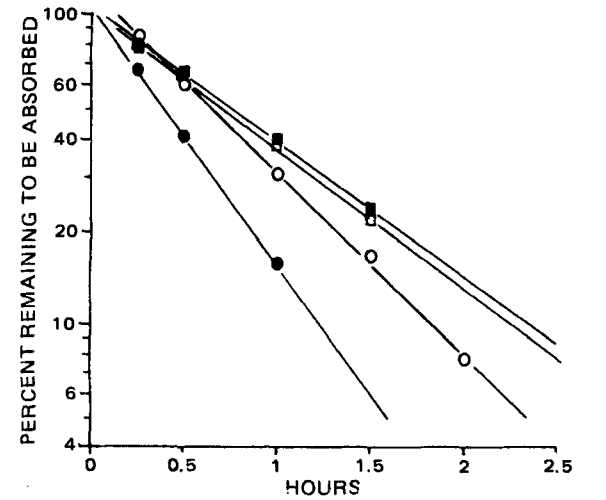

Figure 5—*Absorption profiles from Study 2 data. Key:* ●, *125-mg dose;* ○, *250-mg dose;* ■, *500-mg dose; and* □, *750-mg dose.*

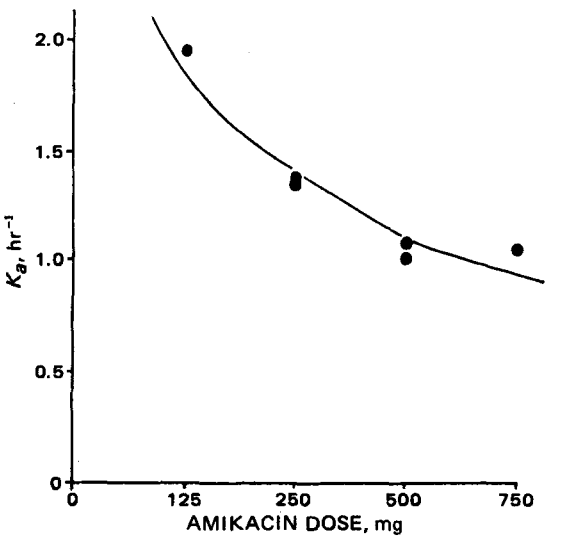

Figure 6—*Relationship between amikacin absorption rate constant and doses.*

According to Ballard (11), K_a, a first-order rate constant, is directly proportional to the dosing solution surface area to volume ratio (SA/V_s) when the membrane thickness and the drug's diffusion and partition coefficients are constant. If the physical distribution of injected dosing solutions is approximated as a sphere, then:

$$SA = 4\pi r^2 \qquad \text{(Eq. 7}a\text{)}$$

$$V_s = 4\pi r^3/3 \qquad \text{(Eq. 7}b\text{)}$$

where r is the sphere's radius. If 1 ml = 1 cm^3 (Eq. 7*b*), then the sphere's area in square centimeters can be determined from the volumes using Eqs. 7*a* and 7*b*. A plot of K_a *versus* SA/V_s should be a straight line that passes through the origin.

Amikacin for intramuscular injection was supplied as a formulated 250-mg/ml solution in a buffered aqueous vehicle. Therefore, a constant concentration was injected, but V_s varied with the dose. For the 125-, 250-, 500-, and 750-mg doses, the respective values of V_s were 0.5, 1, 2, and 3 cm^3; the respective values of SA/V_s were 6.09, 4.84, 3.84, and 3.35 $cm^{2/3}$. Least-squares linear regression analysis of K_a *versus* SA/V_s yielded a statistically significant ($df = 4$, $r = 0.968$, $p < 0.01$) straight line which passed through the origin (Fig. 7):

$$K_a = 0.294(SA/V_s) \qquad \text{(Eq. 8)}$$

Apparently, the variations in K_a were a purely physical result of the variation in the dosing volume. The best strategy for maximizing absorption rates is to administer a dose as several smaller volumes rather than a single larger volume. It may be difficult to detect absorption nonlinearities on the basis of the peak serum level and time to peak serum level unless sufficiently short serum sampling intervals are used.

Differences in the intrinsic absorbability of other aminoglycosides may exist due to differences in their diffusion and partition coefficients; but

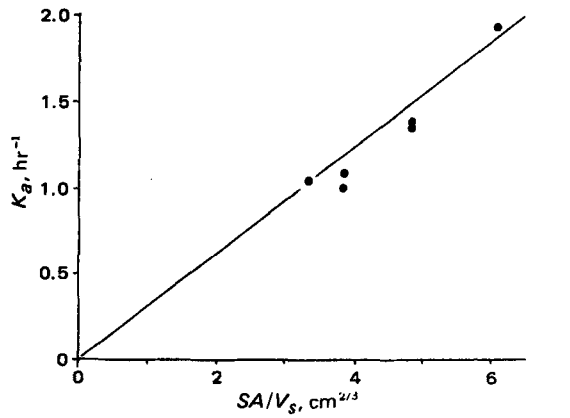

Figure 7—*Relationship between amikacin absorption rate constant and surface area to volume ratio.*

since they are marketed as preformulated dosing solutions, the same variations in K_a with dosing volume might be expected. Part of the variations noted earlier may be due to the effects of the different recommended dosing schedules and different dosing solution volumes on the volumes administered. The question of variations in aminoglycoside absorption rates is an interesting phenomenon that warrants further investigation.

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Theophylline Absorption and Disposition in Rabbits: Oral, Intravenous, and Concentration-Dependent Kinetic Studies

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Abstract □ Theophylline pharmacokinetics following oral and intravenous administration were studied, and the absolute bioavailability of five commercially available products was determined using the rabbit as an *in vivo* model. Concentration-dependent clearance studies were performed by multiple constant-rate infusion and multiple bolus dose administration of aminophylline. Theophylline pharmacokinetics following the oral adminstration of these products obeyed the one-compartment open model adequately. However, the data obtained following rapid intravenous aminophylline administration in the rabbit fit either the one-compartment model (half-life = 2.8 hr and the volume of distribution = 0.586 liter/kg) or the two-compartment model (β-phase half-life = 4.4 hr and $V_{d(\beta)}$ = 0.708 liter/kg). There were no significant product-to-product differences in the time to peak (t_{max}), the rate constant of absorption (k_a), or the percent of dose absorbed at 1 hr (F_1); however, differences in the absolute bioavailability (F), dose-normalized peak serum concentration ($C_{max(n)}$), and percent of dose absorbed at 6 hr (F_6) were significant. There was no evidence of concentration-dependent clearance for theophylline in the rabbit in the serum concentration range studied, but the results of the multiple constant-rate infusion study suggest that total clearance decreases at higher serum theophylline concentrations.

Keyphrases □ Theophylline—pharmacokinetics, oral and intravenous administration, concentration-dependent clearance □ Pharmacokinetics—theophylline, oral and intravenous administration, concentration-dependent clearance □ Bioavailability—theophylline, oral administration, aminophylline, intravenous administration

Several pharmacokinetic studies have investigated theophylline bioavailability from various dosage forms (1–5). Two studies (3, 5) determined the absolute theophylline bioavailability from oral dosage forms. However, additional research is needed to examine the intrasubject variability in theophylline elimination kinetics (6).

The present work concerned theophylline pharmacokinetics in the rabbit following intravenous and oral administration to determine the absolute bioavailability of theophylline from five commercially available dosage forms using the rabbit as an *in vivo* model and to examine the possibility of concentration-dependent clearance of this drug. Although no reports suggested dose-dependent kinetics for theophylline in the rabbit, it may be that at sufficiently high dosages the relationship between the dose and the serum concentration–time integral becomes nonlinear. Such a finding would preclude the use of traditional approaches for quantitating absolute or relative bioavailability.

EXPERIMENTAL

Materials and Methods—All dosage forms studied (A[1], B[2], C[3], D[4], and E[5]) were purchased commercially. Anhydrous theophylline[6] was used as supplied. Aminophylline[7] injection, 250 mg (25 mg/ml), was used for intravenous administration.

Animals—Male New Zealand White rabbits, 2.4–3.9 kg, were maintained on commercial rabbit food[8] and tap water and were fasted overnight prior to each oral experiment. Water was allowed *ad libitum* during fasting and throughout the experiment. Each animal received one oral dosage form of theophylline, followed 24 hr later by an intravenous dose of aminophylline.

Oral Administration of Drugs—The animal was restrained, and its mouth was opened by inserting hemostatic forceps from the side into the oral cavity immediately behind the rabbit's incisors. A small animal capsule administration device, with the tablet or capsule in its slotted end, was placed over the rabbit's tongue and advanced ~4–5 cm into the pharynx. The capsule or tablet then was released by pushing the plunger rapidly and completely. This administration was followed by ~10 ml of water. After the device and forceps were removed, the rabbit's mouth and nostrils were held closed until swallowing occurred.

Rapid Intravenous Administration of Aminophylline—A dose of 24.33

[1] Tablets (aminophylline, 200 mg), lot 776-991, Searle, Chicago, Ill.
[2] Capsules (theophylline, 200 mg), lot 1W60531, Cooper, Wayne, N.J.
[3] Tablets (theophylline, 125 mg), lot 68308, Riker Laboratories, St. Paul, Minn.
[4] S. R. Capsules (theophylline, 2 gr), lot 6090204, Fleming, Fenton, Mo.
[5] S. R. Capsules (theophylline, 1 gr), lot 6090204, Fleming, Fenton, Mo.
[6] Anhydrous theophylline, Nutritional Biochemicals, Cleveland, Ohio.
[7] Aminophylline Injection, Searle, Chicago, Ill.
[8] Purina Laboratory Rabbit Chow, Ralston-Purina, St. Louis, Mo.

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