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Pharmacokinetics of Amikacin and Chloramphenicol in the Aqueous Humor of Rabbits

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Composite data describing ocular pharmacokinetics are unreliable because of intersubject variation. To address this problem, an animal model was developed in which multiple aqueous samples from single subjects were obtained. Following direct anterior chamber or intravenous administration of amikacin or chloramphenicol, pharmacokinetic analysis of drug concentrations in the serum and anterior chamber was performed by using a nonlinear least-squares regression program. The number of anterior chamber paracenteses performed did not alter the beta elimination rates or percent penetration into the anterior chamber. The aqueous humor and peripheral-compartment terminal slopes were identical. These data indicate that complete ocular concentration-time curves can be obtained without altering antibiotic pharmacokinetics. Following direct injection into the anterior chamber, the elimination rates for both antibiotics followed a one-compartment model, whereas those following intravenous administration best fit an open, first-order, two-compartment model. Following intravenous administration, the anterior chamber elimination rate constants for both drugs were equal to that of the serum and significantly longer than that following direct injection. The elimination rates of both drugs following direct injection were similar. Systemic administration resulted in drug levels in aqueous humor that persisted longer than those following direct injection. Chloramphenicol, a lipophilic compound, gave higher mean concentrations in aqueous humor than did amikacin. Our model provides a new approach which rigorously examines ocular pharmacokinetics and provides data which suggest that for selected compounds the parenteral route of administration is preferable.

Ocular pharmacokinetics, the study of drug absorption, distribution, and elimination, has focused primarily on description of kinetic events following local drug administration (22, 24). Despite the potential benefits of parenteral drug administration in selected ocular diseases (6, 23a, 27), there are limited data characterizing ocular pharmacokinetics following systemic drug administration.

Previous studies in our laboratory examined the pharmacokinetics of 5-fluorocytosine (5FC) in the aqueous and vitreous humors and the sera of rabbits following systemic or subconjunctival administration (27). Kinetic modeling permitted objective comparison of 5FC pharmacokinetics following administration by both routes. These data suggested that for lipophilic compounds such as 5FC, the duration of therapeutic concentrations in the aqueous humor was significantly longer when the drug was systemically administered. We showed that the rate of elimination from the aqueous humor following oral administration was similar to that in serum, with terminal elimination half-lives of 3.0 and 3.2 h, respectively. Drug elimination following subconjunctival injection was significantly more rapid. Moreover, following subconjunctival injection no vitreal penetration was observed whereas following oral administration the mean concentrations in the aqueous and vitreous humors were equal.

However, it is important to recognize that our data, as well as the ocular pharmacokinetic data of others (4–7, 11, 12, 14–16), are based on a methodologically imprecise approach (26). While pharmacokinetic coefficients can be derived from indirect studies investigating ocular pharmacodynamics or by using fluorescent probes, for most drugs such data cannot be obtained noninvasively. As a result, ocular antibiotic studies generally examine a single level in one or more subjects to determine whether therapeutic concentrations are achieved. Occasionally, pharmacokinetic analysis is performed by combining samples of aqueous humor, each representing a single concentration-time point from different subjects (4, 5, 8, 11, 14, 16). However, the intersubject scatter in these studies is very large so that reliable kinetic parameters are difficult to establish. While there has been a recent attempt to circumvent this problem by obtaining serial aqueous humor samples in the same animal after a washout period (18), the reliability of this approach has not been validated.

Accurate pharmacokinetic information regarding optimal routes of drug administration has fundamental implications for clinical practice. Therefore, to permit more rigorous examination of ocular pharmacokinetics we developed an animal model which permits repeated sampling of the aqueous humor without altering drug kinetics. By using this animal model, we demonstrated that the important differences in the kinetics of drug elimination previously observed with 5FC following systemic versus local drug administration are present for other antimicrobial agents as well.

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MATERIALS AND METHODS

Animal model. Twenty-five adult, male, albino New Zealand rabbits (Hare Maryland Farms, Hewitt, N.J.)

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weighing 2.3 to 3.0 kg were used. Since aminoglycosides may bind to pigment in the iris (11), we used nonpigmented animals to facilitate measurement of low concentrations of amikacin in the aqueous humor. Antibiotic-free feed (Lab Rabbit Chow HF5326; Purina Mills Inc.) and water were provided. The rabbits were divided into five experimental groups of five animals each. The first group was used to test the validity of the animal model, and the subsequent groups were used to test the serum and aqueous humor kinetics of amikacin or chloramphenicol after intravenous or direct anterior-chamber injection.

The animals were anesthetized with an intramuscular dose of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (2.5 mg/kg) approximately 45 min prior to antibiotic administration. Animals were maintained anesthetized throughout the sampling period with supplemental intravenous ketamine as needed. A 24-gauge angiocatheter was inserted into the marginal ear vein to facilitate antibiotic administration, and a second catheter was inserted into the central artery of the contralateral ear. Following intravenous drug administration, each animal received a 2-ml flush of 0.9% NaCl. Prior to paracentesis, 1 drop of 0.5% tetracaine hydrochloride was administered to the experimental eye, followed by a tetracaine-soaked pledget applied to the conjunctiva prior to fixation with forceps. The arterial line was used to obtain blood samples. Both amikacin (13 mg/kg) and chloramphenicol succinate (100 mg/kg) were infused over 15 min (at a rate of 0.2 ml/min). Amikacin was obtained from Bristol Laboratories, Syracuse, N.Y., and chloramphenicol succinate was from Parke, Davis, Morris Plains, N.J. Chloramphenicol was reconstituted with sterile 0.9% NaCl immediately prior to intravenous administration and with balanced salt solution prior to intraocular administration.

Sampling technique. Bilateral slit lamp examination was performed prior to antibiotic administration and again following each paracentesis. This was done to assess the eye for signs of inflammation, leakage of aqueous humor, and any changes in the depth or clarity of the anterior chamber or lens. All aqueous humor samples were taken by using an aseptic technique. Care was taken to avoid tear fluid during sampling. The experimental eye was exposed with a sterile lid retractor and fixated with forceps. A sterile 30-gauge needle fused to a calibrated 25-μl capillary tube was used to obtain a sample of aqueous humor. This needle was gently inserted into the anterior chamber, and approximately 7 μl of aqueous was removed by the combined forces of positive intraocular pressure and capillary action. The paracentesis site was then examined with the slit lamp to ensure that the tract had sealed without leakage of aqueous fluid. The aqueous fluid was then transferred into a 5-μl microcapillary tube prior to determination of antibiotic levels. To permit accurate administration of a small volume of antibiotic into the anterior chamber, we modified our sampling device by fitting a rubber bulb to one end of the microcapillary pipette. Following the designated sampling periods, animals were sacrificed with pentobarbital sodium (125 mg/kg of body weight) intravenously, followed by bilateral pneumothoraces. The total volume of aqueous humor was immediately aspirated, and cell counts were determined by using a Spencer hemacytometer to assess the number of inflammatory cells present at a magnification of ×40. The eyes were subsequently enucleated and stored at −70°C until vitreal antibiotic levels were determined. Vitreous humor samples were obtained as described by Abel et al. (1); however, anterior and posterior vitreous humor samples were combined when assayed for antibiotic levels.

Antibiotic level assays. To determine amikacin levels in the serum and aqueous and vitreous humors, a one-dimensional vertical-diffusion microbiologic assay was performed. This assay, described by Edberg and Sabath (10), was modified to determine antibiotic levels in 5-μl samples. Blood samples were allowed to clot and were immediately centrifuged at 500 × *g* for 15 min. Prior to analysis, all samples were stored at 4°C, for vitreous humor samples, which were stored at −70°C until analysis. Serum and aqueous humor samples were processed on the day they were obtained. For analysis of amikacin levels, the test organism was *Staphylococcus aureus* ATCC 25923. Prior to the assay, the organism was incubated at 37°C for 1 to 2 h to achieve log-phase growth. The medium was then diluted in 0.85% sterile NaCl in a 1:100 dilution to achieve a final inoculum of 10^6 organisms per ml, using a MacFarland 0.5 standard. One percent purified agar (Difco Laboratories, Detroit, Mich.) and a nutrient broth (8 g/liter; Difco) were prepared with distilled water and adjusted to pH 8 with 1 N NaOH. This solution was heated to 52 to 55°C, mixed with *S. aureus* in a 1:10 dilution, poured into Wintrobe tubes (115 by 3 mm; Clay Adams), and allowed to harden. All agar tubes were prepared on the day of use. Five-microliter aliquots of serum or aqueous or vitreous humor were then pipetted onto the surface of the agar and incubated overnight at 37°C in an ambient-air incubator. Zones of inhibition were read with a magnifying eyepiece calibrated to 0.1 mm. Ten standards were prepared in physiologic sterile saline ranging from 50 to 0.04 μg/ml to determine a standard concentration curve. The accuracy of this method is comparable to the standard well diffusion microbiological assay (data not shown) and was sensitive to 0.04 μg/ml, with a coefficient of variation of 9%.

Chloramphenicol levels were assayed by high-pressure liquid chromatography. Serum and aqueous and vitreous humor samples were stored at −70°C until analysis. Samples were run undiluted at 35°C in a column (15.0 cm long by 0.42 cm wide) packed with Nucleosil 5-C18. Drug extraction was performed by using 100 μl of serum or vitreous humor or 10 μl of aqueous humor in 10 μl of zinc sulfate ($ZnSO_4$)–100 μl of benzoic acid–100 μl of acetonitrile (CH_3CN). Samples were then vortexed and centrifuged, and 50 μl of supernatant was injected into the column. The mobile phase (770 ml of 0.1 M ammonium phosphate, 230 ml of acetonitrile, and 0.5 ml of acetic acid in a buffer of 11.5 g of ammonium phosphate per liter) was delivered to the column at a rate of 2 ml/min with a Consta Metric model 111 pump (LDC, Riviera Beach, Fla.). Samples were injected by a 7120 syringe-loading Rheodyne injector. Chloramphenicol was monitored at 280 nm with a Miton Roy SM4000 UV detector. The detector signals were output to a model 4416 data station (Nelson Analytical, Cupertino, Calif.) with a linear strip chart recorder running at a speed of 30 cm/h recording the results. Quantitation of the antibiotic present was done by using the peak height of the concentration. Standards were prepared at 5, 10, 20, 30, 40, and 50 μg/ml, and controls were prepared at 12.5 and 25 μg/ml by using 15 μg of chloramphenicol (UTAK Laboratories, Saugus, Calif.) per ml. The assay sensitivity was 0.02 μg/ml.

Pharmacokinetic analysis. Pharmacokinetic analyses of the plasma and aqueous humor concentration-time relationships after systemic administration and direct ocular administration were performed by using an iterative, nonlinear, weighted, least-squares regression program, RSTRIP (23). The most appropriate pharmacokinetic models for each animal or set of data were determined by using the RSTRIP model selection criterion, which is a modified form of

Akaike's information criterion (3). Estimations for each exponential coefficient and time constants were computed with the standard deviations of each estimate, along with its 95% confidence range, calculated by using both univariate and support plane approximations for the bounds of the 95% confidence range. On the basis of the coefficient of determination and model selection criterion, aqueous humor and serum antibiotic concentration-time data following intravenous administration best fit the biexponential equation $C = Ae^{-at} + Be^{-bt}$, where a and b are the rate constants in the distribution and elimination phases, respectively, and A and B are the coefficients of the exponential terms for a and b, respectively. Concentration-time data obtained following direct anterior-chamber administration was best fit the monoexponential equation describing a one-compartment model. Other standard pharmacokinetic parameters were determined by using computer-generated primary coefficients and standard pharmacokinetic equations (13). Peripheral-compartment concentrations were calculated by using hybrid coefficients and microconstants (13). Observed drug concentrations in serum and aqueous humor were used to determine maximum concentrations (C_{max}), times to reach C_{max} (T_{max}), and areas under the concentration-time curves (AUC) for blood and aqueous humor. We calculated predicted peripheral concentrations on the basis of model-generated data; these were then compared with actual aqueous humor antibiotic concentrations. Overall differences in pharmacokinetic parameters among rabbits were evaluated with analysis of variance. The paired t test was used to determine whether there was any statistically significant difference between the pharmacokinetic parameters of the aqueous humor and serum of the same rabbit. The pooled t test was used to evaluate differences in elimination rate constants between amikacin and chloramphenicol following direct ocular administration. All statistical tests were performed by using the personal computer version of MINITAB (W. W. Norton, New York, N.Y.). The mean and standard deviation of each pharmacokinetic variable among all rabbits in a group were also calculated. In all tests, the level of significance was fixed at $P < 0.05$.

RESULTS

Characterization of the animal model. Our first objective was to determine whether multiple aqueous paracenteses would alter the rate of drug elimination. Several pharmacokinetic parameters were determined, including the terminal (β) elimination rate and extent of aqueous humor penetration.

In this first series of experiments, we examined aqueous humor kinetics following direct drug administration into the anterior chamber. These studies were performed to determine whether increasing the number of paracenteses altered the rate of drug elimination from the anterior chamber. The rationale behind these experiments was as follows: if serial paracenteses cause an alteration in the kinetics of efflux, then the slope of drug elimination would vary as a function of aspiration number. As shown in Fig. 1, there was no change in the elimination slopes for amikacin or chloramphenicol during a 2-h sampling period ($r = 0.999$), suggesting that serial paracenteses did not alter the elimination rate. These observations were significant at the 0.01 level.

Confirmatory data are provided in Fig. 2, which shows the effects of sequentially delaying the time of the first paracentesis on both drug penetration and the terminal elimination rate. The upper panel of Fig. 2 shows the mean serum kinetic data, and the lower panel shows the drug levels in the aqueous humor of both eyes for all five animals. We will refer to the first aqueous humor sample in the right or left eye of each animal as the virgin eye point. The percentage of the drug dose reaching the eye was calculated by comparing the AUC for a curve describing concentration-time data using sequential virgin time points from different animals (arrows) with that of the drug in the serum of the same animal. The $AUC_{aqueous}/AUC_{serum}$ ratio for virgin eye points and the $AUC_{aqueous}/AUC_{serum}$ ratio following multiple paracenteses (Fig. 3) were similar. In fact, there was slightly more penetration (15 versus 10%) in virgin samples; these differences were not statistically significant. Previously, aqueous aspiration has been shown to increase rather than decrease the percent penetration (25). The mean elimination rate constants for serum and right and left eyes (solid lines) were 0.4979 ± 0.165, 0.476 ± 0.147, and 0.3832 ± 0.050 h^{-1}, respectively. Analysis of variance revealed that there were no differences between the serum and aqueous humor elimination rate constants in these five animals ($P > 0.05$, $F = 1.08$). Since the aqueous humor elimination rates represent data from animals in whom the terminal elimination rate curves were sequentially delayed and had three to five paracenteses, they also support direct-injection studies indicating that serial paracenteses do not alter the aqueous humor elimination rate.

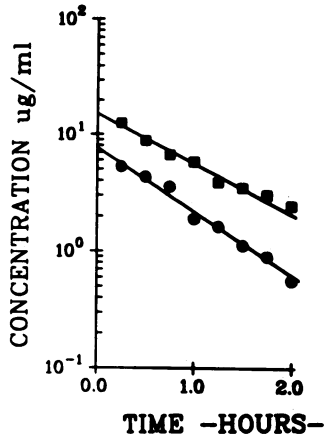

FIG. 1. Aqueous pharmacokinetics following direct injection into the anterior chamber. Following direct anterior-chamber injection of amikacin (20 μg in a volume of 10 μl [●]) or chloramphenicol 50 μg in a volume of 10 μl [■]), aqueous humor samples were obtained at 15-min intervals over a 2-h period. The concentration-time data best fit a one-compartment model (correlation coefficient, >0.99).

Pharmacokinetics following intravenous administration. We next compared the kinetics of amikacin and chloramphenicol in the aqueous humor by measuring sequential drug concentrations in the serum and aqueous humor of two groups of five animals each. These studies were performed to confirm the validity of our animal model further. Moreover, by using this model we wanted to confirm several observations based upon earlier studies with 5FC (27), including the question of whether drug elimination from the aqueous humor following systemic administration is equal to that from plasma. In these experiments, we compared amikacin and chloramphenicol since they have different physicochemical properties which are known to influence penetration of blood-brain and blood-aqueous humor barriers.

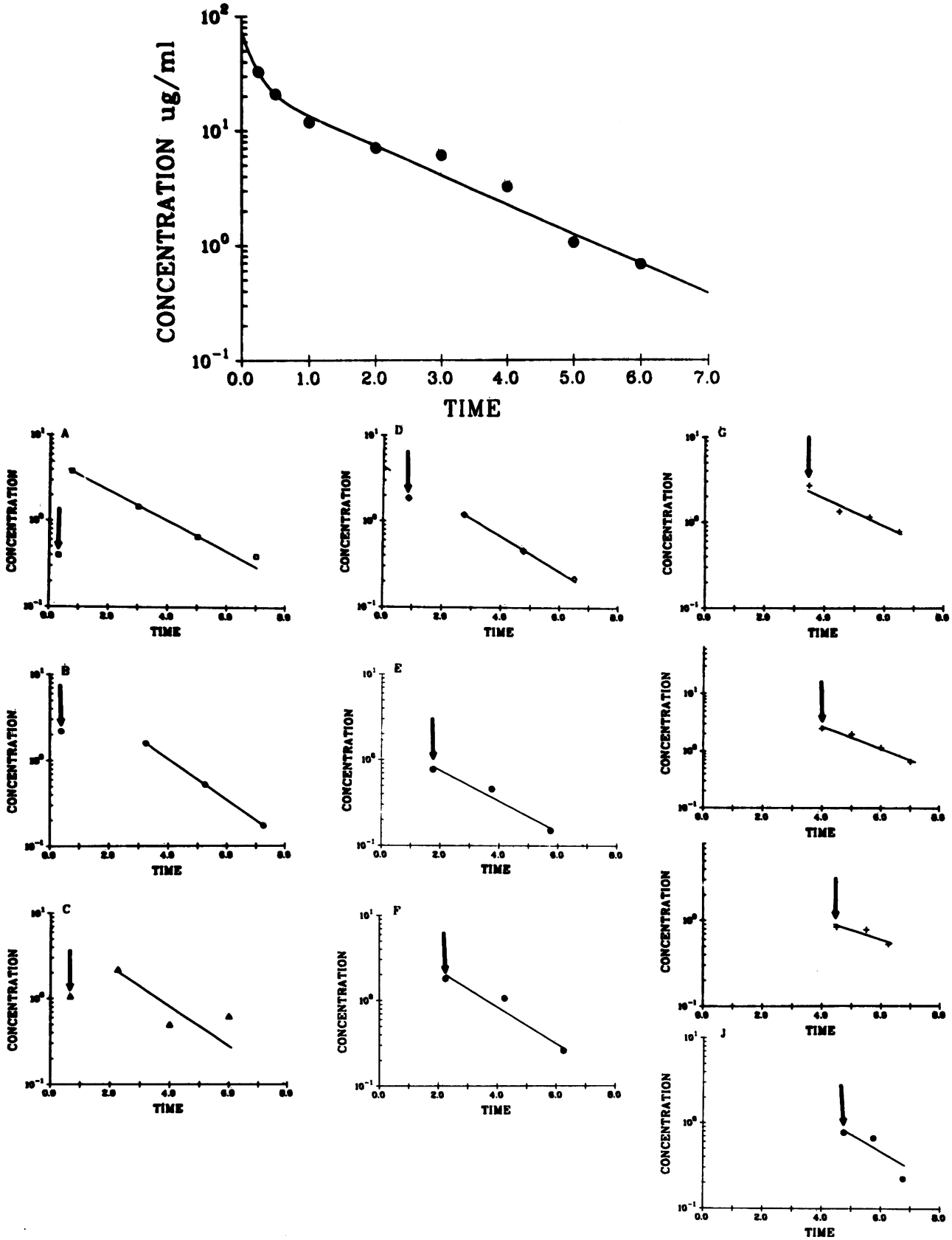

FIG. 2. A comparison of complete serum and sequentially delayed aqueous humor pharmacokinetics following intravenous injection. Five rabbits received amikacin intravenously (13 mg/kg of body weight in a volume of 5 ml administered over 15 min). The upper panel shows semilogarithmically the mean amikacin concentration-time data for serum. The lower panel shows the aqueous humor concentration-time data for all five animals (10 eyes). Staggered initial (virgin) aqueous humor sample time points from 0 to 5 h after completion of intravenous infusion are shown with solid arrows. Also shown are terminal elimination slopes from animals that had sequentially delayed initial samples resulting in progressively fewer paracenteses.

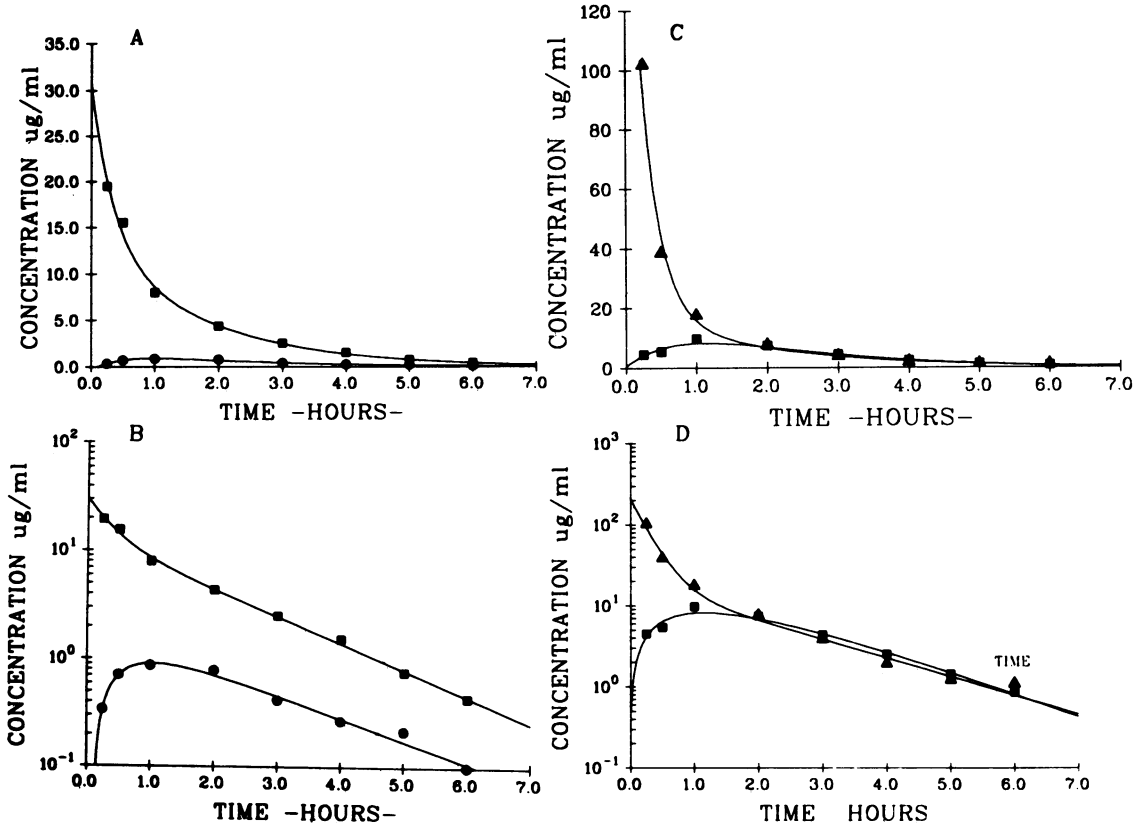

FIG. 3. (A and B) Mean levels of amikacin in serum and aqueous humor following intravenous administration. Panel A is an arithmetic depiction of amikacin concentration-time data of five rabbits (■, serum; ●, aqueous humor). Panel B shows these data presented semilogarithmically. (C and D) Mean levels of chloramphenicol in serum and aqueous humor following intravenous administration. Panel C is an arithmetic depiction of chloramphenicol concentration-time data of five rabbits (▲, serum; ■, aqueous humor). Panel D shows these data presented semilogarithmically. Depiction of data arithmetically facilities comparison of drug penetration (AUCs), whereas the semilogarithmic plots permit comparison of the terminal elimination rates.

Chloramphenicol, which is both lipophilic and uncharged (12), has better penetration of the blood-aqueous humor barrier (2, 9, 17, 20) than does amikacin (8, 17).

Figure 3A and B show the mean drug levels in serum and aqueous humor of five rabbits given amikacin intravenously. These data are plotted arithmetically (Fig. 3A) to better visualize the relative penetration. Figure 3B shows the same data on a semilogarithmic plot to permit graphic comparison of the relationship between serum and aqueous humor terminal elimination rates. Model-derived kinetic parameters are given in Table 1. On the basis of the model selection criteria and the coefficients of determination, the antibiotic concentrations in serum and the aqueous humor over time best fit a two-compartment open model. Amikacin had a mean serum β elimination of 0.5686 h^{-1}, which corresponds to a β half-life of 1.22 h. The corresponding values for the aqueous humor were 0.4794 h^{-1} and 1.42 h, respectively. The mean maximum concentrations in the serum and aqueous humor were 19.46 and 0.87 μg/ml, respectively. Amikacin elimination from the eye equalled that from the serum ($P > 0.05$ with the paired t test). The mean β elimination for serum was 0.5686 ± 0.363 (standard error of the mean [SEM] = 0.16), and that for aqueous humor was 0.479 ± 0.102 (SEM = 0.045; df = 4). There were no measurable amikacin levels (i.e., <0.04 μg/ml) in the vitreous gel at the time of sacrifice. Importantly, the degree of penetration calculated

TABLE 1. Kinetic parameters of amikacin and chloramphenicol following intravenous administration

Drug	β (h^{-1})	$t_{1/2}$ β (h)	K_{el} (h^{-1})	AUC (mg · h/liter)	% Penetration	C_{max} (μg/ml)	T_{max} (h)	t lag (h)	K_{12} (h^{-1})	K_{21} (h^{-1})
Amikacin in:										
Serum	0.5686	1.2190	1.0290	29.8350	NA[a]	30.7050	0	0	0.852	1.6206
Aqueous humor	0.4794	1.4213	NA	2.9349	9.837	0.9073	1.0426	0.1078	NA	NA
Chloramphenicol in:										
Serum	0.6020	1.1575	3.2798	98.0720	NA	109.34	0	0	1.6352	0.9696
Aqueous humor	0.6733	1.0349	NA	27.058	27.58	8.1645	1.548	0.0427	NA	NA

[a] NA, not applicable; constant cannot be analyzed for the specific parameter indicated.

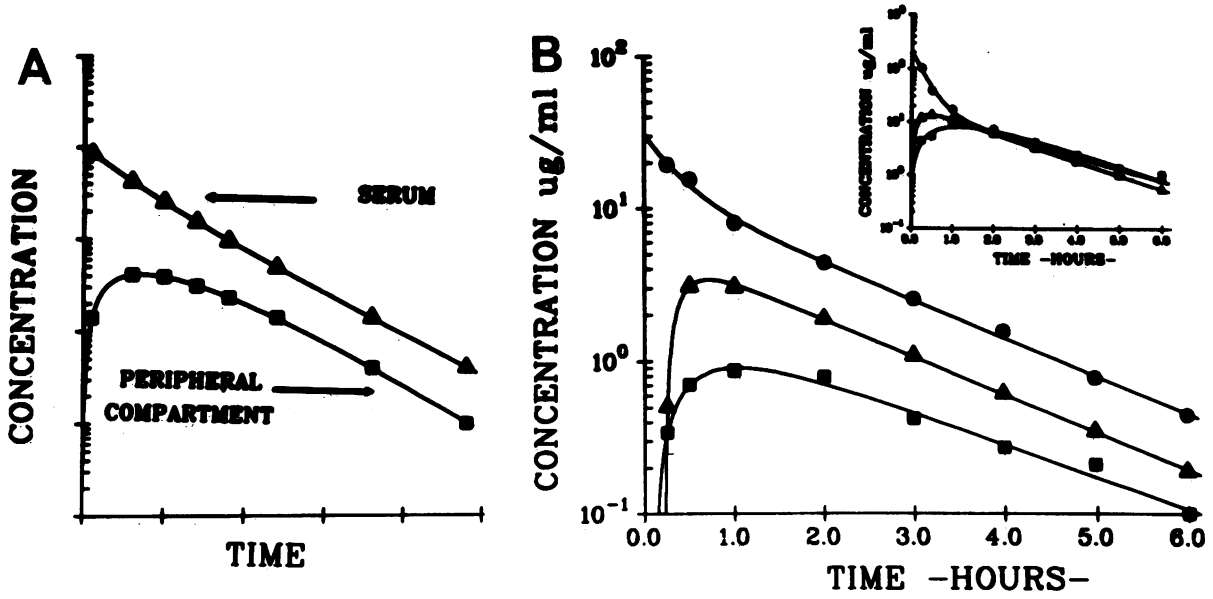

FIG. 4. (A) Semilogarithmic plot of computer-generated concentration-time curves for a two-compartment model; drug concentrations in serum (▲) and the predicted peripheral compartment (■) are shown. (B) Semilogarithmic display of amikacin concentrations in serum (●), aqueous humor (■), and calculated peripheral compartments (▲) following systemic drug administration. (Inset) Semilogarithmic display of chloramphenicol concentrations in serum (●), aqueous humor (■), and the peripheral compartment (▲) following systemic administration.

from the AUC data was 10% which, as noted earlier, is similar to that derived from virgin eye points in the first group of five animals (Fig. 1). While albino rabbits may have higher aminoglycoside concentrations in aqueous humor than do pigmented animals (11), the degree of penetration for amikacin was comparable to that observed for other aminoglycosides in human subjects (6).

Figure 3C and D show the pharmacokinetic data from mean chloramphenicol concentrations in serum and aqueous humor following intravenous administration. As with parenteral amikacin, concentrations in both serum and aqueous humor best fit a two-compartment open model. The β elimination for serum was 0.602 h^{-1}, and the β half-life was 1.1575 h. The corresponding values for aqueous humor were 0.673 h^{-1} and 1.03 h, respectively. The maximum concentrations in serum and aqueous humor were 109.3 and 8.16 μg/ml, respectively, and the degree of penetration calculated from mean concentration data for serum was 28%. The average drug level in vitreous humor at the time of sacrifice was 1.1 μg/ml.

Additional data indicating that multiple paracenteses did not alter the kinetics of drug elimination from aqueous humor are provided by the comparison of the serum, aqueous humor, and peripheral-compartment elimination rates. Figure 4A shows a computer-generated concentration-time curve for a two-compartment model. As shown, in the postdistributive phase concentrations in the serum and peripheral compartment decline at the same rate (13). While terminal elimination in the hypothetical peripheral compartment need not correspond to that in the anterior chamber, the observation that serum and aqueous humor elimination rates were equal was expected since identical results had been noted with 5FC (27). Figure 4B shows serum, peripheral-compartment and aqueous humor concentration-time curves for amikacin. The inset shows the same data for chloramphenicol. Peripheral-compartment data were calculated from serum data by using standard methods (13). The aqueous humor elimination rates for both chloramphenicol and amikacin were identical to those in the peripheral compartment and serum. The peripheral-compartment and aqueous humor concentrations of amikacin were less than that in the serum. However, the elimination slopes were parallel throughout the course of the experiment, although progressively more paracenteses were obtained. If serial paracentesis caused a breakdown in the blood-aqueous humor barrier to amikacin, the levels in the aqueous humor would have approached that in the peripheral compartment and eventually that in the serum. While the terminal aqueous humor and serum levels of chloramphenicol were equal, this was also true for the peripheral compartment. Consistently, previous studies using data pooled from different subjects show excellent penetration for chloramphenicol but not for amikacin into the aqueous humor (2, 8, 9, 17, 20).

Slit lamp examination of both groups following paracenteses after intravenous drug administration showed minimal evidence of inflammation. Evaluation of the aspirated terminal aqueous humor for leukocytes revealed fewer than or equal to 5 cells per mm^3 for the amikacin animals and 7 cells per mm^3 for the chloramphenicol animals, compared with 2 and 13 cells per mm^3 in the respective contralateral controls.

Pharmacokinetics following direct injection. To determine whether the rate of drug elimination following local administration was independent of the physicochemical properties which regulate entry from the serum, we compared the rates of drug elimination from the aqueous humor for both amikacin and chloramphenicol following direct injection. We were particularly interested in confirming data from earlier studies (27) suggesting that elimination rates following direct aqueous humor injection for disparate compounds would be equal to one another and more rapid than those following systemic drug administration. The pooled *t* test (MINITAB) was used to test this prediction. As shown in Fig. 1, the elimination rate constants for amikacin and chloramphenicol were 1.199 and 1.010 h^{-1}, respectively, with corresponding terminal

half-lives of 0.58 and 0.69 h. These values were similar ($P > 0.05$, df = 4). The mean terminal (3-h) chloramphenicol level in the vitreous humor was 0.094 μg/ml, whereas that for amikacin was unmeasurable (<0.05 μg/ml). As shown in Fig. 1 and 3, the β elimination half-life values following intravenous administration for amikacin and chloramphenicol were greater ($P < 0.05$) than those following intraocular administration.

DISCUSSION

Most studies which examine ocular drug penetration simply determine whether therapeutic concentrations are achieved. Such data are of limited value compared with an objective examination of drug pharmacokinetics (22). While there are limitations in using an animal model rather than data obtained from clinical trials, it is ethically impossible to perform elaborate ocular pharmacokinetic studies with humans. Fortunately, the rabbit is a reasonably reliable model for the study of ocular kinetics (6, 21, 22, 24, 25). Since we believe that pharmacokinetic information regarding optimal routes of drug administration has important implications for ocular therapeutics, we have developed a rabbit model which permits objective pharmacokinetic analysis and determination of population kinetic parameters. However, it should be emphasized that while our studies examined the pharmacokinetics of antibiotics, they are intended to describe basic ocular pharmacokinetics rather than provide information which is immediately relevant in the treatment of infections. Furthermore, while direct drug injection into the anterior chamber is not used therapeutically, we employed this route of drug administration to facilitate pharmacokinetic modeling.

Previous studies have shown that traumatic aspiration of large aqueous humor volumes may increase the penetration of drugs (6–8, 21, 22, 25). Moreover, removal of a large volume of aqueous humor may also decrease aqueous humor flow-dependent drug elimination. To develop an in vivo ocular pharmacokinetic model which permitted repeated aqueous humor paracenteses from the same animal but did not alter drug kinetics, we constructed a lancetlike instrument consisting of a 30-gauge needle fused to a calibrated microcapillary tube. This instrument permitted multiple small samples of aqueous humor to be obtained relatively atraumatically. In rabbits, the aqueous humor volume is 350 μl, and the aqueous humor flow rate is 250 μl/h (22). Our sample volume represented only 2% of the total aqueous humor volume, and this procedure was associated with little inflammation.

To determine whether multiple aqueous humor samplings altered the rate of drug elimination, we examined the terminal elimination rates following direct anterior-chamber injection of amikacin. Serial sampling had no effect on the rate of amikacin elimination from the aqueous humor. These observations were corroborated in similar studies with chloramphenicol. To establish that serial paracenteses did not affect the blood-aqueous humor barrier and, hence, penetration of the drug into the aqueous humor from the serum, the percent penetration utilizing only a single virgin aqueous humor concentration-time point per animal (Fig. 2, arrows) was compared with that calculated on the basis of multiple concentration-time points (Fig. 3) from individual rabbits. While the data describing the sequentially obtained virgin eye concentrations in different animals were less reliable than those from multiply sampled animals because of interanimal variation, the degree of amikacin penetration was nevertheless found to be statistically similar in the two groups. Delaying the first paracentesis or increasing the number of paracenteses following systemic administration had no effect on the β elimination rates. Moreover, the ratio of the drug levels in the aqueous humor and peripheral compartment was constant during the elimination phase, despite an increasing number of paracenteses. These studies indicate that there was no alteration in either the degree of penetration or rate of drug elimination from the anterior chamber following serial sampling. The absence of a measurable effect of serial paracenteses on drug pharmacokinetics is likely related to both the absence of a significant inflammatory reaction and the small volume of aqueous humor removed. Moreover, the effects of serial paracenteses are important to our model only insofar as they affect pharmacokinetics; there was none for amikacin or chloramphenicol (see above discussion) or the quinolone fleroxacin (23a).

In the remaining experiments, we compared the pharmacokinetics of amikacin and chloramphenicol following intravenous and direct injections into the anterior chamber. These studies show the utility of standard kinetic models in describing ocular pharmacokinetics. Moreover, use of this animal model allowed us to test several hypotheses based upon early studies with 5FC (27), including the question of whether drug elimination from the aqueous humor following systemic administration is proportional to that from the plasma. In these studies, we used amikacin and chloramphenicol since they possess different physicochemical properties known to influence the ability to cross the blood-aqueous humor and blood-brain barriers. The kinetics of both drugs following intravenous administration were well described by a two-compartment open model in which absorption, distribution, and elimination followed first-order kinetics. Pharmacokinetic data obtained following direct injection into the anterior chamber was described by a one-compartment model.

As predicted by earlier studies with 5FC (27) and pharmacokinetic theory describing drug elimination in a peripheral compartment (13; Fig. 4), drug elimination rates in the aqueous humor were essentially identical to those in the serum. In contrast, the rates of elimination for both compounds following direct injection were significantly more rapid than those following parenteral administration and the half-lives of elimination for amikacin and chloramphenicol were similar (1.20 and 1.01 h, respectively). It appears that drug elimination following direct injection is independent of physicochemical properties which regulate entry from the plasma. This is not surprising in view of the anatomy of the eye and aqueous humor physiology. Aqueous humor is produced in the posterior chamber at a constant rate (approximately 250 μl/h in rabbits [22]) and exits the anterior chamber via Schlemm's canal, in which no membrane barrier exists. Bulk flow of aqueous humor may explain why antibiotics with disparate properties exit the anterior chamber at the same rate following direct injection. However, confirmation of this hypothesis will require studies with additional compounds. Nevertheless, as observed with 5FC (27), it appears that concentrations of compounds which readily penetrate the eye following parenteral drug administration are maintained longer than those following local administration.

We have developed and validated a rabbit model which permits rigorous objective pharmacokinetic analysis and determination of population kinetic parameters. Analysis of population pharmacokinetic data using complete concentra-

tion-time curves not only is theoretically preferable to pooling of data from different subjects (26) but also provides robust kinetic information obtained from a small number of animals. Since pharmacokinetic data are important in the design of clinical trials, we believe that additional studies using our model may have fundamental clinical implications not only for antibiotics but for all classes of ocular pharmaceuticals.

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