Cinoxacin in Female Mongrel Dogs: Effect of Urine pH on Urinary Drug Excretion and Correlation of *In Vitro* Characteristics of Oral Dosage Forms with Bioavailability

J. F. QUAY ˣ, R. F. CHILDERS, D. W. JOHNSON, J. F. NASH, and J. F. STUCKY, II

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Abstract □ Cinoxacin was measured in plasma and in urine of female mongrel dogs after single 10-mg/kg iv doses. The terminal half-life of plasma cinoxacin was 0.873 hr in dogs whose urine had been alkalinized by oral administration of sodium bicarbonate, 3.79 hr in normal dogs, and 15.8 hr in dogs whose urine had been acidified by oral administration of ammonium chloride. Dogs with alkalinized urine were chosen for cinoxacin bioavailability studies to minimize interanimal variability and to maximize recovery of unaltered cinoxacin in the urine. Four lots of crystalline cinoxacin with widely different particle sizes were prepared and characterized with respect to their specific surface areas, particle-size distributions, and dissolution profiles *in vitro*. Capsules of cinoxacin from these lots and aqueous solutions of cinoxacin were administered as single oral doses to dogs with alkalinized urine. Four aspects of the bioavailabilities of the oral preparations, peak plasma cinoxacin concentration, time of peak plasma cinoxacin concentration, area under the plasma cinoxacin concentration–time curve, and urinary excretion of cinoxacin, were determined and found to follow rank-order relationships with the characteristics of the same preparations *in vitro*. Linear relationships were observed between the reciprocal of the dissolution rate constant and the area under the plasma cinoxacin concentration–time curve, between the reciprocal of specific surface area and the reciprocal of the time of peak plasma cinoxacin concentration, and between the reciprocal of specific surface area and the amount of cinoxacin excreted in urine in 24 hr.

Keyphrases □ Cinoxacin—effect of urine pH on urinary excretion, bioavailability correlated with physical characteristics of oral dosage forms, dogs □ Excretion, urinary—cinoxacin, effect of urine pH, dogs □ Bioavailability—cinoxacin, correlated with physical characteristics of oral dosage forms, dogs □ Antibacterials—cinoxacin, effect of urine pH on urinary excretion, bioavailability correlated with physical characteristics of oral dosage forms, dogs

Cinoxacin[1], 1-ethyl-1,4-dihydro-4-oxo[1,3]dioxolo[4,5-*g*]cinnoline-3-carboxylic acid, is a new synthetic compound with an antibacterial spectrum similar to the spectra of nalidixic acid and oxolinic acid (1). Cinoxacin is a weak organic acid[2] with limited solubility under acid conditions[3]. The drug is effective when administered orally to patients with urinary tract infections (2, 3).

BACKGROUND

Nelson (4) and Levy (5) reviewed the literature on the relationship between particle size or surface area of drug preparations and their rates of solution and GI absorption. Smaller particle size (greater surface area) favored rapid dissolution and rapid oral absorption for many drugs. This relationship was demonstrated in human studies of slightly soluble weak organic acids such as sulfadiazine (6), phenytoin (7), aspirin (8), dicumarol (9), and tolbutamide (10).

Moore *et al.* (11) found that the lag time for absorption increased and the absorption rate decreased in the series: sodium nalidixate; nalidixic acid, 4–12 μm; and nalidixic acid, 177–250 μm. The dissolution rates of the same preparations showed rank-order correlation with biological parameters.

The excretion and renal clearance rates of some weak acids and bases are influenced by urinary pH (12, 13). Organic acids with pKa values as widely different as 3.0 (salicylic acid) and 7.0 (phenobarbital) underwent elevated renal clearance in subjects whose urine was alkalinized by sodium bicarbonate administration (14, 15).

Therefore, it appeared likely that, following oral administration, the cinoxacin absorption rate would depend on the surface area or particle size of the preparation. Furthermore, it was expected that the peak blood cinoxacin concentration as well as the rate and extent of excretion of the drug into the urine would depend on both the absorption rate and the renal excretion rate as influenced by the pH of the tubular urine.

The purposes of the present investigation were to establish the relationship between urinary pH and cinoxacin excretion in the dog and then to utilize the appropriate set of experimental conditions in this species to evaluate the relationships between the surface area and dissolution rate of different cinoxacin preparations and their bioavailability.

EXPERIMENTAL

Materials—Samples from one lot of cinoxacin were recrystallized from dimethylformamide at various temperatures and stirring rates to yield three lots of crystals, B, C, and D, with different median particle sizes. Another sample of cinoxacin was sheared in a comminuting mill[4] to prepare the smallest median particle-size material, Lot A. The pure drug substance was assayed by high-pressure liquid chromatography[5] and found to be 100.4, 101.6, 101.7, and 98.4% pure for Lots A, B, C, and D, respectively.

Cinoxacin from each lot was hand filled, without excipients, into No. 2 gelatin capsules. Solutions for oral and intravenous administration consisted of sterile-filtered aqueous solutions of the sodium salt, pH 7.5, containing 100 mg of cinoxacin acid equivalent/ml.

***In Vitro* Characterization of Cinoxacin Preparations**—Specific surface areas of Lots A–D were determined by two Brunauer, Emmett, and Teller (BET) (16) gas adsorption techniques. Surface areas calculated from static volumetric[6] and GC[7] measurements were comparable. Particle-size distributions were determined with an automated counter[8]. The X-ray powder pattern[9] of each lot was identical to that of a reference standard.

Dissolution profiles for 150 mg of each lot in gelatin capsules were generated using a modified[10] USP rotating-basket apparatus (17) at 100 rpm. The medium for dissolution tests was 300 ml of pH 6.5 phosphate buffer USP at 37°. Under these conditions, cinoxacin solubility was 3 mg/ml. During the test, 5-ml aliquots were collected with filter-tipped pipets at 5, 10, 15, 20, 25, 30, 45, and 60 min and at t_∞[11]. The aliquots were diluted by automated analysis equipment[12], and the absorbance due to cinoxacin was measured at 270 nm[13].

[1] Cinobac, Eli Lilly and Co., Indianapolis, Ind.
[2] Aqueous pKa 4.9 at 37°.
[3] The aqueous solubility at 37° is 0.03 mg/ml at pH 1.2, 0.07 mg/ml at pH 4.5, and 1.0 mg/ml at pH 6.0.

[4] Model UD, Fitzpatrick Co., Elmhurst, Ill.
[5] Model 8500 liquid chromatograph, Varian Instrument Division, Palo Alto, Calif.
[6] Micromeritics Instrument Corp., Norcross, Ga.
[7] Quantachrome Corp., Greenvale, N.Y.
[8] Model TA II, Coulter Electronics, Hialeah, Fla.
[9] Cu radiation was used with a Ni filter and diffractometer, North American Phillips Corp., Mt. Vernon, N.Y.
[10] Modifications consisted of the use of a flat-bottom vessel, uncovered.
[11] The t_∞ sample was collected after homogenization of the remaining vessel contents.
[12] Technicon Industrial Systems, Tarrytown, N.Y.
[13] Coleman model 101, Perkin-Elmer Corp., Norwalk, Conn.

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Table I—*In Vitro* Characteristics of Cinoxacin Preparations

Lot	SSA[b], m^2/g	Particle Size[a], μm			K_d[d], min^{-1}
		90%[c]	50%	10%	
A	0.466	15	32	64	0.082
B	0.139	52	83	110	0.045
C	0.0751	84	190	420	0.032
D	0.0259	—	~1000[e]	—	0.016

[a] Equivalent spherical diameter. [b] Specific surface area. [c] Volume percentile, greater than. [d] First-order dissolution rate constant. [e] Visual estimation.

***In Vivo* Evaluation of Cinoxacin**—To prepare dogs with acid urine, female mongrel dogs, 14–20 kg, were treated orally for 2 days preceding the day of the experiment with 2 g of ammonium chloride in gelatin capsules at 7 am, 12 noon, and 4 pm. On the day of the experiment, 2 g was given at 6 am, 7:30 am, and 12 noon.

To prepare dogs with alkaline urine, the same doses of sodium bicarbonate were given on the same schedule. Dogs with unperturbed urine pH were given no premedication but otherwise were treated in an identical manner.

On the night preceding the day of the experiment, the dogs were fasted in their home cages but allowed free access to water. On the day of the experiment, they were transferred to stocks in which they could stand or sit comfortably. Thirty minutes before drug administration, a Foley retention catheter[14] was inserted into the urinary bladder of each dog and secured by inflation of the balloon with 5 ml of water. The dog was then given 500 ml of tap water through a temporarily placed stomach tube.

After 30 min, immediately before the dose of cinoxacin, the bladder was emptied and the urine volume and pH[15] were recorded. The sample was chilled in an ice bath until the end of the experiment and then was frozen and stored at −10° until assayed. Immediately following the collection of the urine sample, a blood sample was withdrawn from one cephalic vein into a heparinized vacuum tube[16]. The plasma fraction was removed, chilled, frozen, and stored in the same manner as the urine sample.

For intravenous studies, 10 mg of cinoxacin/kg as a sterile solution was administered to alkalotic, normal, or acidotic dogs as a bolus by syringe into one cephalic vein. For oral studies, 10 mg of cinoxacin/kg was administered to alkalotic dogs as a sterile solution by stomach tube or as a sample of crystalline powder from Lot A, B, C, or D in a gelatin capsule over the tongue. Both types of oral preparations were followed by 25 ml of water given by the same route as the medication. At specified times, blood and urine samples were collected and treated as described.

On the day of assay, plasma and urine were thawed. A portion of each sample was acidified with 0.1 *N* HCl. Cinoxacin was extracted from the acidified sample into chloroform and then from the chloroform fraction into borate buffer, pH 9.0. The resulting solution was made strongly acidic with concentrated sulfuric acid, and the fluorescence due to cinoxacin was read at 445 nm during excitation at 356 nm[17]. Fluorescence readings were converted to cinoxacin concentrations by use of a calibration curve constructed from extracted plasma samples containing known concentrations of cinoxacin.

Cinoxacin concentrations determined in freshly drawn plasma samples by a fluorometric method (18) were not changed by several months of frozen storage. In addition, several days of cyclic freezing and thawing of plasma samples gave no indication of cinoxacin decomposition. The extraction efficiency of cinoxacin from plasma by this method was determined using ^{14}C-*carboxylic*-cinoxacin.

The radioactive cinoxacin was added to drug-free human plasma in concentrations of 2, 5, 10, 20, and 40 μg/ml, and the samples were analyzed in quintuplicate. The data showed 92–96% recovery of drug in the borate buffer. The fluorescence spectrum of cinoxacin in this buffer coincided with that of extracts (by the described procedure) from both standard spiked plasma and experimentally derived plasma samples of cinoxacin. Furthermore, this method is specific for the parent cinoxacin in either plasma or urine. The principal metabolite, 1-ethyl-1,4-dihydro-7-hydroxy-6-methoxy-4-oxocinnoline-3-carboxylic acid, had 0.1% of the fluorescence of cinoxacin at 445 nm.

Table II—Results of Duncan's Test[a] on the Intravenous Data

t, min	Plasma Cinoxacin, μg/ml		
	Alkalinized	Untreated	Acidified
1.5	86.4[a] (4.9)	83.5[a] (8.4)	103[b] (5.6)
10	52.4[a] (1.4)	1.3[a] (2.0)	54.2[a] (2.0)
20	42.9[a] (1.6)	45.5[ab] (1.7)	51.8[b] (2.4)
30	36.2[a] (1.5)	43.6[b] (1.9)	47.9[b] (1.9)
40	30.8[a] (1.7)	41.6[b] (2.1)	47.3[b] (1.9)
60	24.3[a] (1.6)	39.2[b] (2.3)	46.8[c] (1.9)
80	17.8[a] (1.6)	34.5[b] (2.2)	45.1[c] (2.1)
120	10.6[a] (1.5)	31.4[b] (2.4)	43.3[c] (1.8)
180	3.60[a] (0.40)	25.7[b] (2.9)	41.2[c] (1.9)
240	1.41[a] (0.19)	21.7[b] (3.5)	40.3[c] (2.3)
300	0.53[a] (0.08)	17.7[b] (3.8)	37.0[c] (2.0)
360	0.16[a] (0.05)	15.0[b] (3.6)	36.2[c] (2.1)
420	0.09[a] (0.04)	12.8[b] (3.1)	34.5[c] (1.8)
480	0.07[a] (0.03)	10.1[b] (2.5)	34.1[c] (2.0)
AUC (0–480 min), μg-min/ml	4030[a] (240)	11,600[b] (1300)	19,500[c] (900)
ΣU_{24}, %	98.5[a] (2.7)	81.9[b] (5.7)	7.8[c] (1.1)[b]

[a] Means of the variables within a row are statistically different ($\alpha = 0.05$) if they do not have a superscript letter in common. Numbers in parentheses are standard errors of the mean for eight dogs unless otherwise indicated. [b] Four dogs only.

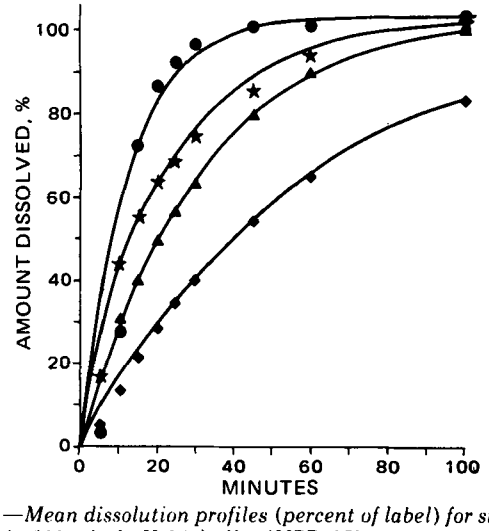

Figure 1—*Mean dissolution profiles (percent of label) for six 150-mg capsules in 300 ml of pH 6.5 buffer (USP, 37°) at 100 rpm (rotating basket). Key: ●, Lot A; ★, Lot B; ▲, Lot C; and ♦, Lot D. Curves were calculated from Eq. 1 using the rate constants of Table I.*

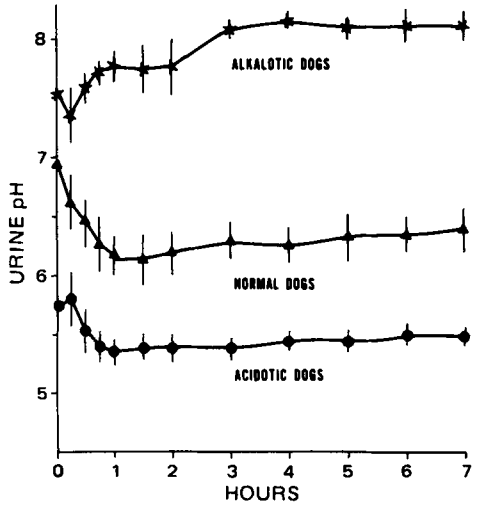

Figure 2—*Mean urine pH (±SEM) in eight dogs following intravenous administration of 10 mg of cinoxacin/kg. Key: ★, alkalotic dogs; ▲, normal dogs; and ●, acidotic dogs.*

[14] French No. 14.
[15] Model 7 pH meter, Corning Scientific Instruments, Medfield, Mass.
[16] Vacutainer, Becton-Dickinson and Co., Rutherford, N.J.
[17] Aminco SPF-125 spectrophotofluorometer, American Instrument Co., Silver Spring, Md.

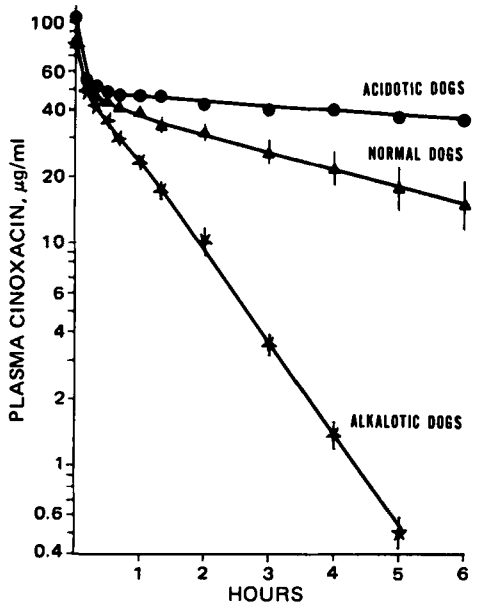

Figure 3—*Mean plasma concentration* (±SEM) *of cinoxacin in eight dogs following 10 mg/kg iv. Key:* ★, *alkalotic dogs;* ▲, *normal dogs; and* ●, *acidotic dogs.*

Eight dogs received 10 mg of cinoxacin/kg by all eight described treatments (five oral preparations and three intravenous protocols). No particular order was followed in the administration sequence of different dosage forms. However, at least 7 days (equivalent to 12 half-lives for cinoxacin in dogs with acidified urine) elapsed between experiments with any given dog. Because only two dogs were used on any given day, the series of experiments required an extended period. Therefore, day effects that may be present in the data are assumed not to influence the evaluation of the bioavailability of the different preparations.

RESULTS AND DISCUSSION

***In Vitro* Characteristics of Cinoxacin Preparations**—Mean dissolution profiles of cinoxacin from capsules of Lots A–D are shown in Fig. 1. Points in Fig. 1 represent the experimentally determined values; the solid lines represent the best fit (weighted nonlinear least-squares regression) to the following equation:

$$D_t = D_\infty(1 - e^{-K_d t}) \qquad \text{(Eq. 1)}$$

where D_t is the amount dissolved at time t in minutes, K_d is the dissolution rate constant in reciprocal minutes, and D_∞ is the amount dissolved at t_∞.

Although Eq. 1 is only rigorously valid for an idealized single-particle system (19), it was chosen to represent the *in vitro* dissolution profiles for its simplicity and because other models sometimes used to describe

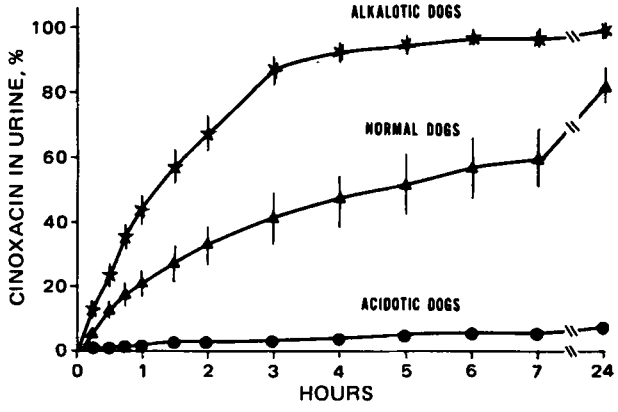

Figure 4—*Mean cumulative cinoxacin* (±SEM) *in the urine of eight dogs following 10 mg/kg iv. Key:* ★, *alkalotic dogs;* ▲, *normal dogs; and* ●, *acidotic dogs.*

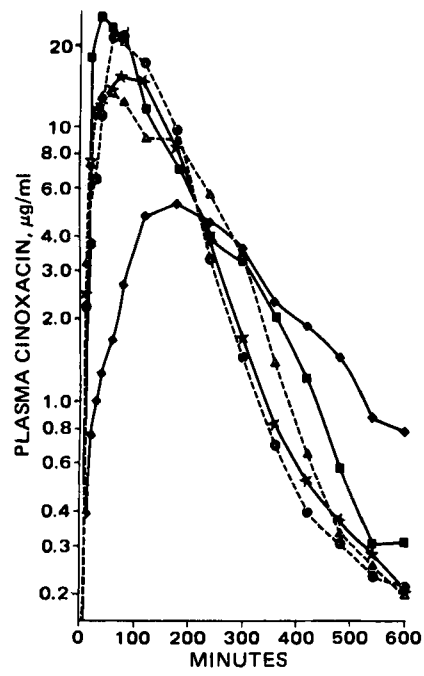

Figure 5—*Mean plasma concentration of cinoxacin in eight alkalotic dogs following 10 mg/kg po. Key:* ■, *solution;* ●, *Lot A;* ★, *Lot B;* ▲, *Lot C; and* ♦, *Lot D.*

dissolution from a multiparticulate system under nonsink conditions, such as the negative two-thirds root law (20), failed to provide a better fit. Best estimates of K_d for the four lots of cinoxacin, along with their specific surface areas and particle-size distributions, are listed in Table I. The observed decrease in surface area in Lots A–D is clearly reflected by the decline in dissolution rate constants calculated for the corresponding lots.

Effect of Urinary pH Perturbations on Cinoxacin Excretion in Dogs—*Urine pH*—The time course of urine pH observed in alkalotic, normal, and acidotic dogs after intravenous administration of 10 mg of cinoxacin/kg is given in Fig. 2. For female dogs rendered alkalotic with sodium bicarbonate, the mean urine pH during the experimental period ranged from 7.4 to 8.2 (overall mean of 7.9).

In normal dogs, the mean urine pH ranged from 6.1 to 6.9 (overall mean of 6.4). For dogs rendered acidotic with ammonium chloride, the mean urine pH ranged from 5.4 to 5.8 (overall mean of 5.5). As shown in Fig.

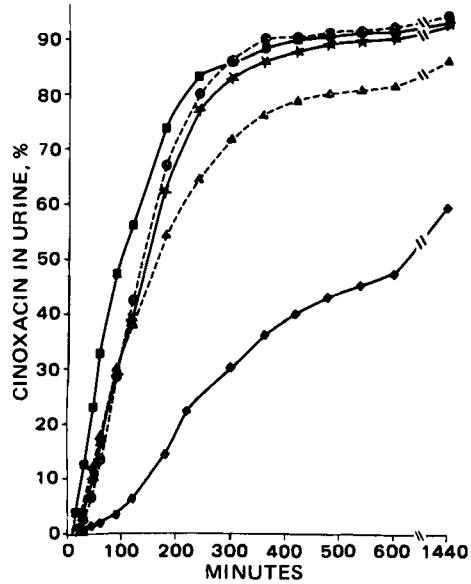

Figure 6—*Mean cumulative cinoxacin in the urine of eight alkalotic dogs following 10 mg/kg po. Key:* ■, *solution;* ●, *Lot A;* ★, *Lot B;* ▲, *Lot C; and* ♦, *Lot D.*

Table III—Results of Duncan's Test [a] on the Oral Data

t, min	Plasma Cinoxacin, μg/ml				
	Lot S	Lot A	Lot B	Lot C	Lot D
10	2.1[a,c]	2.33[a] (2.1)	2.43[a] (1.2)	3.19[a] (1.4)	0.39[a] (0.21)
20	17.6[a] (4.8)	3.72[b] (2.0)	8.41[b] (3.1)	7.39[b] (3.2)	0.75[b] (0.28)
30	18.0[a,c]	6.41[ab] (1.6)	11.5[a] (4.0)	10.8[a] (3.7)	1.02[b] (0.34)
40	24.8[a] (1.8)	10.9[b] (2.3)	12.6[b] (3.9)	12.4[b] (3.3)	1.25[c] (0.42)
60	22.5[a] (1.9)	21.0[ab] (4.0)	14.3[b] (2.5)	13.3[b] (2.9)	1.67[c] (0.47)
80	20.2[a] (3.4)	21.5[a] (2.4)	16.6[ab] (2.0)	12.0[b] (2.2)	2.64[c] (0.62)
120	13.0[ab] (3.0)	17.0[a] (0.86)	15.1[a] (1.8)	8.90[bc] (1.5)	4.71[c] (0.86)
180	6.93[a] (2.0)	9.69[a] (2.1)	8.55[a] (1.9)	8.88[a] (2.8)	5.19[a] (0.85)
240	3.99[a] (1.5)	3.29[a] (0.88)	3.73[a] (0.86)	5.61[a] (1.9)	4.45[a] (1.1)
300	3.20[a] (1.5)	1.44[a] (0.31)	1.70[a] (0.43)	3.38[a] (1.6)	3.59[a] (0.94)
360	2.01[a] (1.1)	0.689[a] (0.14)	0.830[a] (0.15)	1.36[a] (0.59)	2.28[a] (0.57)
420	1.05[ab] (0.51)	0.395[b] (0.046)	0.516[b] (0.11)	0.636[ab] (0.22)	1.86[a] (0.74)
480	0.565[ab] (0.24)[d]	0.306[b] (0.039)	0.373[b] (0.052)	0.327[b] (0.069)	1.42[a] (0.51)
540	0.306[a] (0.12)[d]	0.229[a] (0.025)	0.275[a] (0.041)	0.253[a] (0.040)	0.864[b] (0.20)
600	0.303[a] (0.098)[d]	0.210[a] (0.035)	0.206[a] (0.041)	0.209[a] (0.035)	0.770[b] (0.13)
AUC (0–600 min), μg-min/ml	3460[a] (520)	3160[a] (150)	2910[a] (210)	2690[a] (220)	1580[b] (200)
C_{max}, μg/ml	30.1[a] (2.2)	25.1[ab] (2.5)	20.8[b] (1.7)	20.8[b] (1.9)	7.33[c] (0.65)
T_{max}, min	42.5[a]	75.0[a]	80.0[a]	92.5[a]	173[b]
ΣU_{24}, %	93.4[a] (3.7)	95.0[a] (3.9)	93.1[a] (2.1)	86.2[a] (2.9)	59.8[b] (8.5)

[a] Means of the variables within a row are statistically different ($\alpha = 0.05$) if they do not have a superscript letter in common. Numbers in parentheses are standard errors of the mean for eight dogs unless otherwise indicated. [b] Aqueous solution. [c] Two dogs only. [d] Five dogs only.

2, the variation of urine pH between dogs for individual time points was generally smaller for alkalotic and acidotic dogs than for normals.

Persistence of Cinoxacin in Plasma after Intravenous Administration—The cinoxacin concentrations in the plasma of alkalotic, normal, and acidotic dogs as a function of time after intravenous administration of 10 mg/kg are shown in Fig. 3. As indicated by analysis of variance, significant differences in plasma cinoxacin concentrations appeared among the three groups of dogs at 20 min. At 60 min, the results of all three protocols were different from each other [Duncan's test (21), Table II].

In addition, the rate of decline of mean plasma cinoxacin concentration approximated a first-order decay process, as judged by the apparent linearity of the semilogarithmic plots of the data after 60 min. Therefore, the slopes of the linear regression lines were calculated through the points after 60 min. For alkalotic dogs, the slope was -0.345 ± 0.030 (SD) hr^{-1} with a corresponding half-life of 0.873 hr. For normal dogs, the slope was -0.0795 ± 0.0013 hr^{-1} with a corresponding half-life of 3.79 hr. For acidotic dogs, the slope was -0.0190 ± 0.0011 hr^{-1} with a half-life of 15.8 hr. The correlation coefficients associated with the linear regression of the log mean plasma values on time were 0.978, 0.999, and 0.989 for alkalotic, normal, and acidotic dogs, respectively.

Urinary Excretion of Cinoxacin after Intravenous Administration—The amounts of cinoxacin, expressed as percent of the administered dose, recovered in the urine of alkalotic, normal, and acidotic female mongrel dogs after 10 mg/kg iv are illustrated in Fig. 4. Alkalotic dogs rapidly excreted essentially all of the dose in the urine. Ninety-five percent of the dose appeared in the urine within 5 hr and 98.5% of the dose appeared within 24 hr. Normal dogs excreted appreciably less, 51.7% of the dose, in the same 5 hr. By 24 hr, normal dogs had excreted 81.9% of the dose.

Dogs maintained in the acidotic state excreted very little cinoxacin in the urine, reaching a mean of 4.5% of the dose in 5 hr and 7.8% in 24 hr. However, urinary excretion in six acidotic dogs followed for 72 hr increased to 39.1% as they began to recover from the acidotic state. Variation between dogs in the recovery of cinoxacin in the urine was larger for normal dogs than for either acidotic or alkalotic dogs.

From the results of the preceding intravenous studies, it is clear that, judged by the interanimal variability in urine pH, plasma cinoxacin concentration, and urinary cinoxacin excretion, either acidified or alkalinized dogs would provide a more reproducible test system than would normal dogs. The alkalinized dogs also excreted essentially all of the dose unchanged in the urine within 24 hr, thus minimizing any uncertainty associated with interanimal differences in metabolic degradation. Moreover, the cinoxacin elimination half-life from the blood of the alkalotic dog (0.873 hr) was more representative of that of normal humans (0.96 hr) (22) than was the elimination half-life of either normal or acidotic dogs.

In addition, comparative metabolism studies showed that both humans and dogs excrete primarily unchanged drug in the urine[18]. Although fewer metabolites are formed in dogs than in humans, those found in dogs generally coincide with those formed in humans. Therefore, the alkalinized dog was selected as an animal model to evaluate the bioavailability of different orally administered cinoxacin preparations.

***In Vivo* Characterization of Cinoxacin Oral Preparations in Alkalotic Dogs**—The plasma cinoxacin concentrations of alkalotic dogs at various times after oral administration of a single 10-mg/kg dose as an aqueous solution or as a capsule of Lot A, B, C, or D are shown in Fig. 5. Oral administration of the solution produced the earliest and highest peak mean plasma cinoxacin concentration, 24.8 μg/ml at 40 min. Progressively lower peak mean concentrations were produced by administration of a Lot A, B, C, or D capsule. The most striking difference for most time points was between Lot D, the lot with the smallest surface area (largest particle size), and all of the other preparations.

Analyses of variance were performed on the data represented in Fig. 5. Significant differences in plasma concentrations existed among the five preparations from 20 min to 2 hr and from 8 to 10 hr. Significant differences also existed among the given preparations in urinary recovery, area under the plasma cinoxacin concentration–time curve, peak plasma cinoxacin concentration, and time of peak plasma cinoxacin concentration.

Table III lists the results of Duncan's test applied to the data to ascertain which individual comparisons between groups yield significant differences. As these results indicate, for 24-hr urinary recovery of cinoxacin (ΣU_{24}), for observed area under the plasma cinoxacin con-

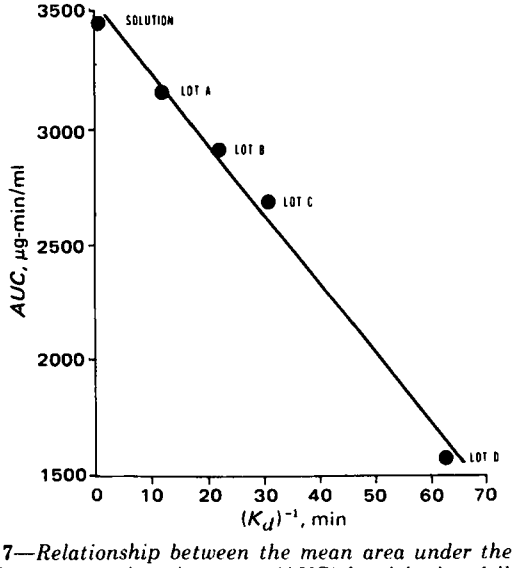

Figure 7—*Relationship between the mean area under the plasma cinoxacin concentration–time curve* (AUC) *for eight dogs following 10 mg/kg po and the mean dissolution rate constant* (K_d) *with a linear correlation coefficient of −0.995.*

[18] Dr. R. L. Wolen, Eli Lilly and Co., Indianapolis, Ind., personal communication.

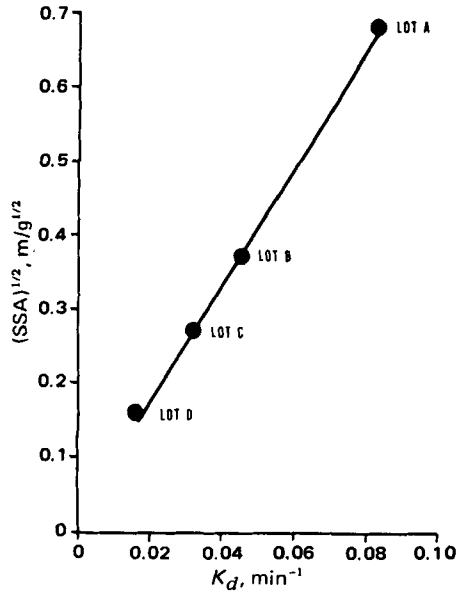

Figure 8—*Relationship between specific surface area (SSA) and the mean dissolution rate constant* (K_d) *for cinoxacin with a linear correlation coefficient of 0.999.*

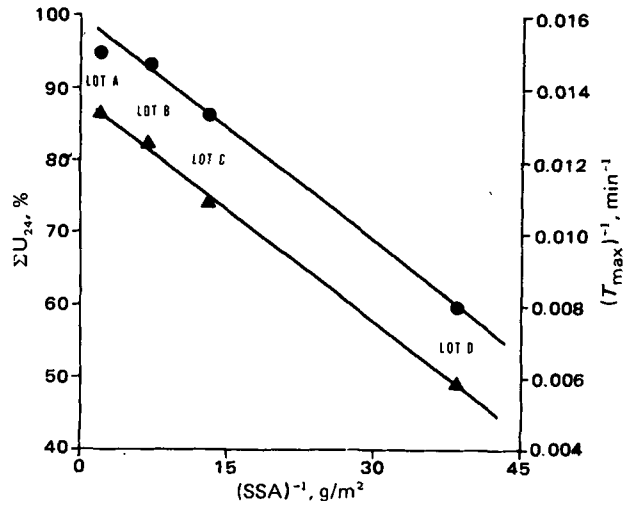

Figure 9—*Relationships between specific surface area (SSA) and both the mean cumulative cinoxacin in the urine after 24 hr* (ΣU_{24}) *and the mean time of peak plasma cinoxacin concentration* (T_{max}) *in eight dogs following 10 mg/kg po. Key:* ▲, T_{max}^{-1} *and* SSA^{-1} *with a linear correlation coefficient of −0.999; and* ●, ΣU_{24} *and* SSA^{-1} *with a linear correlation coefficient of −0.996.*

centration–time curve (*AUC*, 0–10 hr), and for time of peak plasma cinoxacin concentration (T_{max}), the only significant differences ($\alpha = 0.05$) were those between Lot D and the other four preparations. For peak plasma cinoxacin concentration (C_{max}), Lot D was again distinct from the other four preparations. Additionally, the solution was different from Lots B and C. Further comparisons at specific time points can be made by reference to Table III.

The amounts of cinoxacin recovered in the urine of individual alkalotic dogs at various times after administration of a single 10-mg/kg dose as an aqueous solution or as a capsule of Lot A, B, C, or D are shown in Fig. 6.

Correlations of *In Vivo* and *In Vitro* Cinoxacin Data—Numerous correlations for the oral cinoxacin preparations can be formed from the pairwise treatment of the *in vitro* (Table I) and *in vivo* (Table III) data. Even though statistically significant differences did not exist among all *in vivo* parameters for the five different oral preparations, near perfect rank-order correlations (23) existed between the mean values of these *in vivo* and *in vitro* parameters. For predictive purposes, functional relationships were derived from pairs of these parameters. Four such relationships are presented in Figs. 7–9.

The relationship between *AUC* and K_d was linearized (Fig. 7) by plotting the reciprocal of the mean K_d *versus* the mean *AUC*, with a resulting correlation coefficient of −0.995. The dissolution rate constant, K_d, for the oral solution, was assumed to be large relative to that for Lot A, so its reciprocal was assigned a value of zero. This *in vivo–in vitro* correlation relates two parameters, each of which is a function of the entire dose response *versus* time curve. Therefore, the relationship should be a more valid predictor of the performance of formulations over a broader range (24) than any correlation between single points taken from the dose response *versus* time curves. In this case, *AUC* is a measure of the extent of drug absorption and K_d represents the *in vitro* drug dissolution rate. The relationship depicted in Fig. 7 has been used in the generation of a dissolution test specification for cinoxacin formulations.

In Fig. 8, an *in vitro–in vitro* relationship is quantitated for cinoxacin. The dissolution rate constant is correlated with the square root of the specific surface area as measured by the BET gas adsorption technique. In general, specific surface areas generated by the BET method are more accurate measures of the bulk sample surface, independent of either assumed particle geometry or size distribution, than are various sieve, particle-size, or permeametric measurements (25–27). The correlation coefficient associated with the linear regression of K_d on the square root of the specific surface area is 0.999. This relationship is useful in the selection of bulk raw material lots of cinoxacin to help assure that the finished product will have suitable dissolution characteristics.

Relationships between two measures of the absorption of cinoxacin, *i.e.*, the mean T_{max} and the mean ΣU_{24}, and the specific surface area of the drug are presented in Fig. 9. Linear regression of the reciprocal of the specific surface area with ΣU_{24} and with the reciprocal of T_{max} yielded correlation coefficients of −0.996 and −0.999, respectively. This pair of correlations quantitatively relates measures of both the rate and extent of absorption of cinoxacin to a physical property of the raw material.

These correlations have been used to help establish product specifications for the finished dosage forms, 250- and 500-mg capsules. A comparative bioavailability study of these two capsule formulations and an oral solution is being conducted in humans.

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Rapid High-Performance Liquid Chromatographic Determination of Bleomycin A_2 in Plasma

GERALD K. SHIU *, THOMAS J. GOEHL *x, and WILLIAM H. PITLICK ‡

Received April 10, 1978, from the **Department of Medicinal Chemistry and the* ‡*Department of Pharmaceutics, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261.* Accepted for publication July 26, 1978.

Abstract □ A rapid and specific method for the determination of bleomycin A_2 is described. A 50-μl aliquot of 20% trichloroacetic acid was added to 200 μl of plasma. The sample was vortexed and centrifuged, and 50 μl of the clear supernate was injected into a liquid chromatograph equipped with a microparticulate reversed-phase column and a fixed wavelength detector. Elution was carried out using methanol–acetonitrile–0.0085 *M* heptanesulfonic acid–acetic acid. A linear calibration curve was found in the 0.05–5-μg/ml range with an estimated precision of ±6% (*CV*). Preliminary pharmacokinetic data in the rabbit also are reported.

Keyphrases □ Bleomycin A_2—high-performance liquid chromatographic analysis in plasma □ High-performance liquid chromatography—analysis, bleomycin A_2 in plasma □ Antineoplastic agents—bleomycin A_2, high-performance liquid chromatographic analysis in plasma

The antibiotic–antineoplastic agent bleomycin is actually a mixture of cationic polypeptides. The complex was isolated from fermentation products of *Streptomyces verticillus* (1). It is effective against human neoplasms, particularly squamous cell carcinoma, lymphoma, and testicular carcinoma (2–4). The most common toxic side effects are minor cutaneous reactions. However, about 10% of the patients develop pulmonary toxicity, occurring as pneumonitis and pulmonary fibrosis (5).

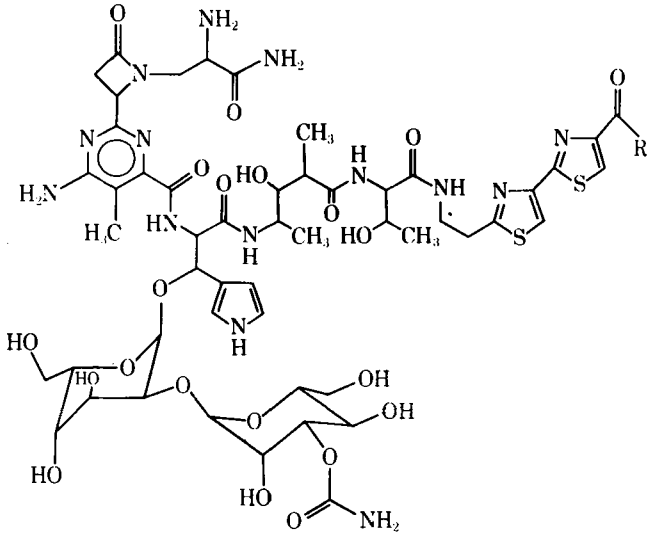
BACKGROUND

Crude bleomycin has been purified by chromatographic methods into two large fractions, A and B (6). Further fractionation provided six bleomycin A fractions and five bleomycin B fractions. Of the various highly hydrophilic polypeptides, 11 components have been completely purified. Each component consists of a carboxy terminal glycopeptide (~1300 daltons), designated as bleomycinic acid, which is substituted in an amide-type linkage with 3-(dimethylthionium)propylamine (bleomycin A_2, II), agmantine (bleomycin B_2, III), or 3-(methylsulfinyl)propylamine (bleomycin A_1, I).

The bleomycin administered clinically consists of, by weight, 55–70% bleomycin A_2, 25–32% bleomycin B_2, and the remaining percentage divided among the other subfractions (7). In all cases, at least 65% is from the bleomycin A group and less than 35% is from the bleomycin B group. The most commonly used regimen has been 15 mg/m^2 iv twice weekly (8).

Although almost all clinical work has been done using this formulation, there are indications that the individual bleomycin fractions should be studied. For example, bleomycin A_5 was more toxic to *Escherichia coli* than the more abundant bleomycin A_2 present in the formulation currently in use (9). Bleomycin acid was devoid of activity against *E. coli* (9). The possible effectiveness of bleomycin A_5 was reported by others (10, 11). Bleomycin B_4 caused kidney damage in dogs and had weak antitumor activity (12). However, few studies on individual components of the bleomycin mixture have been presented (12–14).

To elucidate the pharmacokinetics of individual bleomycins, a specific and sensitive assay must be available. Analytical techniques to estimate plasma levels of the bleomycin mixture include microbiological assays (11, 12, 15, 16), radioactive labeling assays (2, 17–19), and radioimmunoassays (20, 21). Unfortunately, all of these methods do not distinguish between the various components of the bleomycin mixture or their metabolites. Thus, specificity is a problem with all presently available techniques. In this report, a sensitive and specific paired-ion high-performance liquid chromatographic (HPLC) assay for one component of the bleomycin mixture, bleomycin A_2, is presented.



I: R = $NH(CH_2)_3SOCH_3$
II: R = $NH(CH_2)_3\overset{+}{S}{=}(CH_3)_2$
III: R = $NH(CH_2)_4NHC(=NH)NH_2$

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