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Influence of Urinary pH on the Pharmacokinetics of Cinoxacin in Humans and on Antibacterial Activity In Vitro

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The impact of acidification and alkalinization of the urine on the pharmacokinetics of cinoxacin was examined after single 500-mg oral doses were administered to nine healthy male volunteers. Acidic and alkaline conditions were achieved by repeated oral doses of ammonium chloride or sodium bicarbonate, respectively. Plasma cinoxacin levels in all subjects were adequately described in terms of one-compartment-model kinetics with first-order absorption and elimination. Acidification and alkalinization treatment had no effect on cinoxacin absorption or distribution. The mean elimination half-life of cinoxacin in plasma was 1.1, 2.0, and 0.6 h in control subjects and with acidification and alkalinization of urine, respectively. Recovery of intact cinoxacin in samples of urine collected 0 to 36 h after cinoxacin administration represented 65% of the dose in control subjects and urine acidification and 80% of the dose with alkalinization of urine. The mean renal clearance of cinoxacin was 76, 118, and 278 ml/min with acidification, control, and alkalinization, respectively, and renal clearance was highly correlated with urinary pH. Urine concentrations of cinoxacin were significantly higher with alkalinization compared with control values during the first 4 h after drug administration. Urine cinoxacin concentrations were reduced somewhat by acidification, but these tended not to be significantly different from control values. Changes in cinoxacin elimination owing to urine pH are less pronounced in humans than in dogs. The antibacterial activity of cinoxacin against some common urinary tract pathogens was pH dependent. A four- to eightfold reduction in cinoxacin activity was generally observed at pH 8 compared with lower pH values. However, in view of the high levels of cinoxacin which are obtained in both acidic and basic urine, the impact of urine pH on cinoxacin antibacterial efficacy would be of minor clinical importance.

Cinoxacin has been shown to exhibit antibacterial activity against most gram-negative organisms commonly encountered in urinary tract infections (4, 11). It is well absorbed from oral doses and is rapidly excreted from the body under normal conditions with an elimination half-life of 1 to 1.5 h (2, 13).

It is well established that renal elimination of weak acids or weak bases may be urinary pH dependent (8). Cinoxacin is a weak organic acid (pK_a, 4.7) and has low aqueous solubility under acidic conditions. Experiments in dogs have shown that urinary excretion of cinoxacin is pH dependent (12). Under induced alkalotic conditions, the elimination half-life was reduced from a normal value of 3.8 to 0.87 h, whereas in acidotic dogs the half-life was increased to 15.8 h.

In view of the importance of urinary concentrations of cinoxacin for the treatment of urinary tract infections (4, 11, 16) and of the major role exerted by the kidneys in cinoxacin elimination (2, 7, 13), it is necessary to examine the effect of urinary pH on the pharmacokinetic and antibacterial properties of cinoxacin in humans.

In this study, a new direct fluorimetric assay procedure was used to examine the pharmacokinetics of cinoxacin in healthy male volunteers under normal (control), alkaline, and acidic conditions. The antibacterial activity of cinoxacin was also measured against common urinary tract pathogens at various pH values.

MATERIALS AND METHODS

Clinical study. Subjects. Nine healthy male volunteers, 22 to 32 years of age (mean, 26 years) weighing 66 to 91 kg (mean, 80 kg), participated in the study after passing a complete physical examination and giving written informed consent. Subjects were instructed to take no other medication for 2 weeks before or during the study.

Medication. Cinoxacin (500-mg capsules; lot CT-

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2969-7K), ammonium chloride (500-mg enteric coated tablets; lot 3 NY 82A), and sodium bicarbonate (650-mg tablets; lot 4 AE 40A) were supplied by Eli Lilly & Co., Indianapolis, Ind.

Protocol. The nine subjects were divided into three groups of three, and the groups received single oral doses of 500 mg of cinoxacin as three different treatments by a Latin square design. Treatments were administered 1 week apart. On one occasion, cinoxacin was administered alone, whereas on the other two occasions, subjects received cinoxacin with ammonium chloride to acidify the urine or sodium bicarbonate to alkalinize the urine.

For the ammonium chloride treatment, subjects received 2 g of ammonium chloride at 8 a.m., noon, 4 p.m., and 8 p.m. on the day preceding the cinoxacin dose. The next day subjects received 4 g of ammonium chloride with 240 ml of water at 6 a.m. At 8 a.m., 500 mg of cinoxacin was administered with 120 ml of water. Additional 2-g doses of ammonium chloride were given with 40 ml of water each hour from 9 a.m. to 8 p.m. of that day and also at 8 a.m., noon, and 4 p.m. of the next day. In all, subjects received 42 g of ammonium chloride in 3 days and 28 g on the day of cinoxacin administration.

For the sodium bicarbonate treatment, subjects received 2.6 g of sodium bicarbonate at 8 a.m., noon, 4 p.m., and 8 p.m. on the day preceding the cinoxacin dose. The next day, subjects received 3.9 g of sodium bicarbonate with 240 ml of water at 6 a.m. At 8 a.m., 500 mg of cinoxacin was administered with 120 ml of water. Additional 1.95-g doses of sodium bicarbonate were given with 40 ml of water each hour from 9 a.m. to 8 p.m. of that day and also at 8 a.m., noon, and 4 p.m. of the next day. In all, subjects received 45.5 g of sodium bicarbonate in 3 days and 27.3 g on the day of cinoxacin administration.

On the occasions when cinoxacin was administered alone, the frequency and volume of water ingestion were identical to those in the other treatments. For all three treatments, subjects were not permitted to eat food from 8 p.m. on the day before until noon on the day of cinoxacin administration.

Blood samples (10 ml) were taken from a forearm vein into heparinized glass tubes (Vacutainer) immediately before and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h after cinoxacin administration. Plasma was separated by centrifugation and stored at −20°C until assayed. Complete urine collections were obtained at −1 to 0, 0 to 1, 1 to 2, 2 to 3, 3 to 4, 4 to 6, 6 to 8, 8 to 12, 12 to 24, and 24 to 36 h after cinoxacin administration. Urine volumes and pH values were recorded immediately after each collection period, and 10 ml of both undiluted and 1:10 diluted (water) urine was stored at −20°C until assayed. Urine pH values were determined with an analog pH meter (model 301, Orion Research, Inc., Cambridge, Mass.). Assays were uniformly carried out within 1 week of sampling, and there was no discernible degradation of cinoxacin during that period.

Analytical procedures. Cinoxacin concentrations in plasma and urine were determined by a direct fluorimetric method developed in this laboratory. The method is more simple than previously described fluorimetric procedures which involve solvent extraction (1, 9).

Plasma (0.5 ml) or urine, diluted 1:10 or 1:25, respectively, with distilled water (0.5 ml) and 10% trichloroacetic acid (4.5 ml), was blended in a Vortex mixer for 1 min and centrifuged at 3,000 × *g* for 5 min. The fluorescence of the supernatant was determined at excitation and emission wavelengths of 358 and 422 nm (uncorrected), respectively (Perkin-Elmer Corp. MPF 4 spectrophotofluorimeter). To compensate for day-to-day variation in fluorimeter response, cinoxacin standard curves were run in heparinized plasma and in urine, together with each batch of unknown samples, and all values were normalized to the fluorescence of a standard solution of 1 μg of quinine sulfate per ml in 0.1 N sulfuric acid.

Assay validation. The plasma and urine cinoxacin levels obtained by the above method were compared in three individuals who received single 500-mg oral doses of cinoxacin with those obtained by a solvent extraction method (1).

In a separate study, the fluorescence was determined of plasma containing cinoxacin alone and also in the presence of therapeutic concentrations of acetaminophen, aspirin, chlorothiazide, hydrochlorothiazide, levodopa, phenobarbital, phenytoin, probenecid, sulfadiazine, and sulfisoxazole. Fluorescence was determined also in plasma containing approximately double-normal concentrations of creatinine and four to five times normal concentrations of urea after addition of exogenous creatinine and urea to normal plasma.

Pharmacokinetic analysis of data. Individual cinoxacin plasma profiles were analyzed by using a single-compartment kinetic body model with first-order drug absorption and elimination and an absorption lag time (15). Estimates of pharmacokinetic parameters were obtained by using the nonlinear regression computer program NREG (6) on a Univac 1100 digital computer. For the regression analysis, all cinoxacin concentrations were weighted according to their reciprocals.

Areas under cinoxacin concentrations curves in plasma from zero to infinite time (AUC) were measured by trapezoidal rule, with end correction where necessary. Renal clearance (Cl_R) was calculated by dividing the total quantity of cinoxacin voided in urine by the AUC.

Cinoxacin absorption efficiency from the different treatments could not be measured directly because of the induced changes in cinoxacin elimination rates. Absorption could be determined indirectly however by taking advantage of pH-related perturbations in Cl_R (5). The method is summarized as follows:

$$F = \left[\frac{\Delta Cl_R}{D}\right]\left[\frac{(AUC')\,(AUC)}{(AUC') - (AUC)}\right]$$

where F is the fraction of dose D which is absorbed into the systemic circulation, ΔCl_R is the treatment-related change in Cl_R, and AUC and AUC′ are the areas under cinoxacin plasma curves after different treatments.

Statistical analysis. Plasma and urine concentrations at each sampling time and pharmacokinetic parameter values were compared by analysis of variance for cross over design. When a significant treatment effect was observed, differences between individual treatments were examined by Tukey's test (10). The F values, calculated from the three pairs of treatments by means of the equation above, were compared by Student's t test.

In vitro antimicrobial studies. Organisms. *Escherich-*

ia coli ATCC 25922 was the main study organism; 33 clinical isolates of *E. coli*, 15 isolates of *Klebsiella pneumoniae*, 11 strains of *Enterobacter* species, 12 isolates of *Proteus mirabilis*, 18 strains of *Pseudomonas aeruginosa*, 4 strains of *Morganella morganii*, 2 isolates of *Providencia stuartii*, 2 strains of *Serratia* species, and 2 strains of *Citrobacter freundii* were also used.

Media. Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.), and Mueller-Hinton agar (Difco) were used for minimum inhibitory concentration (MIC) determinations. The pH of the media was adjusted to 5, 6, 7, and 8 by dropwise addition of 1.0 N hydrochloric acid or sodium hydroxide.

Inocula. For MIC determinations on agar plates, 10-ml bacterial cultures were diluted with isotonic sodium chloride solution, based on optical density determination, to yield 10^4 to 10^5 organisms per spot. For MIC and minimal bactericidal concentrations in broth, the optical density-adjusted cultures were diluted into a broth of the appropriate pH to produce a final inoculum of 10^5 to 10^6 colony-forming units per ml. For urine dilution tests, the organisms were diluted into Mueller-Hinton broth adjusted to the pH of the test subject's urine.

Determination of MIC and minimal bactericidal concentrations. The agar dilution method (Steers replicator) was used (14). Some bacterial strains were also tested by the microtiter broth dilution method (3). The minimal bactericidal concentration was determined by streaking 10 μl of clear wells onto octants of Mueller-Hinton agar plates. Three or less colonies from these subcultures, representing a ≥99.9% killing, were taken as the breakpoint.

Urine dilution tests were done on Millipore-filtered urine from one subject with alkaline urine and two individuals with acidic urine. The subject's urine voided before cinoxacin administration was used as diluent. Serial twofold dilutions of the test urines were performed in microtiter plates, with *E. coli* ATCC 25922 and a clinical isolate each of *P. mirabilis* and *K. pneumoniae* as indicator organisms.

All MIC and minimal bacterial concentration determinations and urine dilution tests were done in duplicate, the higher value of duplicate determinations being taken as the breakpoint.

Killing curves of *E. coli* ATCC 25922 at pH 5, 6, 7, and 8 were obtained at cinoxacin concentrations of 1, 2, 4, 8, 10, 100, and 1,000 μg per ml, using cultures in the logarithmic growth phase. Cultures were sampled for colony-forming unit determination at zero time and at 1- to 2-h intervals thereafter by plating in duplicate 10 μl of appropriate dilutions onto octants of Mueller-Hinton agar plates.

Materials. Cinoxacin powder (lot S1-73-8c) for assay standardization and microbiological procedures was reference standard quality, and was supplied by Eli Lilly & Co., Indianapolis, Ind. Compounds used in the assay validation studies were obtained from various sources and were of reference standard quality. All other chemicals and solvents used were analytical reagent grade and were used as received.

RESULTS

Assay for cinoxacin in plasma and urine. The direct fluorimetric assay for cinoxacin was linearly sensitive for drug concentrations between 0.1 and 30.0 μg/ml in plasma and between 1 and 10^3 μg/ml after the appropriate dilution in urine. The standard deviation from multiple replicate determinations was within 6% of the mean at the higher drug concentrations and within 10% at the lowest concentration. The fluorescence intensity of cinoxacin in plasma was unaffected by the presence of the other drugs examined and also by the presence of increased levels of creatinine or urea.

No differences were observed in the plasma and urine concentrations of cinoxacin as measured by the direct fluorimetric assay or by the solvent extraction method (1, 9) in three individuals. The excellent agreement between the two methods is illustrated for one individual in Fig. 1.

Clinical studies. Urine pH values. The mean urine pH values obtained after the three treatments are summarized in Fig. 2. Under control conditions, the mean pH varied between 5.8 and 6.2. After ammonium chloride treatment, the pH decreased to 5.1 to 5.3, whereas sodium bicarbonate treatment resulted in an increase in pH to 7.4 to 7.9.

Plasma levels and urinary excretion of cinoxacin. The mean concentrations of cinoxacin in plasma and urine are shown in Fig. 3 and 4, and cumulative recovery of cinoxacin in urine is summarized in Fig. 5. The pharmacokinetic values obtained from the plasma and urine data are given in Table 1.

The rate of cinoxacin absorption from the oral doses, peak drug levels in plasma, and times of peak levels were independent of treatment. After sodium bicarbonate treatment plasma levels of cinoxacin declined rapidly and were below the limit of detection at 6 h after the administration of the doses (Fig. 3). After ammonium chloride treatment, cinoxacin levels declined more slowly and were detectable at 12 h in eight subjects. Cinoxacin was still detectable in six of nine control subjects at 10 h. The mean cinoxacin elimination half-life increased in the order of 0.6, 1.1, and 2.0 h after the alkali, control, and acid treatments, respectively, and the differences between all treatment pairs were significant ($P < 0.05$). Areas under cinoxacin plasma curves were also significantly increased by acid treatment and decreased by alkali treatment, relative to control values, but the value of FD/V, which is equivalent to $AUC \cdot k_{el}$ (Table 1; 15) was treatment independent. The high coefficients of determination (Table 1) indicate that plasma cinoxacin profiles from all treatments were adequately described by simple one-compartment model kinetics.

The concentration of cinoxacin in urine was high during the first 4 h after the sodium bicar-

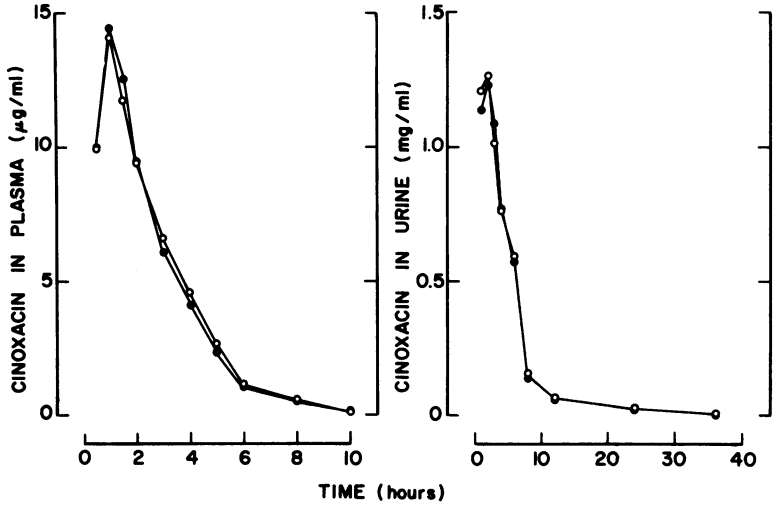

FIG. 1. Plasma and urine levels of cinoxacin measured by direct fluorimetry and by fluorimetry after solvent extraction (1) in a male subject after a 500-mg oral dose of cinoxacin. Symbols: ○, direct fluorescence; ●, solvent extraction.

bonate treatment compared with the other treatments (Fig. 4). After the ammonium chloride treatment, urine cinoxacin levels were lower initially, but were more prolonged. Statistical comparison of the data showed that urine cinoxacin levels were significantly higher under alkaline conditions compared with both other conditions during 0 to 4 h, whereas the reverse was true during most subsequent sampling periods. Mean total urinary recovery of cinoxacin under alkaline conditions was 80% of the dose, this value being significantly higher than the 65%

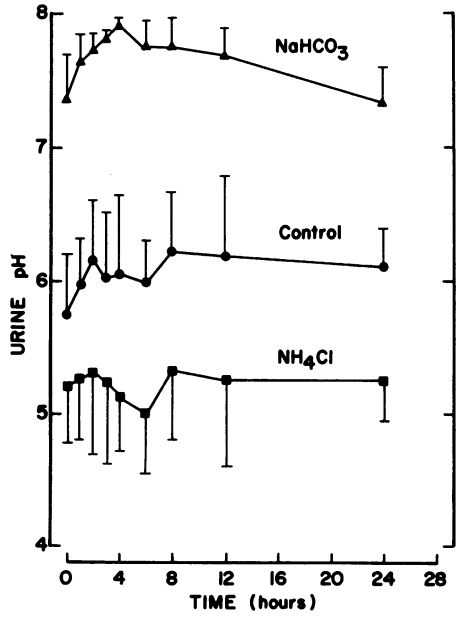

FIG. 2. Mean urine pH values after administration of cinoxacin alone (control; ●) and after cinoxacin plus ammonium chloride (■) and sodium bicarbonate (▲) treatments. Error bars indicate one standard deviation (n = 9).

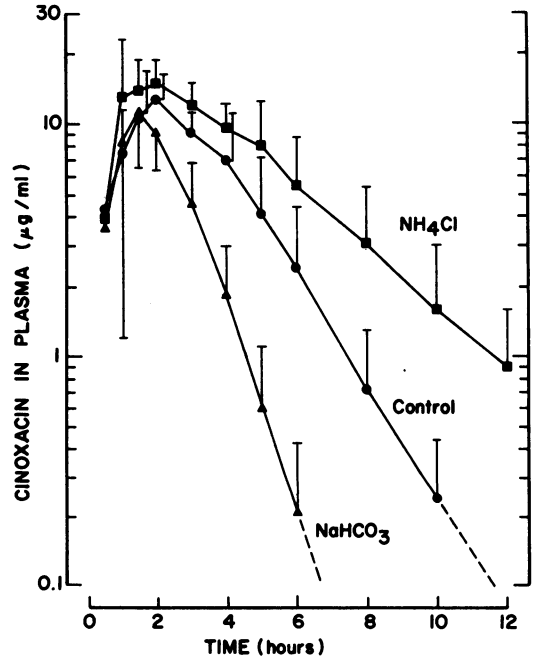

FIG. 3. Mean concentrations of cinoxacin in plasma after control (●), ammonium chloride (■), and sodium bicarbonate (▲) treatments. Error bars indicate one standard deviation (n = 9).

TABLE 1. Mean pharmacokinetic values for cinoxacin after control, ammonium chloride, and sodium bicarbonate treatments[a]

Treatment	C_{max} (μg/ml)	t_{max} (h)	k_a (h^{-1})	t_0 (h)	k_{el} (h^{-1})	$t_{1/2}$ (h)	AUC (μg·h/ml)	FD/V (μg/ml)	Au^{∞} (%)	Cl_R (ml/min)	r^2
NH_4Cl (A)	17.5 ± 3.2	2.3 ± 1.4	1.8 ± 1.1	0.56 ± 0.32	0.39 ± 0.13	2.0 ± 0.6	77.5 ± 20.8	28.4 ± 5.9	65.0 ± 7.0	76 ± 20	0.98 ± 0.04
$NaHCO_3$ (B)	13.2 ± 3.2	1.4 ± 0.5	2.2 ± 0.7	0.61 ± 0.44	1.2 ± 0.1	0.60 ± 0.05	25.6 ± 6.6	27.2 ± 7.6	79.7 ± 9.9	278 ± 79	0.99 ± 0.02
Control (C)	16.3 ± 3.7	1.8 ± 1.0	2.4 ± 1.5	0.81 ± 0.66	0.68 ± 0.21	1.1 ± 0.4	50.1 ± 12.3	29.0 ± 5.0	64.9 ± 7.5	118 ± 38	0.98 ± 0.02
Statistic	NSD	NSD	NSD	NSD	B > C > A	A > C > B	A > C > B	NSD	B > AC	B > CA	NSD

[a] Values are means ± SD (n = 9). Abbreviations: C_{max}, maximum plasma concentration of cinoxacin; t_{max}, time of C_{max}; k_a, first-order rate constant for appearance of cinoxacin in plasma; t_0, time interval between doses and the time of measurable cinoxacin in plasma obtained from computer fitting; k_{el}, first-order rate constant for loss of cinoxacin from plasma; $t_{1/2}$, elimination half-life ($t_{1/2} = \ln 2/k_{el}$); AUC, area under cinoxacin plasma curve from zero to infinite time, calculated by trapezoidal rule with end correction where necessary; FD/V, fraction F of the dose D absorbed, expressed as a concentration in its volume of distribution V (FD/V = AUC k_{el}); Au^{∞}, cumulative percentage of dose recovered in urine samples collected from 0 to 36 h; Cl_R, renal clearance obtained by dividing the total amount of unchanged cinoxacin recovered in urine by AUC; r^2, coefficient of determination from nonlinear regression analysis of individual cinoxacin profiles i.v. plasma to the kinetic one-compartment open model (12) ($r^2 = [\Sigma obs^2 - dev^2]/\Sigma obs^2$).

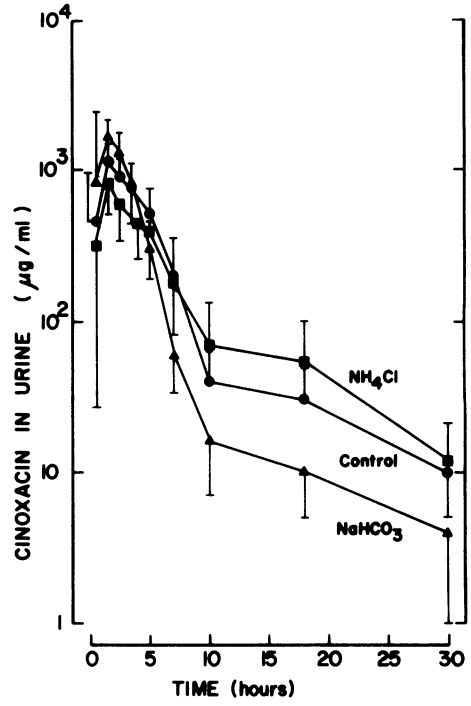

FIG. 4. Mean concentrations of cinoxacin in urine after control (●), ammonium chloride (■), and sodium bicarbonate (▲) treatments. Error bars indicate one standard deviation (n = 9).

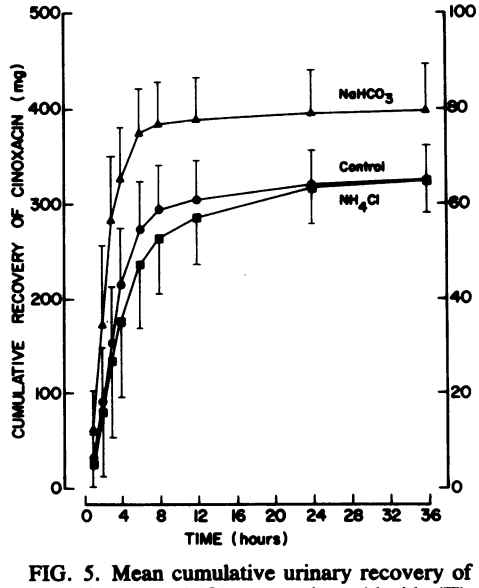

FIG. 5. Mean cumulative urinary recovery of cinoxacin after control (●), ammonium chloride (■), and sodium bicarbonate (▲) treatments. Error bars indicate one standard deviation (n = 9).

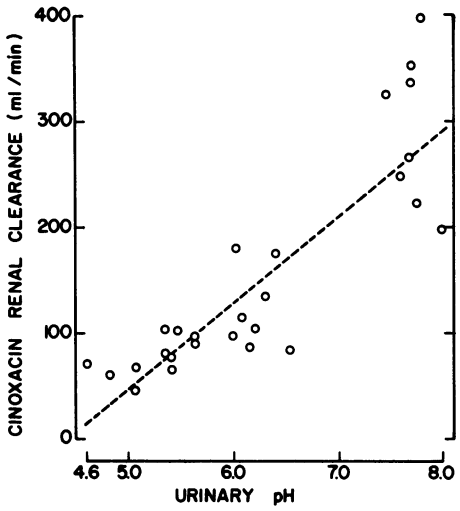

FIG. 6. Linear regression of cinoxacin renal clearance versus urinary pH. $Y = 80.7X - 354$ ($r = +0.851$; $n = 27$; $P < 0.05$).

recovery from the other treatments (Fig. 5). Cl_R correlated highly with urine pH (Fig. 6). The high Cl_R of cinoxacin (mean 278 ml/min) after sodium bicarbonate indicates renal tubular secretion, whereas the low clearance (mean 76 ml/min) after ammonium chloride suggests extensive tubular reabsorption. Tubular reabsorption also occurs in control subjects as Cl_R in these individuals was only one-half of that after sodium bicarbonate treatment.

Bioavailability and distribution of cinoxacin. Since Cl_R and AUC values were obtained under three different conditions, three different parameter combinations could be used to calculate fraction F by means of the equation above, i.e., acidic versus control, alkaline versus control, and acidic versus alkaline. Incorporation of the appropriate values from the three treatment combinations into the equation yielded mean values of 0.78 ± 0.20 (standard deviation), 1.0 ± 0.27, and 0.88 ± 0.18, respectively. These values were not significantly different from each other by t test ($P > 0.05$), so the values from the three treatment pairs were combined to yield an overall F value of 0.89 ± 0.24 ($n = 27$). These calculations show that cinoxacin was approximately 90% absorbed from oral doses and that its bioavailability was not significantly influenced by the acidification or alkalinization treatments. The values of FD/V in Table 1 indicate also that cinoxacin distribution was unaffected by either acidification or alkalinization.

In vitro antimicrobial studies. Influence of pH on cinoxacin MIC values. The 50% MIC (MIC_{50}) and MIC_{90} values for strains of *E. coli*, *K. pneumoniae*, *Enterobacter* species, *Proteus mirabilis*, *P. aeruginosa*, *M. morganii*, *P. stuartii*, *Serratia* species, and *C. freundii* at pH 5, 6, 7, and 8 are shown in Table 2. A twofold increase in MIC values was observed for most species with each unit increase in pH. Thus, an eightfold reduction in cinoxacin activity was generally found at pH 8 compared with pH 5. The increase in pH had its greatest effect on the cinoxacin susceptibility of strains of *P. aeruginosa*. Although the strains tested were uniformly susceptible at pH 5 to 8 μg of cinoxacin per ml, the MIC_{50} and MIC_{90} at pH 8 were 16-fold and at least 64-fold higher, respectively. The increase in MIC values at higher pH values was independent of the base-line MIC. The MIC for *E. coli* ATCC 25922 was determined also in 50% urine, pH 6, of a normal volunteer. The values found from multiple determinations (1.6 to 6.3 μg/ml) were similar to those obtained in Mueller-Hinton broth at the same pH (2 to 4 μg/ml).

Urine dilution tests. The results of the urine dilution tests in one alkaline and two acidic urines are given in Table 3. Although the urinary concentration of cinoxacin was highest in the alkaline urine, the activity was reduced at least fourfold compared with that in acidic urine. The minimal bactericidal concentration (>99.9% killing by 18 to 24 h) was barely reached for *E. coli* ATCC 25922, but not for the other two test organisms in either acidic or alkaline urine.

TABLE 2. Effect of pH on the susceptibility of gram-negative bacilli to cinoxacin[a]

Strain	No. of strains tested	pH 5		pH 6		pH 7		pH 8	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
E. coli	33	2	8	4	8	8	16	16	32
K. pneumoniae	15	4	8	8	16	16	32	16	64
Enterobacter sp.	11	4	8	4	16	16	16	32	32
P. mirabilis	12	2	4	4	4	8	8	16	16
P. seruginosa	18	8	8	16	32	64	256	128	>256
Other[b]	10	2	4	4	8	8	32	16	64

[a] MIC is expressed in micrograms per milliliter.

[b] Includes four strains of *M. morganii*, two strains of *P. stuartii*, two strains of *Serratia* species, and two strains of *C. freundii*.

TABLE 3. Urine dilution tests from three subjects after a single 500-mg dose of cinoxacin

Subject	Treatment	Urine collection period (h)	pH	Cinoxacin concn (μg/ml)	Reciprocal of maximum inhibitory dilution for[a]:		
					E. coli ATCC 25922	*P. mirabilis* 486	*K. pneumoniae* 1575
1	$NaHCO_3$	2–3	7.9	1,261	32 (2)[b]	4 (<2)	32 (<2)
5	NH_4Cl	2–3	5.9	558	128 (2)	128 (<2)	64 (<2)
9	NH_4Cl	2–3	5.2	783	64 (ND)[c]	128 (ND)	128 (ND)

[a] MIC (MBC) determinations in broth at pH 7 for *E. coli* ATCC 25922, *P. mirabilis* 486, and *K. pneumoniae* 1575 were 4 (>128), 8 (>128), and 8 (32) μg/ml, respectively.
[b] Reciprocal of maximal bactericidal dilution is given in parentheses.
[c] ND, Not determined.

Effect of pH on cinoxacin killing curves. The killing pattern for cinoxacin against *E. coli* ATCC 25922 at pH 5, 6, 7, and 8 is shown in Fig. 7. An initial inoculum of $10^{5.5}$ colony-forming units per ml was used. At a drug concentration of 8 μg/ml, killing was rapid at all pH values, resulting in 90 to 99% cell death at 1 to 2 h. In contrast, at cinoxacin concentrations close to the MIC (2 to 4 μg/ml), there was a marked pH effect, the bactericidal action of cinoxacin observed at acidic or neutral pH being partially or completely abolished in alkaline medium.

Figure 8 shows the killing curve of *E. coli* ATCC 25922 when a 10-fold-higher inoculum ($10^{6.6}$ colony-forming units per ml) was exposed to cinoxacin concentrations between 1 and 1,000 μg/ml at pH 7 and 8. At cinoxacin concentrations of 100 and 1,000 μg/ml, the rate of cell death was independent of pH but was slower than with the smaller inoculum. Erradication of the test organisms was not obtained at 24 h. The cinoxacin activity was pH dependent at a concentration of 10 μg/ml, the killing rate being faster at the lower pH value with evidence of bacterial recovery at later sampling times. The cinoxacin concentration at 1 μg/ml was without bactericidal effect at both pH values.

DISCUSSION

One of the original objectives at the onset of this study was to develop a simple yet sensitive assay for cinoxacin in biological fluids which would be suitable for routine laboratory use. The method which was finally used takes advantage of the natural fluorescence of cinoxacin at acidic pH (1, 9). The deproteinizing agent added to plasma thus serves the dual purpose of denaturing plasma proteins and providing an acidic, aqueous environment in which to determine cinoxacin fluorescence. Negligible fluorescence was observed with drug-free plasma and with

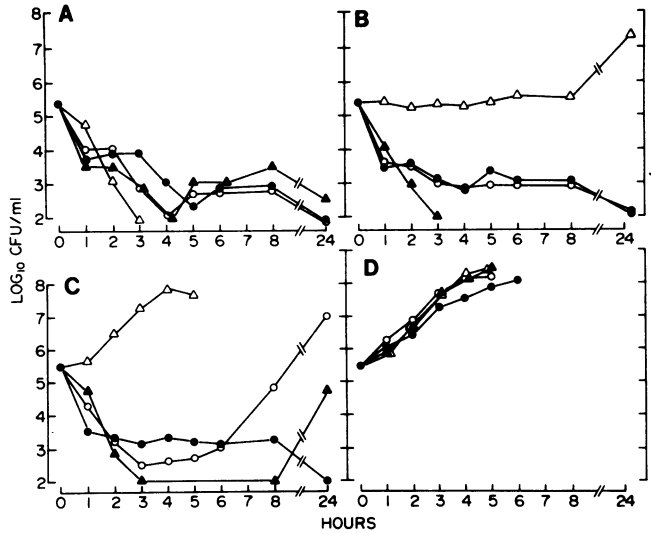

FIG. 7. Killing pattern of *E. coli* ATCC 25922 at pH 5 (●), 6 (○), 7 (▲), and 8 (△) for cinoxacin concentrations of 2, 4, and 8 μg/ml (panels C, B, and A, respectively) and an inoculum of $10^{5.5}$ colony-forming units per ml (control; panel D).

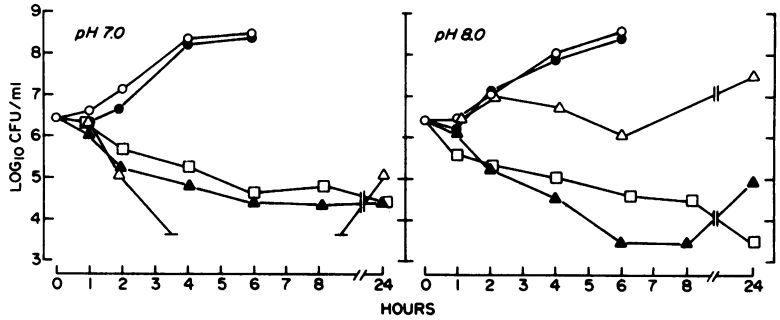

FIG. 8. Killing pattern for cinoxacin concentrations of 1 (●), 10 (△), 100 (▲), and 1,000 (□) μg/ml against *E. coli* ATCC 25922 at pH 7 and 8 and an inoculum of $10^{6.6}$ colony-forming units per ml (control; ○).

plasma containing several other drugs or elevated levels of creatinine or urea. It was convenient to use the same method for plasma and urine, and no interference was noted from urinary components at the dilutions used.

The direct fluorimetric assay is faster than, and equally sensitive to, assays which incorporate a solvent extraction step (1, 9). However, if other substances with fluorescing capability were present in plasma or urine, an appropriate separation step would be necessary for accurate cinoxacin determination.

Before the clinical phase of the study was initiated, a pilot study was conducted to determine the doses of ammonium chloride and sodium bicarbonate necessary to maintain urine pH during the sampling period. In all, 42 g of ammonium chloride and 45.5 g of sodium bicarbonate were required during a 3-day period. These doses are almost identical on a gram per kilogram per day basis to those used previously to maintain similar urine pH ranges in dogs (12). Calculated values for cinoxacin absorption efficiency (see the equation above) and the *FD/V* values in Table 2 indicate that the doses of ammonium chloride and sodium bicarbonate had little or no effect on cinoxacin absorption or distribution.

Changes in urinary pH had a marked effect on cinoxacin elimination. The drug half-life in plasma increased from a mean normal value of 1.1 to 2.0 h with acidification and decreased to 0.6 h with alkalinization. The pharmacokinetic values for cinoxacin in control subjects were in good agreement with those reported previously (2, 13).

Urinary levels of cinoxacin during alkali treatment were significantly higher during the initial 4 h than those after the other treatments. During subsequent collections, however, acidified urine contained higher drug levels which were in excess of 100 μg/ml for 8 h compared with only 6 h after alkalinization. After all three treatments, urine levels of cinoxacin generally exceeded effective values against susceptible organisms for at least 6 to 8 h (4, 11, 16).

Changes in urinary pH appear to influence the elimination rate of cinoxacin to a smaller degree in humans than in dogs (12). In this study, the cinoxacin elimination half-life was increased approximately 3-fold under acidotic compared with alkalotic conditions, whereas in the dog the difference in cinoxacin half-life between these conditions was 18-fold.

Why cinoxacin elimination should be less sensitive to urine pH in humans is not clear. Cinoxacin is a weak acid (pK_a, 4.7). At a urinary pH of 5.5, approximately 13.7% of the drug is in the un-ionized form. At pH 6, this value drops to 4.8%, and at pH 7, this value drops to 0.5%. There is thus a marked decrease in the un-ionized fraction, which is presumably the form capable of undergoing kidney tubular reabsorption with increasing pH. It appears that cinoxacin excretion in humans is less sensitive to these changes than in the dog. However, the slower elimination of cinoxacin in dogs compared with humans under normal conditions (elimination half-life of 3.8 h versus 1.1 h) suggests that renal handling of cinoxacin may be different in the two species.

The in vitro studies supplement an earlier report (4) and show that the activity of cinoxacin against some common urinary tract pathogens is reduced at alkaline pH. This finding was not unexpected and is possibly related to the very small percentage of un-ionized drug present at alkaline pH. The impact of pH on cinoxacin activity could only be demonstrated at drug concentrations close to the MIC of the organisms.

The in vitro results for *P. aeruginosa* were not expected. This organism is generally considered resistant to cinoxacin (4). Although our in vitro studies at pH 7 and 8 support this conclusion, the MIC of cinoxacin for the strains tested was only 4 to 8 μg/ml at pH 5. Whether acidification of the urine would be useful in the treatment of

P. aeruginosa urinary tract infections with cinoxacin can only be properly evaluated in clinical trials.

We conclude that the pH effect on the antibacterial activity and therapeutic efficacy of cinoxacin would be of minor importance in most clinical situations, since cinoxacin concentrations far above the MIC for common urinary tract pathogens can readily be obtained in alkaline and acidic urine. Alkalinization may nevertheless be a useful measure to increase cinoxacin elimination in cases of overdose or of undue drug accumulation in patients with compromised renal function (7).

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