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Pharmacodynamic Evaluation of Ofloxacin and Trimethoprim-Sulfamethoxazole in Vaginal Fluid of Women Treated for Acute Cystitis

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Vaginal colonization with *Escherichia coli* is an integral step in the development of acute cystitis, and persistent vaginal coliform colonization may also be a predisposing step to recurrent urinary tract infections. For this reason, we evaluated antibiotic concentrations in the vaginal fluid, serum, and urine and the vaginal colonization by *E. coli* of 56 women receiving either ofloxacin (200 mg orally twice a day) or trimethoprim-sulfamethoxazole (TMP-SMX) (160/800 mg orally twice a day) for the treatment of acute cystitis. Ofloxacin and trimethoprim both penetrated into vaginal fluid to a considerably greater extent than sulfamethoxazole. Among 33 patients given ofloxacin, the concentration of the drug in vaginal fluid during one dosage interval ranged from 1.6 to 21.6 μg/ml. In 21 women given TMP-SMX the range of drug concentrations in vaginal fluid was 2.6 to 32.5 μg/ml for TMP and 1.0 to 6.2 μg/ml for SMX. Treatment with both ofloxacin and TMP-SMX remarkably reduced vaginal colonization by *E. coli* during and up to 30 days after therapy. For the ofloxacin-treated women, eradication of vaginal *E. coli* was associated with a high ratio of drug concentration in vaginal fluid to that in serum. We conclude that ofloxacin and TMP both achieve high concentrations in vaginal fluid and are equally successful in eradicating *E. coli* from the vagina.

Vaginal colonization with *Escherichia coli* is an integral first step in the pathogenesis of acute cystitis, and persistent vaginal colonization with *E. coli* has been associated with the development of recurrent urinary infections in women (2, 7, 13). However, few studies have examined the pharmacodynamic properties of antibiotics in vaginal fluid or their effect on vaginal *E. coli* colonization. We therefore undertook this study to evaluate the pharmacokinetics and antibacterial activity of ofloxacin, a new quinolone antibiotic, and trimethoprim-sulfamethoxazole (TMP-SMX) in the vaginal fluid of women with acute cystitis.

MATERIALS AND METHODS

Patient population and specimen collection. Between October 1985 and December 1986, patients enrolled in a treatment trial comparing the efficacy of oral ofloxacin with oral TMP-SMX for the treatment of culture-documented acute cystitis were eligible for this study (3). Fifty-six women with ages between 18 and 39 (mean age, 24) agreed to participate.

Before antibiotics were administered, vaginal fluid, serum, and midstream urine samples were obtained for base-line drug concentration measurements. Vaginal fluid was obtained by using a sterile preweighed 25-mm-diameter filter paper disk (no. 42; Whatman, Inc., Clifton, N.J.) that was stored in a sealed test tube. During the speculum examination, the disk was placed high on the vaginal wall for 15 s, with care taken to avoid introital contamination. The disk was removed with sterile forceps and placed into the original test tube, and the tube was sealed tightly and stored at −70°C until analysis. A vaginal culture was also obtained by using a sterile cotton-tipped swab.

Antibiotic therapy. As previously described (3), patients were randomly assigned to receive either oral ofloxacin (200 mg) or TMP-SMX (160/800 mg) twice a day for 3 to 7 days. Patients were given the first dose in the clinic and were instructed to take their assigned doses approximately every 12 h. Diaries were used by the patients to record the time of each dose.

Follow-up visits. Patients returned to the clinic during therapy at day 2 or 3 and at days 5 to 9 and 30 posttherapy. During these visits, vaginal and urine cultures were again obtained. Blood and vaginal fluid samples for determination of antibiotic concentrations were collected at the first follow-up visit. All specimens were collected within 15 min of each other for each patient.

Bacteriology. All cultures and susceptibility tests were performed in our laboratory as previously described (8). Vaginal growth of *E. coli* was reported semiquantitatively as none, few, light, moderate, or heavy.

Analysis of drug concentrations. Concentrations of TMP-SMX in serum, urine, and vaginal fluid were measured by high-performance liquid chromatography as described by Weber et al. (15) with the following modifications. The internal standard was *p*-nitrobenzenesulfonamide (Aldrich Chemical Co., Inc., Milwaukee, Wis.). The mobile phase consisted of potassium phosphate buffer (0.067 M; pH 4.8) and ethanol (82:18, vol/vol). No interferences from either endogenous compounds or other commonly administered drugs were noted with these conditions. Urine specimens were diluted 1:10 with deionized water and assayed similarly as serum specimens. Filter paper disks containing vaginal fluid were transferred to tared 15-ml conical centrifuge tubes and eluted with 0.5 ml of deionized water containing 0.05 ml of internal standard solution. The tubes were vortexed and then centrifuged at 1,000 × *g*. The clear supernatants were removed and concentrated under a stream of air to 0.1 ml before being injected into the high-performance liquid chromatograph. The tubes containing the extracted filter papers

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TABLE 1. Drug concentrations on day 2 or 3 in women with acute cystitis treated with ofloxacin (200 mg) or TMP-SMX (160/180 mg)

Drug	No. of patients	Time since last dose (h)	Mean drug concn (μg/ml) in [a]:			Ratio[b]
			Serum	Vaginal fluid	Urine	
Ofloxacin	10	0–1.9	1.8 ± 1.1 (0.4–3.6)	9.6 ± 5.7 (3.6–21.3)	334 ± 315 (41–1,140)	8.8 ± 8.5 (1.7–30.4)
	5	2.0–3.9	3.0 ± 0.7 (2.4–4.4)	8.2 ± 3.5 (5.3–14.1)	386 ± 203 (184–666)	3.0 ± 1.5 (1.2–5.4)
	10	4.0–7.9	1.5 ± 0.6 (0.4–2.4)	8.7 ± 6.0 (1.6–21.6)	286 ± 262 (41–904)	5.1 ± 3.0 (1.6–10.5)
	8	≥8.0	1.1 ± 0.4 (0.6–1.7)	6.9 ± 3.7 (2.2–11.7)	262 ± 125 (48–441)	7.1 ± 3.4 (1.4–12.3)
TMP	8	0–1.9	3.5 ± 1.0 (1.6–4.5)	12.3 ± 9.0 (5.5–32.5)	182 ± 79 (106–328)	4.8 ± 6.0 (1.2–20.3)
	8	2.0–3.9	3.9 ± 1.1 (1.5–5.1)	8.8 ± 4.9 (2.6–18.0)	138 ± 57 (44–237)	2.3 ± 0.9 (0.5–3.5)
	4	4.0–7.9	2.9 ± 0.3 (2.7–3.4)	8.0 ± 4.3 (3.1–14.1)	147 ± 64 (53–208)	2.8 ± 1.5 (1.2–5.2)
	1	≥8.0	3.1	7.7	705	3.5
SMX	8	0–1.9	78 ± 27 (37–107)	3.2 ± 2.0 (1.0–5.9)	119 ± 127 (22–398)	0.05 ± 0.05 (0.01–0.16)
	8	2.0–3.9	90 ± 17 (65–114)	2.9 ± 1.7 (1.2–5.9)	179 ± 92 (73–381)	0.03 ± 0.01 (0.01–0.05)
	4	4.0–7.9	72 ± 8 (61–81)	3.2 ± 1.9 (1.2–6.2)	362 ± 299 (124–860)	0.04 ± 0.03 (0.02–0.09)
	1	≥8.0	65	1.0	32	0.015

[a] Values are means ± standard deviations. Values in parentheses are ranges.
[b] Ratio of drug concentration in vaginal fluid to that in serum.

were dried and weighed to obtain the weight of vaginal fluid. Recovery of TMP added to filter paper disks (4.2 μg/ml) was 99%; recovery of SMX (1.5 μg/ml) added in a similar manner was 93%. Between-run precision of the assay (as percent coefficient of variation) was 2.4% for TMP and 1.5% for SMX.

Ofloxacin concentrations in serum, urine, and vaginal fluid were assayed by a modification of the published procedure for the analysis of TMP-SMX (15). The following changes were made.

Single-point calibration standards, 25-μg/ml solutions of ofloxacin (Ortho Pharmaceutical Co., Raritan, N.J.) in serum, urine, and potassium phosphate buffer, were used to calibrate the assays for serum, urine, and vaginal fluid, respectively. Propoxyphene (80 μg/ml in acetonitrile) was the internal standard. The column used was a Nova Pak (8NVC18 5u; Waters Associates, Inc., Milford, Mass.), and the mobile phase was a mixture of potassium phosphate buffer (0.25 M; pH 2.5) and acetonitrile (84:16, vol/vol). The column effluent was monitored at 254 nm (0.005 absorbance units, full scale) and 295 nm (0.2 absorbance units, full scale). At a flow rate of 2.0 ml/min, the retention times for ofloxacin and the internal standard were 5.0 and 7.5 min, respectively. No interferences from either endogenous compounds or other commonly administered drugs were noted in this assay. Serum, urine, and vaginal fluid samples were assayed by using the procedure for TMP-SMX described above. With a 0.05-ml sample size, the method showed a linear detector response from 0.1 to 326 μg/ml. With two levels of ofloxacin controls (1.6 and 24.4 μg/ml), recoveries of ofloxacin from serum, urine, and vaginal fluid ranged from 96 to 102%; between-run precision (percent coefficient of variation) of multiple assays at these two levels of control (1.6 and 24.4 μg/ml) were 11.3 and 5.5%, respectively.

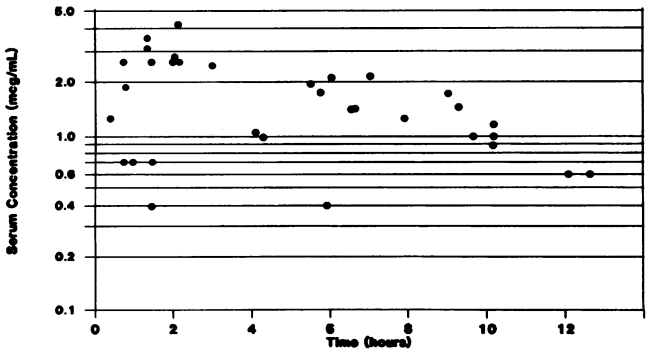

FIG. 1. Ofloxacin concentrations in serum on day 2 or 3 of multiple oral dose (200 mg) therapy in women with acute cystitis.

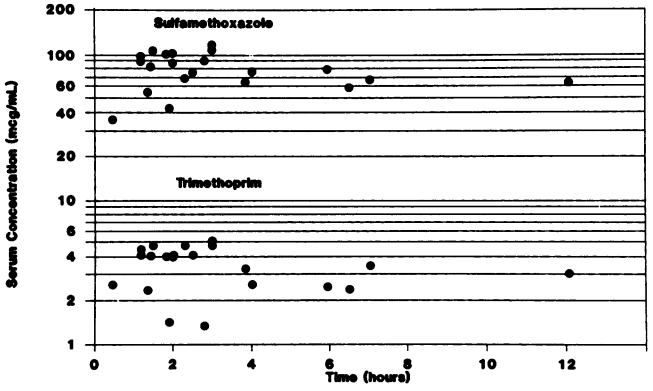

FIG. 2. TMP-SMX concentrations in serum on day 2 or 3 of multiple oral dose (160/800 mg) therapy in women with acute cystitis.

TABLE 2. *E. coli* colonization in vaginas of women treated with oral ofloxacin (200 mg every 12 h) for 3 to 7 days

Time of vaginal culture (no. of patients)	No. (%) of women with indicated colonization					Total no. (%) of women colonized
	Heavy	Moderate	Light	Few	None	
Pretherapy (31)	8 (26)	3 (10)	10 (32)	3 (10)	7 (22)	24 (77)
Day 2 or 3 of therapy (31)	0 (0)	1 (3)	5 (16)	0 (0)	25 (81)	6 (19)
Days 5 to 9 posttherapy (29)	3 (10)	0 (0)	5 (17)	1 (3)	20 (70)	9 (31)
Day 30 posttherapy (29)	4 (15)	1 (4)	4 (15)	0 (0)	17 (66)	9 (35)

Pharmacokinetic and pharmacodynamic analyses. Pooled data for all patients for ofloxacin and TMP-SMX concentrations in serum, urine, and vaginal fluid were plotted separately on semilogarithm paper versus the time since the last antibiotic dose. Additionally, Cartesian plots of drug concentration in serum versus that in vaginal fluid, in serum versus that in urine, and in vaginal fluid versus that in urine were constructed for each antibiotic. Ratios of drug concentration in vaginal fluid to that in serum were calculated for each subject. For this study, we assumed that 1 g of vaginal fluid is equal in density to 1 ml of water.

Concentrations in serum and vaginal fluid and ratios of concentration in vaginal fluid to that in serum were correlated with the vaginal bacteriologic outcomes (i.e., eradicated, modified, or persistent vaginal *E. coli*).

Statistical analysis. The statistical significance of differences in proportions of vaginal coliform eradication in groups of patients was determined by the Fisher exact test.

RESULTS

Drug concentrations in vaginal fluid, serum, and urine. Ofloxacin and TMP penetrated into vaginal fluid to a much greater extent than did SMX, with mean peak concentrations in vaginal fluid (0 to 2 h postdose) for ofloxacin, TMP, and SMX of 9.6 ± 5.7, 12.3 ± 9.0, and 3.2 ± 2.0 μg/ml, respectively (Table 1). The better penetration of ofloxacin and TMP was also demonstrated by calculating the ratios of drug concentration in vaginal fluid to that in serum for each drug. The mean peak ratio for SMX (0.05) was much lower than that for ofloxacin (8.8) and TMP (4.8) (Table 1). Mean peak concentrations in urine for ofloxacin, TMP, and SMX were 386, 182, and 362 μg/ml, respectively, but were highly variable.

Pharmacokinetic analysis. Ofloxacin (Fig. 1) and TMP-SMX (Fig. 2) concentrations in serum declined linearly with time and appeared to follow first-order kinetics. The concentrations of TMP and ofloxacin in serum were similar, whereas SMX levels in serum were 10- to 20-fold greater.

There was no apparent relationship between the drug concentration in serum and that in vaginal fluid for any of the three drugs (r^2 = 0.002, 0.01, and 0.002 for ofloxacin, TMP, and SMX, respectively).

Relationship of vaginal *E. coli* colonization and drug concentration in vaginal fluid. For the ofloxacin-treated group, 77% of women had vaginal colonization with *E. coli* prior to therapy; 30% were heavily colonized. At day 2 or 3 of therapy and at days 5 to 9 and 30 after therapy, colonization dropped significantly to 19, 31, and 35%, respectively (Table 2). At the follow-up visit at 1 month posttherapy, about 18% of women remained heavily colonized with *E. coli* in the vagina. Eradication of vaginal *E. coli* was associated with a high ratio of ofloxacin concentration in vaginal fluid to that in serum; there was eradication for 13 of 13 (100%) women for whom the ratio was ≥5.0, compared with eradication for 10 of 15 (67%) women for whom the ratio was <5.0 (P = 0.04) (Fig. 3). Additionally, for those women who did not have complete eradication of vaginal *E. coli*, ofloxacin concentrations in the vaginal fluid were low (<9 μg/ml). Of those women not colonized with vaginal *E. coli* prior to therapy, none were colonized with coliforms during ofloxacin treatment or at the posttherapy follow-up visits.

Among women treated with TMP-SMX, 79% had vaginal colonization with *E. coli* prior to therapy. Vaginal colonization with *E. coli* significantly decreased during and for up to 1 month after TMP-SMX treatment (Table 3). There was no association between TMP-SMX concentrations in vaginal fluid or ratios of TMP-SMX concentration in vaginal fluid to that in serum and eradication of vaginal *E. coli*. Unlike ofloxacin-treated women, three patients treated with TMP-SMX who were not colonized before therapy developed vaginal *E. coli* colonization during therapy. Concentrations of TMP-SMX in the vaginal fluid of these three patients during therapy were relatively low: 2.6/1.0, 7.7/1.0, and 9.7/6.2 μg/ml.

DISCUSSION

Although the healthy vagina is infrequently colonized by members of the family *Enterobacteriaceae*, colonization of the vaginal introitus with *E. coli* usually precedes acute cystitis (2, 7, 13). Additionally, women who experience recurrent urinary tract infections often have persistent vaginal colonization with coliforms (1, 6, 12), as do women who experience early recurrent infection after single-dose antibiotic therapy. Thus, effective treatment of urinary tract infections may well depend in part upon eradication of *E. coli* colonizing the vagina. Most antimicrobial studies on treatment of urinary tract infections, however, rarely evaluate eradication of vaginal coliforms, and characteristic antibiotic concentrations in vaginal fluid have been determined

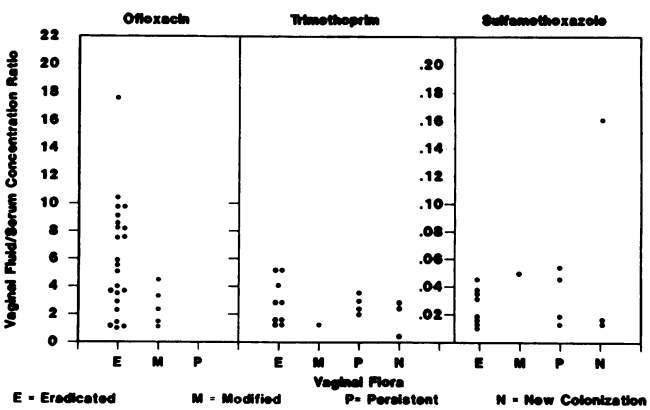

FIG. 3 Vaginal *E. coli* colonization versus ratio of drug concentration in vaginal fluid to that in serum on day 2 or 3 of antibiotic therapy for acute cystitis.

TABLE 3. *E. coli* colonization in vaginas of women treated with oral TMP-SMX (160/800 mg every 12 h) for 7 days

Time of vaginal culture (no. of patients)	No. (%) of women with indicated colonization						Total no. (%) of women colonized
	Heavy	Moderate	Light	Few	None	New	
Pretherapy (21)	4 (19)	1 (5)	7 (33)	3 (14)	6 (29)		15 (71)
Day 2 or 3 of therapy (21)	1 (4)	2 (10)	0 (0)	2 (10)	14 (66)	2 (10)	7 (33)
Days 5 to 9 posttherapy (19)	1 (5)	0 (0)	2 (11)	1 (5)	15 (79)		4 (21)
Day 30 posttherapy (16)	0 (0)	1 (6)	1 (6)	1 (6)	13 (82)		3 (19)

for only a few antibiotics (4, 5, 11, 14). Moreover, the influence of the new fluorinated quinolone antibiotics, such as ofloxacin, on vaginal colonization with *E. coli* has not been previously reported.

In our study, ofloxacin was shown to penetrate well into vaginal fluid. The concentration of ofloxacin in the vaginal fluid of all patients was greater than 1.5 μg/ml, which is considerably above the MIC for most *E. coli* infecting the urinary tract. Although the vaginal fluid samples were not collected in a strictly timed fashion, there was no apparent relationship between concentration and time postingestion; over the interval we studied, bactericidal concentrations persisted throughout the dosage interval. The concentrations in serum determined at various times following a 200-mg oral dose for our patients are similar to previously reported concentrations determined during controlled pharmacokinetic studies (9). Compared with penetration into serum, ofloxacin appeared to penetrate into vaginal fluid to a significant extent (i.e., by ≥120%). Concentrations of TMP in vaginal fluid were very similar to those of ofloxacin in our population and generally far exceeded the MIC for most *E. coli*. In contrast, concentrations of SMX in vaginal fluid were much lower, often less than the expected MICs for *E. coli* (i.e., 4.5 μg/ml). Stamey and Condy (11) previously reported similar findings, noting that TMP concentrations in vaginal fluid exceed simultaneous concentrations in serum by severalfold whereas SMX often does not achieve detectable concentrations in vaginal fluid. These observations are not surprising because basic compounds such as TMP and ofloxacin tend to concentrate in acidic fluids such as vaginal secretions at concentrations greater than that in blood, possibly because of ion trapping. The reverse is observed for weak acids, such as SMX (10).

Prior to initiation of treatment with either ofloxacin or TMP-SMX, approximately 75% of women in each group had vaginal colonization with *E. coli*. Ofloxacin therapy reduced vaginal coliform colonization to 19% by day 2 or 3 of therapy and caused persistent reduction in vaginal colonization up to 30 days after cessation of therapy. These observations suggest that ofloxacin, as previously reported for TMP, effectively eradicates vaginal *E. coli*. Of additional interest was the fact that low concentrations in vaginal fluid or ratios of concentration in vaginal fluid to that in serum during therapy with ofloxacin were associated with persistent vaginal colonization. This relationship deserves further systematic study with various ofloxacin doses. In addition, the importance of posttreatment vaginal coliform colonization as a predictor of early recurrence and the necessity of bactericidal concentrations in vaginal fluid in the management of urinary tract infections should be evaluated further by subsequent treatment studies.

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