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Recovery of Norfloxacin in Feces after Administration of a Single Oral Dose to Human Volunteers

RICHARD D. COFSKY, LORRAINE DUBOUCHET, AND SHELDON H. LANDESMAN*

Division of Infectious Diseases, Downstate Medical Center, Brooklyn, New York 11203

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Twelve healthy volunteers received single 400-mg oral doses of norfloxacin. During the ensuing 48 h, from 8.3 to 53.3% (mean, 28%) of this dose was recovered in the feces. Peak drug concentrations in fecal specimens ranged from 207 to 2,716 μg/g.

Norfloxacin (MK-0366), an orally administered quinolinecarboxylic acid derivative chemically related to nalidixic acid, has substantial activity in vitro against members of the family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Campylobacter fetus* subsp. *jejuni*, *Vibrios* spp., and *Yersinia enterocolitica* (2–6) and somewhat less activity against gram-positive organisms such as streptococci, *Staphylococci aureus*, the enterococci, and *Clostridium difficile* (2–6). If adequate concentrations of the drug can be maintained in the large bowel, this spectrum of activity suggests that norfloxacin might be clinically useful in gut decontamination in neutropenic patients, prophylaxis in burn patients, prophylaxis and treatment of traveler's diarrhea, and surgical prophylaxis before elective colonic surgery. In this study concentrations of norfloxacin in fecal specimens were measured for 48 h after oral administration of a single dose.

After an overnight fast, 12 healthy male volunteers with a mean age of 34 years (range, 22 to 52 years) and a mean weight of 74 kg (range, 64 to 85 kg) were each given a single 400-mg oral dose of norfloxacin. Volunteers consumed their usual diets during the study. One fecal specimen was obtained before drug administration, and all specimens for the next 48 h were collected. These were frozen at −70°C until assayed.

After the procedure which was recommended by the manufacturer to increase the solubility of norfloxacin (Merck Sharp & Dohme Research Laboratories, West Point, Pa.), the entire fecal specimen was combined with twice its weight of 0.1 N NaOH and homogenized in a Waring blender. From 1 to 2 ml of this homogenate was centrifuged at 3,000 rpm for 20 min. The supernatant (pH ≥9) was measured and assayed for norfloxacin content (see below). The pellet was resuspended in 2 ml of 0.1 N NaOH, mixed, and centrifuged; the resultant supernatant (pH ≥12) was assayed for norfloxacin. This process was repeated until the supernatant no longer contained a measurable amount of norfloxacin. In most cases three washes sufficed. These analyses were always performed in triplicate. Tests showed that norfloxacin concentrations as low as 4.5 μg/g of feces could be detected by this method.

An agar well diffusion method (1) was used to assay the norfloxacin content of fecal specimens. The test organism *E. coli* (ATCC 25922) was incubated for 18 h in 10 ml of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) to a population of ca. 10^9 CFU/ml. Molten Trypticase soy agar (22 ml) was seeded with 0.5 ml of this culture and allowed to harden in petri dishes. Four-millimeter wells were cut into the agar. Standards of norfloxacin in concentrations of 1.5, 5.0, and 20.0 μg were prepared in 0.1 N NaOH, and the pH was adjusted to match that of the supernatant being tested. Tests showed that there was no difference in the zones of inhibition when norfloxacin was added to solutions at an acidic, neutral, or basic pH. Samples of 0.01 ml of each standard and of the supernatant being assayed were pipetted into the wells with microcapillary tubes (in triplicate). The supernatants were tested undiluted and at dilutions of 1:2, 1:4, and 1:8. After overnight incubation at 35°C, the diameter of the zone of inhibition around each well was measured. Graphs of the zone of inhibition versus drug concentration were plotted for each standard. Appropriate calculations were then made to determine the concentration of norfloxacin in the supernatant being tested.

Two types of controls were employed (each in triplicate) to verify this method. In one, NaOH of the same normality as that used for the standards was pipetted into the wells to determine whether NaOH alone had inhibitory effects on the test organism. No inhibition occurred. The other control employed was the addition of norfloxacin to fecal specimens at concentrations of 0, 16, 32, 64, 128, and 256 μg/ml; an assay of the drug levels in this mixture was then performed. Calculations based on microbiological assay showed that activity equal to 80% (range, 70 to 100%) of the known quantity of norfloxacin could be recovered from the fecal specimens. This biological activity may be that of norfloxacin itself or that of a yet unidentified metabolite.

The mean number of stool specimens obtained during the 48 h after drug ingestion was 3.75 (range, 2 to 7) per volunteer (Table 1).

Recovery of norfloxacin from these volunteers over the 48-h period ranged from 8.3 to 53.3% (mean, 29%) of the 400-mg dose (Table 1). In 10 of the 12 volunteers fecal norfloxacin concentrations were highest between 23 and 36 h after norfloxacin administration (Table 2). Peak norfloxacin concentrations ranged from 207 to 2,716 μg/g of feces (Table 2).

The mean recovery of 29% of the oral dose may be lower than the actual percentage of norfloxacin in the stool since

* Corresponding author.

TABLE 1. Norfloxacin elimination in fecal specimens

Volunteer	No. of Specimens during study period	Norfloxacin eliminated (mg)[a] at the following h after dose:				Total amt eliminated (mg)[a] during study period
		0–12	>12–24	>24–36	>36–48	
1	4		6.2 (1.6)	7.5 (1.9)	19.6 (4.9)	33.3 (8.3)
2	5	36.1 (9.0)		22.8 (5.7)		58.9 (14.7)
3	3	7.3 (1.8)	9.2 (2.3)	46.0 (11.5)		62.5 (15.6)
4	3	14.0 (3.5)		82.4 (20.6)		96.4 (24.1)
5	3		64.8 (16.2)	30.7 (7.7)	1.9 (0.5)	97.4 (24.4)
6	3	28.1 (7.0)		68.1 (17.0)	8.7 (2.2)	104.9 (26.2)
7	7	13.5 (3.4)	63.9 (16.0)	26.7 (6.8)	5.8 (1.5)	109.9 (27.5)
8	3			118.0 (29.5)		118.0 (29.5)
9	4	26.8 (6.7)		103.6 (25.9)	1.7 (0.4)	132.1 (33.0)
10	2			156.0 (39.0)		156.0 (39.0)
11	3		39.3 (9.6)	92.3 (23.1)	26.6 (6.7)	157.2 (39.3)
12	4			213.2 (53.3)		213.2 (53.3)

[a] Values within parentheses are the percentages of the dose.

our assay method demonstrated activity equal to 80% of a known dose of norfloxacin added to fecal samples. Possible explanations for this phenomenon include metabolism of drug by stool components or binding of drug to stool components or both.

We noted a substantial variation in the percentage of norfloxacin recovered from different volunteers (8.3 to 53.3%). Possible reasons for this variation include differences in the absorption, metabolism (if it is metabolized in feces), and binding of drug in the gut. These variations may have been accentuated by different diets consumed by individual volunteers. We did not attempt to control diet as we were interested in levels of the drug during normal usage.

The average of 3.75 fecal specimens over 48 h and especially the fact that one volunteer had 7 specimens suggest that this drug may have a mild diarrheal action. This is worthy of further investigation.

The peak levels of norfloxacin were at least 290 μg/g of feces in each volunteer. Also, most volunteers had drug levels of greater than 50 μg/g for at least 24 h. Since the norfloxacin MIC that inhibited 50% of members of the family *Enterobacteriaceae* is less than 1 μg/ml and the 50% MIC for *P. aeruginosa* is 5 μg/ml (2–6), a single 400-mg oral dose of norfloxacin should produce sustained drug concentrations in feces many times higher than these MICs. Although in vivo activity of antibiotics in stool may not correlate with in vitro activity, the drug levels obtained in this study appear to be high enough to warrant clinical evaluation.

TABLE 2. Concentrations of norfloxacin in fecal specimens

Volunteer	Norfloxacin (μg/g of feces)[a] at the following h after dose:			
	0–12	>12–24	>24–36	>36–48
1		48 (13)	174 (30)	306 (47)
2	14 (3) 207 (9)		183 (25) 64 (29)	0 (48)
3	206 (6)	301 (14)	728 (25)	
4	55 (3)		144 (25) 290 (33)	
5		499 (23)	247 (33)	15 (47)
6	639 (9)		563 (27)	102 (47)
7	0 (3) 210 (8)	605 (23)	318 (26) 436 (30) 232 (35)	128 (46)
8	0 (10)		796 (25) 261 (30)	
9	200 (5)		1,598 (28) 1,60 (33)	51 (38)
10			478 (25) 364 (29)	
11		440 (13)	1,025 (26)	280 (47)
12	0 (2) 0 (11)		2,716 (27) 1,870 (30)	

[a] Values within parentheses are the number of hours after norfloxacin administration.

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