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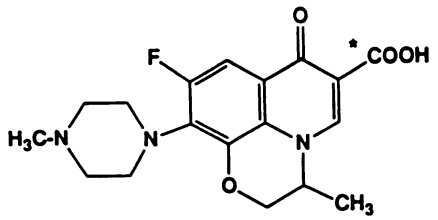
Short Communication

The Metabolism of Ofloxacin in Humans

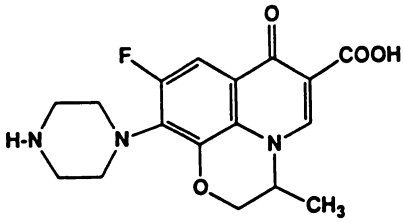
Ofloxacin (fig. 1), (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-cmethyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid, is a new broad spectrum antibacterial drug active against most Gram-negative bacteria, many Gram-positive bacteria, and some anaerobes. It is currently under investigation for urinary tract infections and other indications (1). Ofloxacin is a fluorinated quinolone and is structurally related to nalidixic acid, which exerts its bactericidal activity through selective inhibition of bacterial DNA synthesis in the presence of competent RNA and protein synthesis (2). The extent to which the quinolones are metabolized appears to be quite variable (3). This report describes the metabolic fate of [^{14}C]ofloxacin following administration of single oral 400 mg doses to human volunteers.

For this study, [^{14}C]ofloxacin was synthesized with a specific activity of 5.2 μCi/mg. Chemical and radiochemical purity (>95%) was established by HPLC. Unlabeled ofloxacin, desmethyl ofloxacin [(±)-9-fluoro-2,3-dihydro-3-methyl-10-(1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid], and ofloxacin *N*-oxide [(±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)- 7-oxo-7H - pyrido -[1,2, 3-de][1,4]benzoxazine-6-carboxylic acid piperazine-4-oxide] were supplied by Daiichi Seiyaku Co., Tokyo, Japan. Six normal male volunteers were administered single oral doses (100 μCi, 400 mg) of [^{14}C]ofloxacin as a peppermint-flavored solution in water (Peninsula Testing Corporation, Miami, FL). Smoking and the intake of caffeinated beverages were not allowed for 12 hr before and 12 hr after drug administration. Alcohol intake was denied for 24 hr before and 72 hr after administration. Serial plasma samples were collected for 48 hr after the dose. Plasma concentrations of ofloxacin were measured by HPLC using a previously described method (4), and these data were analyzed by noncompartmental linear pharmacokinetic methods. Urine and feces were collected in 12- or 24-hr intervals after dosing. Radioactivity in the urine samples was determined by liquid scintillation counting. Feces were homogenized after dilution with methanol/water (50:50, v/v). Aliquots of fecal homogenates were combusted in a sample oxidizer and analyzed by liquid scintillation counting. Radioactivity in all samples was measured in Scinti-Verse Bio HP scintillation fluid (Fisher Scientific, Fairlawn, NJ). To obtain metabolite profiles, samples of urine and fecal homogenate collected up to 48 hr after administration of [^{14}C]ofloxacin were analyzed by direct injection onto a Lichrosorb SI-100 column (4.6 mm × 25 cm) and isocratic elution with tetrahydrofuran/ethyl acetate/methanol/phosphoric acid (44:30:18:8, v/v/v/v) at a flow rate of 1.0 ml/min. The eluent was monitored at 254 nm and passed directly from the UV detector to a model HS radioactivity flow detector (Radiomatics Instrument Corp., Tampa, FL). Radioactivity in the eluent was measured in Flo-Scint II scintillation fluid (Radiomatics). To confirm the structures of the principal metabolites, pooled urine samples (0–48 hr) were percolated through a Chemtube CT-2030 column (Analytichem International, Harbor City, CA). Quantitative recovery of the radioactivity was achieved by washing the column successively with methylene chloride, methylene chloride/methanol (80:20, v/v), and methanol. Fractionation of the radioactivity was carried out by preparative TLC on 1-mm silica plates (Whatman, Clifton, NJ) using methylene chloride/methanol/ammonia (66:33:5, v/v/v) as the developing solvent. Cochromatographic comparisons of synthetic standards with ofloxacin and its metabolites confirmed the absence or presence of these compounds. The more polar urinary metabolites were further purified by normal phase HPLC on an Alltech SI-100 column (4.6 mm × 25 cm) and eluted isocratically with ethyl acetate/methanol/trifluoroacetic acid (50:50:1, v/v/v) at a flow rate of 1.5 ml/min. Metabolites isolated from urine were analyzed on a Finnigan model 8230 mass spectrometer. Negative ion direct chemical ionization experiments were run at 1.1 torr using 1% ammonia in methane. After molecular ion spectra were generated for the standards, the purified metabolites were subjected to the same ionization techniques to confirm their identity.

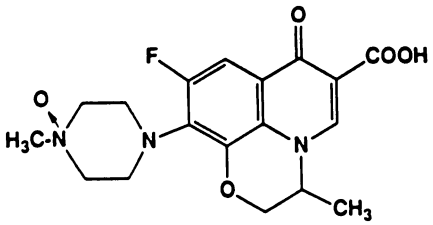
The mean peak plasma concentration (C_{max}) of ofloxacin was



OFLOXACIN (*I*)



DESMETHYL OFLOXACIN (*II*)



OFLOXACIN N-OXIDE (*III*)

FIG. 1. *The structure of ofloxacin and its metabolites.*
*, Indicates position of the ^{14}C-label.

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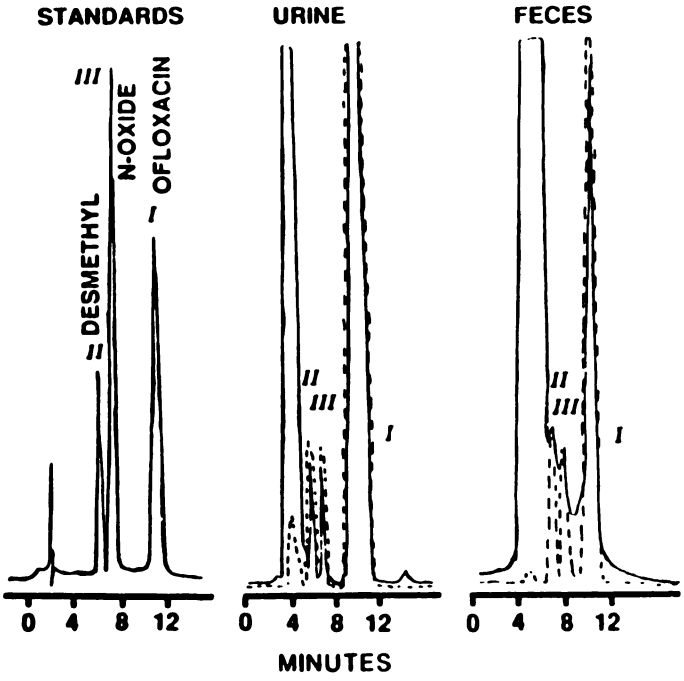

FIG. 2. *Representative HPLC profiles of the radioactivity in urine and fecal samples collected 4–8 and 24–48 hr, respectively, after oral administration of [^{14}C]ofloxacin to male volunteers.*

Solid lines represent UV response; *broken lines* represent radioactivity response.

3.4 μg/ml, and the time of maximum plasma concentration (t_{max}) occurred from 0.5 to 1.5 hr after drug administration. The harmonic mean $t_{½}$ value for the disappearance of ofloxacin from plasma was 6.4 hr, while the mean AUC value was 28.3 μg·hr/ml, consistent with the results of a previous investigation (5). Approximately 79% of the dose was recovered in the urine and 8% in the feces during the 7 days following drug administration. Nearly 71% of the dose was eliminated in urine within the first day. HPLC analysis of urine samples indicated that 90–95% of the radioactivity was associated with intact ofloxacin, while the desmethyl and *N*-oxide metabolites each accounted for about 1%. In the fecal samples, ofloxacin generally accounted for about 80% of the radioactivity while desmethyl ofloxacin and ofloxacin *N*-oxide each accounted for nearly 7%. The remainder of the radioactivity in both the urine and feces was associated with a more polar, unknown metabolite. Representative HPLC elution profiles from the urinary and fecal samples are shown in fig. 2. Upon percolation of the 0–48 hr urine pool through the Chemtube column, 94% of radioactivity was recovered in the methylene chloride eluate. Radiochromatography indicated that nearly all of this radioactivity (R_F, 0.5) was associated with ofloxacin. Chemical ionization mass spectral analysis of this material yielded an intense molecular ion at *m/z* 361 and diagnostic fragment ions at *m/z* 319, 294, and 127. This spectrum was identical to that of standard compound. Radiochromatography of the methylene chloride/methanol eluate from the Chemtube column showed the presence of two peaks, each accounting for 1% of the urinary radioactivity. These peaks migrated at R_F 0.4 and 0.2, respectively, and were associated with the desmethyl and *N*-oxide metabolites. Mass spectral analysis of the material at R_F 0.4 yielded an intense molecular ion at *m/z* 347 as well as a prominent fragment ion at *m/z* 305 (resulting from loss of propene), consistent with the structure of desmethyl ofloxacin, while analysis of material at R_F 0.2 yielded a molecular ion at *m/z* 377 as well as prominent fragment ions at *m/z* 361 (resulting from the loss of oxygen) and 319 (resulting from loss of propene), which were consistent with the structure of ofloxacin *N*-oxide. The remainder of the urinary radioactivity (3%), obtained in the methanolic eluate from the Chemtube column, was associated with a polar metabolite, which, upon mass spectral analysis, yielded a strong fragment at *m/z* 361, the molecular ion of ofloxacin. Furthermore, treatment of this material with β-glucuronidase (type H-5; *Helix pomatia*; 400,000–600,000 units/g, Sigma Chemical Co., St. Louis, MO) resulted in the quantitative recovery of radioactivity as intact ofloxacin. Thus, it would appear that this metabolite is a glucuronide conjugate of parent drug.

In conclusion, this study has shown that following a single oral dose to humans, ofloxacin is rapidly absorbed and eliminated, primarily in the urine. In both urine and feces, the drug is eliminated mostly unchanged. Oxidative *N*-dealkylation, *N*-oxidation, and glucuronidation, the only observable pathways, yielded relatively small amounts of metabolites. The biotransformation of the drug in humans appears to be nearly identical to that in animals (rats, dogs, and monkeys), where it has been extensively investigated (6, 7).

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