Difloxacin Metabolism and Pharmacokinetics in Humans after Single Oral Doses

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By using high-performance liquid chromatography, the metabolism and pharmacokinetics of difloxacin were characterized in humans after single oral doses of 200, 400, and 600 mg. Group mean peak levels in plasma were obtained 4 h after administration. The means of the individual peak levels for the 200-, 400-, and 600-mg groups were 2.17, 4.09, and 6.12 µg/ml, respectively. The mean respective terminal-phase half-lives were 20.6, 27.1, and 28.8 h; the mean half-life for all subjects was 25.7 h. Within the dose range studied, the behavior of difloxacin could be well described by a set of linear pharmacokinetic parameters with a one-compartment open model. Levels of unconjugated metabolites in plasma were negligible. The major urinary components were difloxacin and its glucuronide, each accounting for roughly 10% of the dose. Also present were the N-desmethyl and N-oxide metabolites, accounting for 2 to 4%. Trace levels of other metabolites were observed. Group mean renal clearances ranged from 4.1 to 5.6 ml/min, indicating extensive reabsorption from the glomerular filtrate. As a result, the terminal phase half-life and the dose-normalized area under the curve were substantially greater than those of other members of the class.

Difloxacin (A-56619) is a quinolone carboxylic acid antimicrobial agent with high in vitro activity against a wide range of gram-positive and gram-negative aerobes and anaerobes (4, 10, 22). It is reported to be superior to norfloxacin in numerous in vivo murine infections, including various protection tests, pyelonephritis models, immunosuppressed host models, intracellular infection with Salmonella typhimurium, and subcutaneous abscesses caused by Bacillus fragilis (12).

In animals, difloxacin was well absorbed after oral administration and was extensively metabolized, primarily by glucuronidation (6). Most of the glucuronide ester was secreted in the bile, subsequently to be hydrolyzed in the intestines, resulting in either partial reabsorption of the aglycone or its elimination in the feces. After both oral and intravenous administration of [14C]difloxacin to dogs, over 80% of the administered radiolabel was recovered in the feces.

The pharmacokinetics and metabolism of difloxacin in normal volunteers have been determined in the course of a single-dose phase I study, and the results are the subject of the present report.

MATERIALS AND METHODS

Study description. The study involved oral administration of 200 (n = 6 subjects), 400 (n = 6), and 600 (n = 11) mg of difloxacin (as the hydrochloride salt) in capsules to healthy male adult volunteers in a randomized, double-blinded, placebo-controlled experiment. Mean (±standard deviation) subject body weights for the groups receiving 200, 400, and 600 mg were 70.4 ± 7.7, 71.7 ± 8.7, and 70.6 ± 6.9 kg, respectively. The mean subject age was 20.3 ± 2.7 years. Subjects fasted for 8 h before and 4 h after administration. With the dose and at the end of each urine collection interval, the subjects were required to drink 200 ml of water.

Heparinized blood for harvest of plasma was obtained before and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 28, 32, and 48 h after administration. The urine collection intervals were 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 32, and 32 to 48 h. All samples were stored frozen until analysis. After administration of the 200-mg dose, it became apparent that the half-life of difloxacin was much greater than anticipated; hence, sampling was extended to 96 h postadministration for the subsequent 400- and 600-mg dose groups.

Analytical procedures. All samples were assayed by high-performance liquid chromatography with an Adsorbosphere HS C18 analytical column (Alltech Associates, Inc.; 250 mm by 4.6 mm [inner diameter]) in conjunction with fluorescence (389-nm emission cutoff filter) or tandem fluorescence-UV detectors operated at a wavelength of 280 nm (G. R. Granemann and L. T. Sennello, J. Chromatogr., in press). For plasma analyses, the mobile phase (pH ~2.7) contained 0.05 M phosphate, 0.2% sodium dodecyl sulfate, and approximately 50% acetonitrile. Plasma samples were treated with a displacing reagent containing 0.5% sodium dodecyl sulfate and either A-57084 or A-56681 (analogs of difloxacin in which the phenyl para-substituents are bromo and methyl, respectively) as an internal standard. The mixtures were ultrafiltered with an Amicon Centrifree apparatus (30 min at 1,500 × g) before chromatography. With this procedure, recoveries of difloxacin and its metabolites were greater than 98%. Intrarassay coefficients of variation for standards ranging from 10 ng/ml (limit of quantitation for this study) to 10 µg/ml averaged 0.5% (range, 0.2 to 1.4%). The interassay coefficient of variation, determined from repeated analysis of a quality control reference of 1.5 µg/ml, averaged 2.5%. Calibration curve correlation coefficients averaged 0.9997 (range, 0.9994 to 0.9999).

For analysis of urine, the phosphate concentration of the mobile phase was increased to approximately 0.08 M, and the pH was increased to 5.3. After dilution with the mobile phase, urine specimens were analyzed without further treatment. Urine pools were prepared for assessment of the

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extent of conjugation of difloxacin and its metabolites. Duplicate samples were either diluted or subjected to alkaline hydrolysis followed by neutralization and dilution. The base treatment was previously established to hydrolyze the glucuronide conjugates while not degrading difloxacin or its major oxidative metabolites. Incubation with β-glucuronidase was not employed because it was inefficient in the hydrolysis of the minor isomeric glucuronide of difloxacin formed in alkaline urine by intramolecular acyl migration (11, 21).

References for potential oxidative metabolites were synthesized and used in the calibration curves. The criteria for identification of metabolites were (i) cochromatography with references, (ii) relative fluorescence efficiencies equivalent to references, and (iii) isolation, with mass spectral confirmation. Identical retention characteristics of metabolites and references were observed in mobile phases containing cation-pairing (sodium dodecyl sulfate) and anion-pairing (tetrabutylammonium) reagents and no ion-pairing reagents. The relative fluorescence efficiencies of the metabolites vary over a 100-fold range; thus, comparison of UV/fluorescence measurements provided a highly discriminating means of assessing high-performance liquid chromatographic peak identity.

The major metabolites were also isolated from biological samples with subsequent chemical and mass spectral confirmation. Difloxacin glucuronide had the following characteristics ascribed to ester glucuronides: (i) hydrolysis by β-glucuronidase, (ii) hydrolysis by 0.5 N NaOH (20 min at 50°C), and (iii) base-catalyzed rearrangement by O-acyl migration to form β-glucuronidase-resistant isomers. In addition, elemental microanalysis and Dische (8) analysis were consistent with the proposed structure. Fast atom bombardment mass spectroscopy gave prominent signals at m/z 576 (M+H)+ and 400 (M-C6H4O2)-. The N-desmethyl and N-oxide metabolites were isolated from urine (100 ml, pH 7) by methylene chloride (500 ml) extraction, followed by preparative Adsorbosphere HS C18 high-performance liquid chromatography. Structures were confirmed by fast atom bombardment, electron impact, and ammonia desorption chemical ionization mass spectral comparisons to the references. For N-desmethyl difloxacin, prominent high-mass ions by fast atom bombardment were m/z 408 (M+Na)+, 386 (M+H)+, and 368 (M+H−H2O)+; those by electron impact were m/z 385 (M)+, 343 (M-C6H4N)+, 341 (M-Co2)+, and 299 (M-C6H4N−CO2)2+. For difloxacin N-oxide, prominent high-mass ions by electron impact were m/z 415 (M)+; 399 (M-O)+, 397 (M-H2O)+, and 355 (M-O−CO2)+; those by desorption chemical ionization were m/z 416 (M+H)+, 400 (M+H−H2O)−, and 398 (M+H−H2O)−.

Pharmacokinetics. Pharmacokinetic linearity was evaluated by using both model-free and curve-fitting approaches. In the model-free approach, times elapsed to peak drug concentrations (Tmax; hours), urinary metabolic ratios, renal clearances (CLR; milliliters per minute), dose-normalized values for drug concentrations at each sampling time, maximum drug concentration in plasma (Cmax; micrograms per milliliter per 100 mg) and area under the 0- to 48-h plasma drug concentration curve (AUC0−48; microgram hours per milliliter per 100 mg) were compared among groups. The AUC was calculated by trapezoidal rule (13). Renal clearance (CLR; ml/min) was calculated as Xr/AUC for the 0 to 48-h interval where Xr is the amount of drug excreted in the urine. The mean residence time (MRT) was calculated as described by Yamaoka et al. (27) and required regression estimates of k4. The amount of drug and metabolites excreted in the urine at infinite time were also extrapolated using k4 (13). In the curve-fitting approach, data sets for each subject were fitted by the NONLIN computer program (16) with four models, all including lag time as a parameter: (i) first-order adsorption and elimination, (ii) first-order adsorption and biexponential elimination, and zero-order input with (iii) monoeponential or (iv) biexponential disposition. The results of the regressions were compared by using the Akaike information criterion (1, 26); with this approach the biexponential equation (one-compartment open model) best represented the data. Estimated parameters were the absorption rate constant (ka; per hour), the elimination rate constant (k4; per hour), the apparent volume of distribution (Vf, where f is the fraction of the dose absorbed [liters]), and lag time (hours). The apparent body clearance (CL/f; milliliters per minute) was calculated as the product of k4 and Vf/13). For the purpose of data consolidation, the mean data for the three groups were fitted simultaneously with NONLIN by using a common parameter set.

Statistical analysis. The equivalence among the three dosing groups in the pharmacokinetic profile of difloxacin was examined by a one-way analysis of variance with dosage as the factor. Calculations were performed with PROC GLM of SAS (19). When the P value for dosage was less than 0.05, indicating differences among the dosages, pairwise comparisons were made at the 0.05 significance level using the t test (Fisher least significant difference test).

RESULTS

Mean concentrations of difloxacin in plasma after various oral doses are presented in Figure 1. Various kinetic parameters and associated statistics are given in Table 1. Observed times elapsed to peak levels were not statistically different among groups, averaging 3.9, 5.2, and 4.7 h for the 200-, 400-, and 600-mg dose groups. Group mean peak levels all occurred at 4 h. The corresponding respective means (± standard deviations) of the individual peak levels were 2.17 ± 0.28, 4.09 ± 0.61, and 6.12 ± 0.68 µg/ml. These values, after dose normalization, were not significantly different by one-way analysis of variance. The drug concentrations at each collection time from 0.25 to 48 h were also dose normalized. No differences among the groups were detected,
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FIG. 2. Chemical structures of difloxacin and synthesized metabolites.

except at 28 and 48 h, when statistical differences were found between the 200- and 600-mg dose groups. The dose-normalized, trapezoidal 0 to 48-h areas under the plasma drug level curves (AUC_{0-48}) for the 200-, 400-, and 600-mg dose groups were 26.6 ± 3.1, 28.1 ± 2.4, and 28.3 ± 2.6 μg · h/ml per 100 mg administered. No statistically significant intergroup differences were found in this measure of the kinetic linearity of systemic exposure.

Pharmacokinetics. The results of the NONLIN analyses are also presented in Table 1. The absorption half-life and lag time averaged 23 and 32 min, respectively, and showed the greatest intersubject variabilities of all the parameters. The mean elimination half-lives for the 200-, 400-, and 600-mg dose groups were 20.6 ± 1.4, 27.1 ± 3.3, and 28.8 ± 4.2 h. By one-way analysis of variance, the higher mean elimination rate constant for the 200-mg dose group was statistically significant compared to the other groups. Similarly, the apparent total body clearance (CL/f) and mean residence time (MRT) of the 200-mg dose group statistically differed from those of the 400- and 600-mg dose groups; however, intergroup differences were not demonstrable with the apparent volumes of distribution (V/f). Intersubject variability in half-life, MRT, CL/f, and V/f within each group was small, with coefficients of variation ranging from 7 to 21%. Normalization of V/f by the subjects' body weights did not reduce the variability. Intergroup pooling of half-life, MRT, CL/f, and V/f data produced mean values with coefficients of variation of 20% or less. The results of the NONLIN simultaneous regression for the three groups, using a common set of parameters with the presumption of strictly linear pharmacokinetics, are plotted with the experimental data in Fig. 1.

Metabolism and excretion. Approximately one-third of the administered dose was eliminated in the urine as parent drug and metabolites. The major components found in unhydrolyzed urine were difloxacin and its glucuronide ester, which together accounted for roughly 75% of the identified drug material. Five oxidative metabolites were discernable...
in urine (Fig. 2). The N-desmethyl (M1) and N-oxide (M4) analogs were the major components of the group. Trace levels of metabolites M3 and M5, produced in the sequential oxidation of the piperazine moiety of M1, were also observed. Both were present as the free and conjugated forms. Trace metabolite M2 could not be identified. Reference standards of other potential metabolites, expected by analogy to the metabolism of other quinolones, were synthesized, but were not detectable in urine. With the possible exception of M1, which is roughly twice as active as difloxacin, low concentrations, high MICs, or combinations thereof resulted in negligible contributions of the metabolites to the activity derived from difloxacin.

Recoveries of difloxacin and metabolites from the urine for the 200-, 400-, and 600-mg dose groups were 26.8 ± 4.0, 28.4 ± 5.5, and 28.3 ± 6.3% of the dose, respectively. Extrapolations to infinite time, based on the terminal-phase half-lives, resulted in respective recovery estimates of 11.4, 9.6, and 10.0% for difloxacin and 33.8, 30.9, and 31.3% for difloxacin and metabolites. The metabolic patterns for the dose groups were also quite similar (Fig. 3). When relative abundances of the metabolites in urine were compared, intergroup differences were not established for the major components, difloxacin and its conjugate. Differences for the oxidative metabolites M1 and M3 (and conjugated M3) were detected, but were not systematically dose related. In contrast, the presence of the N-oxide (M4) appeared to increase uniformly as a function of dose.

The concentrations of difloxacin in urine were typically 3 to 6 times the corresponding concentrations in plasma. Uncorrected renal clearances averaged 5.62 ± 0.95, 4.32 ± 1.43, and 4.10 ± 0.88 ml/min for the 200-, 400-, and 600-mg dose groups, respectively. The plasma protein binding, determined by ultrafiltration, was approximately 42%; thus, extensive reabsorption of drug from the glomerular filtrate may be inferred. The urine pH did not have an appreciable effect on renal clearance. The slope from regression of the parameters (100 samples; pH range, 5.5 to 8.5) was not statistically different from zero ($r = 0.112$).

**FIG. 3.** Urinary recoveries of (left to right) difloxacin, difloxacin glucuronide, and oxidative metabolites M1, M4, and M3 after oral doses of 200, 400, and 600 mg in humans.

Discussion

After single oral doses ranging from 200 to 600 mg, the intersubject coefficients of variation of key parameters were 20% or less, and the kinetics of difloxacin approximated those of a linear system. The absence of intergroup differences in $T_{\text{max}}$, dose-normalized drug levels in plasma during the first day, and AUC_{0-48} indicate linearity in the extent and rate of absorption. Although the extent of absorption is unknown, it is inferred to be high, since it is nearly quantitative in mice, rats, rabbits, and dogs, and since dose-normalized peak levels (mean, 1.04 μg/ml per 100 mg) match or exceed those reported for other recent quinolones (2, 3, 7, 9, 14, 15, 23–25). Recoveries from urine do not reflect the extent of absorption because parenteral studies in animals have shown glucuronidation and subsequent biliary secretion to be the dominant elimination route (6).

The indicators of linearity in the dispositional kinetics were mixed. Intergroup differences in the parameters above and in V/F were not detected; however, the elimination rate constant and the derived value for CL/f were significantly greater in the 200-mg dose group, compared to the higher dose groups. In part, procedural differences may account for these results. The unexpectedly long half-life, discovered upon analysis of samples from the 200-mg dose group, required extension of the sampling interval from 0 to 48 to 0 to 96 h for subsequent groups; consequently, the half-life, CL/f, and MRT estimates for the 400- and 600-mg dose groups are considered more reliable.

Substantial evidence of a contributive factor to the half-life differences, such as nonlinear metabolism, was lacking. If a saturable metabolic process exists, and if urinary metabolic ratios are representative of relative hepatic clearances, then a dose-related decrease for the affected pathway should be discernable in the urinary patterns; however, this was not the case. Intergroup differences in the relative levels of the major metabolite, difloxacin glucuronide, and of difloxacin were not statistically significant. The only statistically significant trend was an increase in the minor metabolite M4 with increasing dose. Although some statistically significant intergroup differences have been noted, the magnitude of the deviations from values predicted by a linear kinetic model (Fig. 1) was small, considering the suboptimal sampling for the low dose group. Forthcoming intravenous and multiple dose studies will likely resolve these issues.

Five recent quinolone antibacterial agents are sufficiently well described in the literature to allow comparisons to difloxacin: norfloxacin, ciprofloxacin, enoxacin, ofloxacin, and pefloxacin. Metabolic clearance of these quinolones is low compared with hepatic blood flow, and the primary determinant of intraclass differences in total clearance is the mechanism of renal elimination. For all but pefloxacin and difloxacin, renal clearance equals or exceeds (up to 3:1) the glomerular filtration rate; as a result, terminal-phase half-lives are in the 3- to 7-h range (2, 7, 9, 14, 15, 23–25). Tubular secretion sensitive to competition by probenecid appears to be the dominant process (20). In marked contrast, difloxacin and pefloxacin (3), which are not extensively bound to plasma proteins, have renal clearances less than 10% of the glomerular filtration rate. Accordingly, their half-lives are longer (pefloxacin, 12 h; difloxacin, 26 h), and metabolism is the dominant contributor to total clearance. Qualitatively, difloxacin metabolism is similar to that of other group members. For example, N-demethylation and N-oxide formulation occurs, as it does with ofloxacin and, especially with pefloxacin (17). The sequential oxidation of the piperazine moiety observed with difloxacin is a common observation for the class (5, 17, 18). Despite these similarities, difloxacin is the only recently developed quinolone that is extensively glucuronidated in humans. At present, the physiochemical properties of difloxacin responsible for con-
ferring its low clearance and thus its uncharacteristically high dose-normalized AUC have not been established.

LITERATURE CITED