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Pharmacokinetics and Tissue Penetration of Ro 23-6240, a New Trifluoroquinolone

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A 400-mg dose of the trifluorinated quinolone Ro 23-6240 was administered orally to each of six healthy male volunteers, after which the concentrations of this agent in serum and cantharidin-induced inflammatory fluid were measured. Absorption was rapid, with a mean peak level in serum of 6.1 μg/ml, which was attained 0.71 h after administration. The elimination half-life in serum was 11.95 h. The agent penetrated the inflammatory fluid rapidly; the percent penetration was 89.7%. Urinary recovery of Ro 23-6240 was 58.6% by 72 h.

Ro 23-6240 [AM 833; 6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(methyl-1-piperazinyl)-4-oxo-3-quinolone carboxylic acid] is a quinolone derivative. Its activity against members of the family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and staphylococci is similar to that of ofloxacin and is somewhat less than that of ciprofloxacin (4, 6), and it has low protein binding in serum (27%) (6).

The purpose of this study was to investigate the pharmacokinetic properties of Ro 23-6240 after a single oral dose and to study its penetration into chemically induced blister fluid. The composition of the blister fluid is similar to that of an exudate provoked by a mild inflammatory reaction (9).

MATERIALS AND METHODS

Six healthy male volunteers provided written, informed consent for the study, which had been approved by the Dudley Road Hospital Ethical Committee. The mean age of the volunteers was 28 years (range, 21 to 44 years), the mean weight was 78.18 kg (range, 75.4 to 81.4 kg), and the mean height was 175.0 cm (range, 167.5 to 183.8 cm). There was no history of allergy to antimicrobial agents. A complete physical examination, hematology, blood chemistry, and urinalysis were performed on each subject before and after the study. The subjects were also questioned as to any side effects that were apparently caused by the drug.

On the night before the study, two 0.2% cantharides-impregnated plaster squares (1 by 1 cm) were applied to the volar surface of one forearm and taped in place. The subjects fasted from 11 p.m. the night before the study. On the morning of the study, 400 mg of Ro 23-6240 (obtained from Hoffman-La Roche Ltd., Welwyn, England) was given by mouth with 200 ml of water. The subjects drank 200 ml of water in the first 2 h, during which time they were sitting, followed by a light breakfast. No alcohol or beverages containing caffeine were allowed during the trial.

Venous blood was sampled from an indwelling cannula at 15, 30, 45, and 60 min and at 2, 3, 4, 6, 8, 12, 25, 29, and 50 h after drug administration. The blisters were sampled (with a fine needle) at 30 and 60 min after administration and then at 2, 3, 4, 6, 8, 12, and 25 h. The integrity of the blisters was maintained by spraying with a fast-drying plastic dressing. Urine samples were collected at 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, and 48 to 72 h after drug administration. The volume was noted and a portion was saved for assay.

The blister fluid samples were assayed by a microbiological agar diffusion technique (there was insufficient fluid for high-performance liquid chromatography [HPLC]) by using an *Escherichia coli* Sch 12655 strain (from Schering Research, Bloomfield, N.J.) as the indicator organism and Iso-Sensitest agar (pH 7.2; Oxoid Ltd., London, England). Standards were prepared in 70% human serum. All samples were assayed within 1 h of collection, and the plates were incubated in air at 37°C overnight. The coefficient of variation of the assay was 7.8%, and the lower limit of the assay was 0.8 μg/ml. It was not possible to assay blister fluid after 25 h, as the blisters were no longer patent and the expected levels would have been below the limit of sensitivity of the assay.

Serum and urine samples were assayed by HPLC employing a C18 μBondapak Radial Pak column (Waters Chromatography, Harrow, England). The mobile phase consisted of 0.018 M potassium dihydrogen P_i plus 0.13 mM heptane sulfonic acid, methanol, and concentrated phosphoric acid (7:3:0.01); the flow rate was 3 ml/min and a fluorescence detector (excitation, 288 nm; 475-nm band pass emission filter) was used. A total of 200 μl of serum was mixed with 50 μl of norfloxacin (25 μg/ml, as internal standard) and 400 μl of acetonitrile. Samples were then centrifuged for 10 min at 700 × *g*, and 20 μl of supernatant was injected onto the column. Urine samples were assayed by a similar method, except that samples expected to yield high concentrations were diluted as appropriate in phosphate buffer (pH 7). The limits of sensitivity were 0.1 μg/ml for serum samples and 1 μg/ml for urine. The coefficient of variation for serum assays was 6.7%, and the coefficient of variation for urine assays was 2.4%. No statistically significant differences were found between the results of the microbiological and HPLC assay of spiked serum samples. The standard curve for the urine (HPLC) assays was linear from 250 to 1 μg/ml.

Pharmacokinetic analysis was performed on individual data by routine graphical methods (2, 3). The area under the serum (and blister fluid) concentration-time curve (AUC) was calculated by the trapezoidal method from 0 to 50 h (AUC_{0-50}), and the concentration at 50 h (C_{50}) divided by K_{el} was added to give the AUC from time zero to infinity ($AUC_{0-\infty}$).

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TABLE 1. Mean concentration of Ro 23-6240 in serum and blister fluid from six volunteers

Fluid	Concn (μg/ml) of Ro 23-6240 at the following times (h) after administration[a]:											
	0.25	0.5	0.75	1	2	3	4	6	8	12	25	29	50
Serum	1.3 ± 1.0	4.9 ± 2.5	5.6 ± 1.3	4.9 ± 1.0	3.9 ± 0.5	3.7 ± 0.4	3.4 ± 0.4	3.0 ± 0.3	2.7 ± 0.3	2.2 ± 0.2	1.0 ± 0.2	0.8 ± 0.1	0.3 ± 0.1
Blister	NT[b]	0.7 ± 0.3	NT	2.4 ± 0.9	3.2 ± 1.1	3.2 ± 0.9	3.1 ± 0.7	3.1 ± 0.5	2.6 ± 0.4	2.3 ± 0.3	1.2 ± 0.3	NT	NT

[a] Values are means ± SD.
[b] NT, Not tested.

TABLE 2. Mean urinary excretion of Ro 23-6240 from six volunteers

Interval (h) postdose	Excretion of Ro 23-6240 from urine[a]	
	Amt (μg)	Cumulative % dose
0–4	32.6 ± 8.7	
4–8	36.7 ± 6.9	
8–12	34.1 ± 6.2	
12–24	66.8 ± 14.6	
24–48	51.3 ± 10.1	
48–72	13.0 ± 5.2	
0–4		8.2 ± 2.2
0–8		17.4 ± 2.9
0–12		25.9 ± 2.2
0–24		42.5 ± 3.9
0–48		55.5 ± 4.7
0–72		58.6 ± 5.0

[a] Values are means ± SD.

RESULTS

The levels of Ro 23-6240 attained in serum and blister fluid are shown in Table 1, the urinary excretion data are shown in Table 2, and the derived pharmacokinetic parameters are shown in Table 3.

Ro 23-6240 was rapidly absorbed following oral administration. Five of the six volunteers had a maximum level by 0.75 h (T_{max}), and one volunteer attained the maximum level at 3 h. The levels in serum at 1 h were in excess of 3.5 μg/ml for all volunteers; thereafter, the serum levels fell, and the mean elimination half-life ($t_{1/2}$) of Ro 23-6240 was 12.0 h, with a range of 10.5 to 13.3 h.

The drug penetrated the blister fluid rapidly. The drug level in the blister at 1 h was 40% of that in serum at the same time, and at 2 h it was 83% of that in serum. After 3 h the drug levels in the two fluids were very similar and declined at a similar rate. The mean $t_{1/2}$ of the compound in blister fluid was 12.7 h. If the percentage penetration is calculated on the basis of the ratio of the individual $AUC_{0-\infty}$ for the blister and $AUC_{0-\infty}$ for serum, then a value of 89.7 ± 6.3% (standard deviation [SD]) is obtained. The maximum level of Ro 23-6240 achieved in blister fluid exceeded 3.1 μg/ml in all volunteers.

TABLE 3. Pharmacokinetic parameters of Ro 23-6240

Fluid and parameter[a]	Value[b]
Serum	
$t_{1/2\beta}$ (h)	12.0 ± 1.0
C_{max} (μg/ml)	6.1 ± 2.2
T_{max} (h)	0.7 ± 0.1
$AUC_{0-\infty}$ (μg/ml · h)	78.3 ± 9.4
Clearance	
Total (ml/min)	89.2 ± 9.6
Renal (ml/min)	52.4 ± 6.7
Blister	
$t_{1/2\beta}$ (h)	12.7 ± 1.6
C_{max} (μg/ml)	3.8 ± 0.61
T_{max} (h)	4.0 ± 1.9
$AUC_{0-\infty}$ (μg/ml · h)	70.4 ± 10.6

[a] Abbreviations: $t_{1/2\beta}$, terminal elimination half-life in serum and blister fluid; T_{max}, time at which the maximum concentration (C_{max}) in serum or blister fluid is achieved; $AUC_{0-\infty}$, area under the serum (or blister fluid) concentration-time curve.
[b] Values are means ± SD.

The clearance of Ro 23-6240 from serum was moderately slow (89.2 ± 9.6 ml/min [SD]), with a renal clearance of 52.4 ± 6.7 ml/min [SD]), assuming 100% bioavailability. Urinary recovery of Ro 23-6240 over 72 h accounted for 58.6 ± 5.0% (SD) of the administered dose when the HPLC method of analysis was used (Table 3). When the microbiological method was used (data not shown), a higher percentage (70.4 ± 9.0 [SD]) of microbiologically active compound was detected in the urine. Results of HPLC tracings strongly suggested that two metabolites were present that could not be identified or characterized. No pure substance was available. No possible metabolites were detected in serum by the HPLC conditions used in this study. The 48-to-72-h urine sample in five of the six volunteers contained levels of Ro 23-6240 that were in excess of 10 μg/ml, and in one volunteer the level was in excess of 4 μg/ml.

The hematological, biochemical, and urinalysis data showed no abnormalities. Five of the six volunteers complained of a mild frontal or parietal headache that started 1 to 2 h after drug administration and that lasted between 5 and 24 h; one of the five volunteers complained of mild nausea for 20 h after drug administration.

DISCUSSION

The pharmacokinetics of Ro 23-6240 appear to accord well with the limited information that is available (F. Weidekamm, K. Stoeckel, and D. Dell, International Symposium of New Quinolones, abstr. no. 83, 1986). Weidekamm et al. have shown that the serum half-life is 9 to 10 h and that the maximum concentration in serum is 4.5 μg/ml following a 400-mg oral dose, they recovered 56% of the administered dose in the urine as Ro 23-6240. Studies in dogs and humans have demonstrated at least two metabolites: the N-oxide (Ro 19-7513) and the N-demethyl (Ro 19-7728) derivatives of Ro 23-6240. These account for about 10 to 15% of the administered dose. While the N-oxide derivative is devoid of antimicrobial activity, the N-demethyl derivative is comparable to Ro 23-6240 in its activity against many bacteria (I. Lenox-Smith, personal communication). This would assist in explaining the difference that we found between the amount of drug recovered from urine at 72 h when measured by HPLC (mean, 58.6%) and by a microbiological assay (mean, 70.4%). Because of the lack of known-potency metabolites, we were not able to confirm these observations in the laboratory.

When given by mouth, Ro 23-6240 was rapidly absorbed in five of the volunteers, giving a peak level in serum by 0.75 h. This property is shared by many other quinolones (1, 8, 10). The $t_{1/2}$ of Ro 23-6240 (mean, 11.95 h) was, however, somewhat longer than that of orally administered cirpofloxacin (mean, 3.9 h) (1), enoxacin (mean, 6.2 h) (10), ofloxacin (mean, 10.7 h) (5), or pefloxacin (mean, 10.1 h) (7). Ro 23-6240 penetrated the inflammatory exudate slightly more slowly than ciprofloxacin, with a mean T_{max} of the former at 4.0 h and of the latter at 2.6 h (1). This penetration was comparable to those of ofloxacin (mean T_{max}, 5.3 h) (5) and enoxacin (mean T_{max}, 3.3 h) (10). The mean percent inflammatory fluid penetration of Ro 23-6240 (as measured by the ratio of $AUC_{0-\infty}$s of 89.7%) was lower than those of enoxacin (113%) (10), ciprofloxacin (116%) (1), and ofloxacin (125%) (5).

Results of studies on antibacterial activity of Ro 23-6240 (6) show that the majority of the members of the family *Enterobacteriaceae* are highly susceptible to the compound, with an MIC for 90% of strains (MIC_{90}) of 5 μg/ml, as are *P. aeruginosa* (MIC_{90}, 2 μg/ml), *Staphylococcus aureus* (MIC_{90}, 0.5 μg/ml), and *H. influenzae* and *Neisseria gonorrhoeae* (MIC_{90}, ≤0.5 μg/ml). The data from our study therefore suggest that 400 mg of Ro 23-6240 administered orally once or twice a day might be sufficient to treat infections caused by such pathogens. *Streptococcus pneumoniae* (MIC_{90}, 8 μg/ml) and *Bacteroides fragilis* (MIC_{90}, 4 μg/ml) might respond to a higher dose. As the levels of Ro 23-6240 in urine at 48 to 72 h after a single dose were in excess of the MIC_{90} for common urinary tract pathogens, the possibility of once daily or less frequent treatment of uncomplicated urinary tract infections is worthy of investigation.

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LITERATURE CITED

1. **Crump, B., R. Wise, and J. Dent.** 1983. Pharmacokinetics and tissue penetration of ciprofloxacin. Antimicrob. Agents Chemother. **24**:784–786.
2. **Gladtke, E., and H. H. Hattinberg.** 1979. Pharmacokinetics—an introduction, p. 58–59 Springer-Verlag, Berlin.
3. **Greenblatt, D. J., and J. Koch-Weser.** 1975. Clinical pharmacokinetics. N. Engl. J. Med. *297*: 702–705.
4. **Hirai, K., H. Aoyama, M. Hosaka, Y. Oomori, Y. Niwata, S. Suzue, and T. Irikurah.** 1986. In vitro and in vivo antibacterial activity of AM 833, a new quinolone derivative. Antimicrob. Agents Chemother. **29**:1059–1066.
5. **Lockley, M. R., R. Wise, and J. Dent.** 1984. The pharmacokinetics and tissue penetration of ofloxacin. J. Antimicrob. Chemother. **14**:647–652.
6. **Manek, N., J. M. Andrews, and R. Wise.** 1986. In vitro activity of Ro 23-6240, a new difluoroquinolone derivative, compared with that of other antimicrobial agents. Antimicrob. Agents Chemother. **30**:330–332.
7. **Montay, G., Y. Goueffaur, and F. Roquet.** 1984. Absorption, distribution, metabolic fate, and elimination of pefloxacin mesylate in mice, rats, dogs, monkeys and humans. Antimicrob. Agents. Chemother. **25**:463–472.
8. **Swanson, B. N., V. K. Boppana, P. H. Vlasses, H. H. Rotmensch, and R. K. Ferguson.** 1983. Norfloxacin disposition after sequentially increasing oral doses. Antimicrob. Agents Chemother. **23**:284–288.
9. **Wise, R., A. P. Gillett, B. Cadge, S. R. Durham, and S. Baker.** 1980. The influence of protein binding upon tissue levels of six β-lactam antibiotics. J. Infect. Dis. **142**:77–82.
10. **Wise, R., M. R. Lockley, J. Dent, and M. Webberly.** 1984. Pharmacokinetics and tissue penetration of enoxacin. Antimicrob. Agents Chemother. **26**:17–19.