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Short Communication

High-performance liquid chromatography and preliminary pharmacokinetics of rufloxacin and its metabolites, N-desmethylrufloxacin and rufloxacinsulfoxide, in urine of Rhesus monkey *Macaca mulatta*

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ABSTRACT

A gradient high-performance liquid chromatographic method for the quantification of rufloxacin and two of its metabolites in urine, N-desmethylrufloxacin and rufloxacinsulfoxide, has been developed and validated. Monkey urine samples were diluted ten times with distilled water and 20 μl were injected onto a Cp Spher 5-ODS column, 5 μm particle size. The mobile phase was a mixture of 4% acetonitrile and 96% buffer at time 0, which changed linearly over 37 min to 26% acetonitrile and 74% buffer. Detection was achieved at 246 nm. The limit of detection of the three compounds was 0.50 μg/ml. An example of a pharmacokinetic study of rufloxacin and its metabolites in monkeys is shown.

INTRODUCTION

Rufloxacin is a new long-acting, once-daily quinolone antibacterial. Its chemical structure is comparable to that of ofloxacin [1]. The drug is highly active *in vitro* against a broad spectrum of Gram-negative and Gram-positive organisms, including those resistant to β-lactam antibiotics

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[2,3]. In animals (rats, dogs and monkey), the drug is absorbed well after oral administration, with an absolute bioavailability of about 60%, is distributed extensively in tissues with high tissue/plasma ratios, has a long half-life of 12–24 h, and about 30–40% is excreted in the urine [4]. In man the half-life is approximately 35 h, 25% is excreted unchanged in the urine, the renal clearance is 20 ml/min and the protein binding approximately 70%. The amount of drug excreted, according to microbiological determination, is 10–20% higher than according to high-performance liquid chromatography (HPLC) data [5]. This means that active metabolites of rufloxacin must be present in the urine.

Possible metabolites are, in analogy with pefloxacin and ofloxacin, N-desmethylrufloxacin, rufloxacin-N-oxide, 3-oxorufloxacin, rufloxacinsulfoxide, N-desmethylrufloxacinsulfoxide and 3-oxorufloxacinsulfoxide (Fig. 1). Reports on the HPLC analysis of rufloxacin do not include analysis of the possible metabolites [6].

The aim of the present investigation is to develop an HPLC analysis for quantitation of the metabolic products in the urine of the Rhesus monkey *Macaca mulatta*.

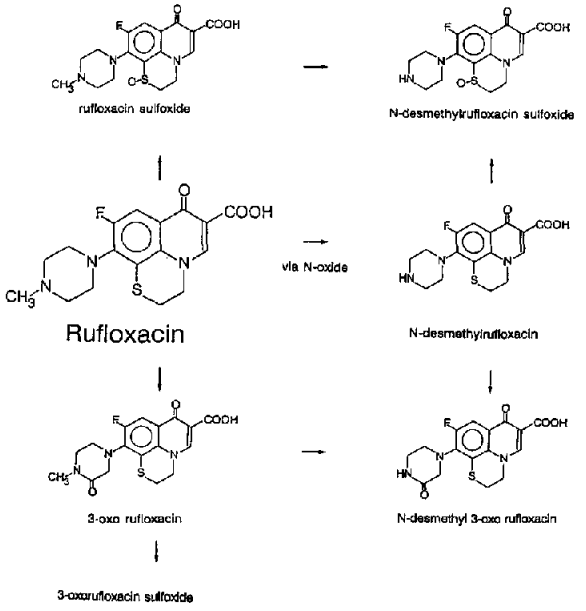

Fig. 1. Structures and possible metabolic pathways of rufloxacin.

EXPERIMENTAL

Drugs

Pure rufloxacin and the metabolites N-desmethylrufloxacin and rufloxacinsulfoxide were obtained from Mediolanum Farmaceutici (Milan, Italy).

Chemicals

The following chemicals were used: HPLC-grade acetonitrile (FSA Laboratory Supplies, Loughborough, UK), analytical-grade orthophosphoric acid, 99% (Merck, Darmstadt, Germany) and analytical-grade diethylamine (Merck-Schuchardt, Darmstadt, Germany).

HPLC analysis

The HPLC system consisted of a Spectra Physics SP 8775 autosampler, a Spectra Physics SP 8800 ternary HPLC pump (Eindhoven, Netherlands), a Kratos Spectroflow 757 UV detector (Applied Biosystems, Maarssen, Netherlands) and a Spectra Physics SP 4290 integrator. The column was CP Spher 5-ODS, 5 μm particle size, 250 mm × 4.6 mm I.D. (Chrompack, Middelburg, Netherlands) with a guard column of 75 mm × 2.1 mm I.D., packed with pellicular reversed phase, 10 μm particle size, (Chrompack) and operated at room temperature. The mobile phase was a mixture of acetonitrile and buffer (6.75 ml of 89% orthophosphoric acid and 2 ml of diethylamine adjusted with distilled water to 1000 ml; pH 2.1). At time (t) = 0, the mobile phase consisted of 4% acetonitrile and 96% buffer. During the following 37 min the mobile phase changed linearly until it was 26% acetonitrile and 74% buffer. At 37 min (t = 37) the mobile phase was changed within 5 min to the initial composition. The flow-rate was 1.5 ml/min. UV detection was achieved at 246 nm.

The capacity factors were: rufloxacinsulfoxide, 6.82; N-desmethylrufloxacin, 8.93; and rufloxacin, 11.91.

Sample preparation

Urine samples were diluted ten times with distilled water and 20 μl were injected onto the column.

Deconjugation

Deglucuronidation was carried out with 100 μl of urine, 100 μl of glucuronidase [50 000 U/ml β-glucuronidase, type LII (lyophilized powder from limpets *Patella vulgata*, Sigma, St. Louis, MO, USA, Cat. No G 8132)] and 800 μl of potassium dihydrogenphosphate buffer, pH 3.8, for 16 h at 37°C.

Concentration

The concentrations of rufloxacin and its N-desmethyl and sulfoxide metabolites were measured using calibration curves where peak heights of the compounds were expressed *versus* spiked concentrations in urine.

Calibration curves

These were constructed by adding a variable quantity of stock solution to blank monkey urine. The correlation coefficient was 0.9999 for rufloxacin, 0.9998 for N-desmethylrufloxacin and 0.9997 for rufloxacinsulfoxide.

Detection limit

The limit of detection of rufloxacin, N-desmethylrufloxacin and rufloxacinsulfoxide was 0.50 μg/ml at a signal-to-noise ratio of 3.

Validation

The intra-day variability and inter-day variability are given in Table I.

Animal study

Two female Rhesus monkeys (*Macaca Mulatta*, Nos. 10 and 99, and weighing 13 and 14 kg, respectively) were obtained from the Central Animal Laboratory of the University of Nijmegen. The monkeys were placed separately in a metabolic cage and had free access to food and water. Two intravenous doses of 333 mg of rufloxacin were administered to the monkeys.

Sampling procedures

Urine was collected three times a day over the periods 8–12 a.m., 0–6 p.m. and overnight, 6 p.m. to 8 a.m. The total time of sample collection was 200 h (seven times the expected half-life of 30–40 h). Urinary pH was measured immediately after collection. Measurements were made with a radiometer (Copenhagen PMH61) instrument. Urine samples of 5 ml were stored at −20°C until analysis.

TABLE I

INTRA-DAY AND INTER-DAY VARIATION

Concentration (μg/ml)	Coefficient of variation (%)	
	Inter-day	Intra-day
Rufloxacin		
60	1.10	1.09
45	3.14	1.12
4	8.92	1.50
3	2.81	1.12
N-Desmethylrufloxacin		
500	0.56	0.06
380	1.18	0.14
13	2.49	0.81
4	3.99	2.64
Rufloxacinsulfoxide		
6.5	3.30	1.60
5.8	4.11	0.30
2.6	5.73	3.84

RESULTS AND DISCUSSION

Fig. 2 shows an HPLC profile of a urine sample of a Rhesus monkey after two intravenous administrations of 333 mg of rufloxacin. Rufloxacin, and its metabolites N-desmethylrufloxacin and refloxacinsulfoxide, are present in the urine. No other metabolites could be distinguished in the chromatogram (compounds with increasing and decreasing concentrations during the experimental time). Rufloxacin and its metabolites are well separated from each other in this linear-gradient HPLC system. Fig. 3 shows the renal excretion rate–time profile of rufloxacin and its two metabolites N-desmethylrufloxacin and rufloxacinsulfoxide in a Rhesus monkey (No. 10) after two intravenous administrations of 333 mg of rufloxacin. Fig. 3 shows that rufloxacin is quickly N-demethylated into N-desmethylrufloxacin (67.1%), and only slowly oxidized at the sulfur atom into rufloxacinsulfoxide (1.3%).

Fig. 4 shows the renal excretion rate–time profile of rufloxacin and its two metabolites N-des-

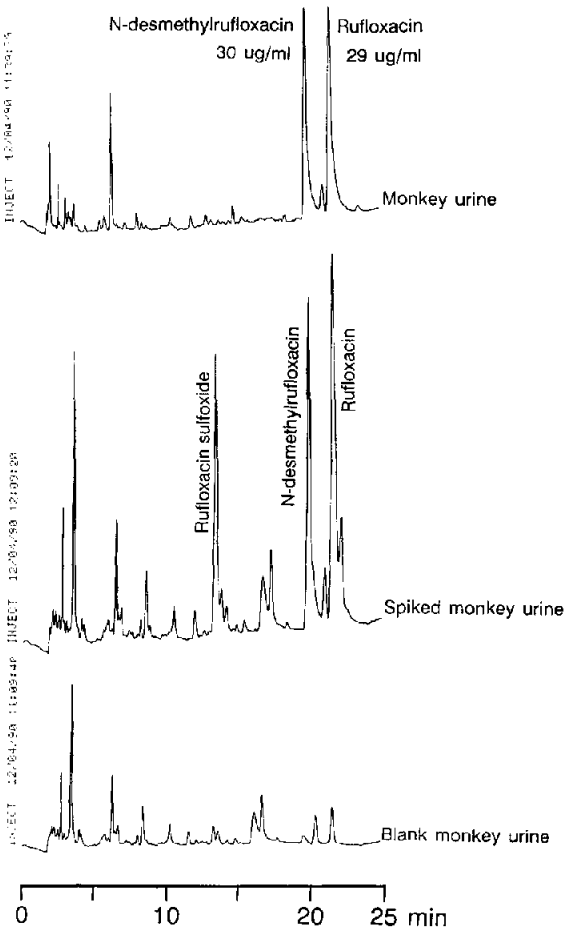

Fig. 2. HPLC of a monkey urine sample after an intravenous dose of 333 mg of rufloxacin.

methylrufloxacin and rufloxacinsulfoxide in another Rhesus monkey (No. 99) after the intravenous administration of 333 mg of rufloxacin. Rufloxacin is in this monkey slowly N-demethylated into N-desmethylrufloxacin (12.3%) and slowly oxidized at the sulfur atom into rufloxacinsulfoxide (0.8%).

Monkey No. 99 had an extremely low urine flow and was almost anuric. In both monkeys the amount of rufloxacinsulfoxide in urine was just above the detection limit and total urinary recovery was approximately 1% of the administered dose. The main metabolite was N-desmethylrufloxacin.

This method enables the HPLC analysis of rufloxacin and two of its possible metabolites in the urine of monkey and other animals and can be used for the investigation of species differences in

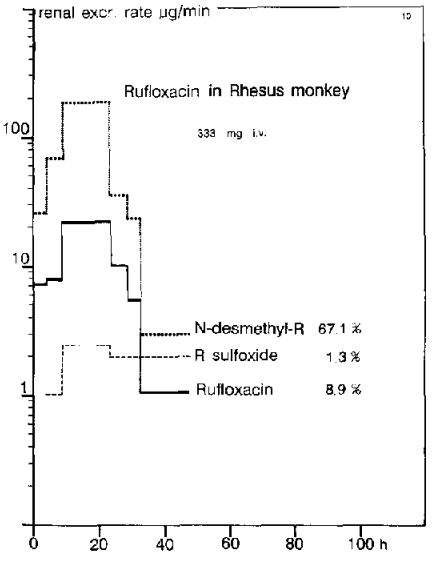

Fig. 3. Renal excretion rate time profile of rufloxacin and its metabolites N-desmethylrufloxacin and rufloxacinsulfoxide in a Rhesus monkey (No. 10) after two intravenous doses of 333 mg of rufloxacin.

the metabolism of rufloxacin. The difference in concentration of the parent drug obtained by the microbiological assay [5] and by HPLC [5] can be explained by the occurrence of rufloxacin metabolites.

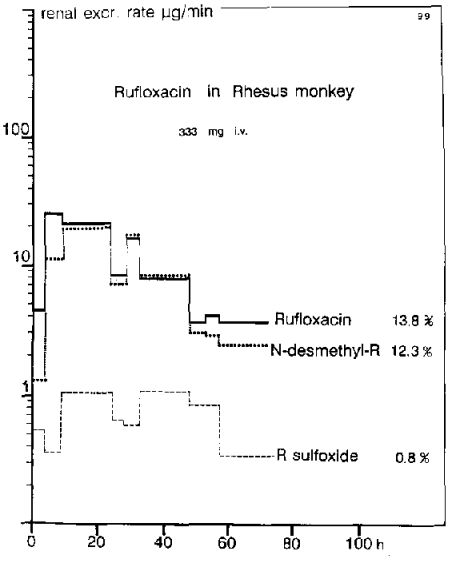

Fig. 4. Renal excretion rate-time profile of rufloxacin and its metabolites N-desmethylrufloxacin and rufloxacinsulfoxide in a Rhesus monkey (No. 99) after two intravenous doses of 333 mg of rufloxacin.

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