Journal of Chromatography, 173 (1979) 155–163

CHROM. 11,694

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF ROSOXACIN AND ITS N-OXIDE METABOLITE IN PLASMA AND URINE

M. P. KULLBERG, R. KOSS, S. O'NEIL and J. EDELSON

Department of Drug Metabolism and Disposition, Sterling-Winthrop Research Institute, Rensselaer, N.Y. 12144 (U.S.A.)

(Received December 28th, 1978)

SUMMARY

A high-pressure liquid chromatographic method for the analysis of rosoxacin and its pyridyl N-oxide metabolite in plasma and urine extracts is described. A statistical evaluation of the assay data has shown acceptable accuracy and precision for 0.5 to 25 μg of rosoxacin or the metabolite per ml of plasma and for 2.5 to 60 μg/ml of either compound in urine. The minimum quantifiable level for rosoxacin was 0.13 μg/ml in plasma and 0.64 μg/ml in urine; for the metabolite in plasma and urine, the corresponding values were 0.21 and 0.60 μg/ml, respectively. The method was applied to plasma and urine from three dogs medicated orally with 5 mg/kg of rosoxacin. The pharmacokinetic parameters calculated for rosoxacin were: plasma half-life, 1.9 h; plasma clearance, 65 ml/min; volume of distribution, 11.3 l. The average total urinary excretion of rosoxacin as free and conjugated rosoxacin and its free N-oxide was 7.7 ± 0.2% over the 48-h collection period.

INTRODUCTION

Rosoxacin, 1-ethyl-1,4-dihydro-4-oxo-7-(4-pyridyl)-3-quinoline-carboxylic acid, is a member of a group of orally active quinolinone and naphthyridine antimicrobial agents intended for the treatment of bacterial infections. The analytical methodology and the metabolic fate of this class of antimicrobial compounds has recently been reviewed[1].

This report describes a high-performance liquid chromatographic (HPLC) method for the quantitation of both rosoxacin and its pyridyl N-oxide metabolite (I; 1-ethyl-1,4-dihydro-4-oxo-7-(4-pyridyl)-3-quinolinecarboxylic acid N-oxide) (see Fig. 1) in plasma and urine. The assay was used to determine rosoxacin and I in plasma and urine of dogs that had received 5 mg/kg of rosoxacin orally.

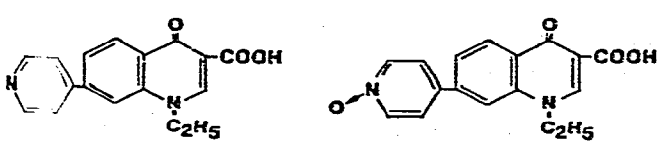

Fig. 1. Structural formulae for rosoxacin (left) and its N-oxide (I; right).

EXPERIMENTAL

Materials

Rosoxacin, its metabolite (I) and the internal standard, 7-(2,6-dimethyl-4-pyridyl)-1-ethyl-1,4-dihydro-4-oxo-3-quinoline-carboxylic acid N-oxide were synthesized at the Sterling-Winthrop Research Institute. The HPLC column was purchased from Whatman (Clifton, N.J., U.S.A.). The acetonitrile was redistilled in all-glass apparatus; other chemicals were obtained commercially (reagent grade) and were used without further purification.

Preparation of samples

Serial dilutions of rosoxacin and I were prepared in 0.05 *M* sodium hydroxide. Appropriate aliquots (*ca.* 200 μl) of the stock solutions were pipetted into 1 ml of control plasma or urine from the appropriate species to produce standards in the biological media. Plasma and urine standards were prepared in duplicate to yield plasma concentrations of 0 and 0.5 to 25 μg/ml and urine concentrations of 0 and 2.5 to 60 μg/ml for both rosoxacin and I.

Two sets of randomized and coded plasma and urine samples, to be analyzed under single-blind conditions, were prepared as described above. Each set of plasma samples consisted of six triplicates at final concentrations of 0, 0.52, 2.4, 8.6, 13 and 21.5 μg/ml for rosoxacin and 0, 0.55, 2.8, 6.1, 12.0 and 23.0 μg/ml for I. Each set of urine samples contained six triplicates at final concentrations of 0, 2.8, 15, 22, 31.5, and 55.2 μg/ml for rosoxacin and 0, 2.4, 13.5, 22, 38 and 50.4 μg/ml for I. The coded samples were so prepared that high and low levels of either compound existed in a set of replicates. One set of samples was analyzed immediately and the other after storage at −4° for 7 days. Freshly prepared plasma or urine standards were extracted and analyzed concomitantly with each set of samples.

Plasma analysis

To 1.0 ml of plasma in a glass conical tube were added 100 μl of the internal standard solution (50 μg/ml in 0.05 *M* sodium hydroxide), 0.5 ml of 0.5 *M* citrate buffer (pH 5.0) and 5.0 ml of chloroform. The tube was shaken mechanically and centrifuged, and the upper (aqueous) phase was discarded. A 3.0-ml aliquot of the chloroform phase was pipetted into a clean conical tube, the chloroform was evaporated to dryness at 40° with the aid of a stream of nitrogen, the residue was dissolved in 250 μl of methanol, and 25 μl of this solution were injected into the HPLC system described below.

Urine analysis

To 1.0 ml of urine in a glass tube were added 100 μl of the internal standard solution (120 μg/ml in 0.05 *M* sodium hydroxide), 1.0 ml of 0.5 *M* citrate buffer (pH 5) and 12 ml of chloroform. The mixture was shaken for 10 min and centrifuged, and 10 ml of the organic phase were pipetted into a clean glass tube. Then 3.0 ml of 0.05 *M* sodium hydroxide were added, the tube was shaken and centrifuged, and to 2.0 ml of the aqueous phase in another glass conical tube were added 1 ml of 0.5 *M* citrate buffer (pH 5) and 5.0 ml of chloroform. The tube was shaken for 10 min and centrifuged, 3.0 ml of the organic phase was pipetted into a conical tube, and the

solvent was evaporated to dryness at 40° with the aid of a stream of nitrogen. The residue was dissolved in 250 μl of methanol, and 25 μl of this solution was analyzed by HPLC as described below.

Hydrolysis of urine samples

Urine (1 ml) was acidified with 1.0 ml of 0.1 *M* hydrochloric acid and placed in a boiling-water bath for 30 min. After cooling to room temperature, the mixture was neutralized with 0.1 ml of 1 *M* sodium hydroxide, and 1.0 ml of 0.5 *M* citrate buffer (pH 5.0) was added. The solution was then extracted as described above.

HPLC system

Pump: LDC Mini-pump®, Model 709. Column: Whatman Partisil-PXS 10/25 PAC, 250 × 4.6 mm I.D., with a Corasil pre-column, 60 × 4 mm I.D. Detector: Altex Model 153 ultraviolet detector with a 280-nm filter. Mobile phase: Redistilled acetonitrile–0.2 *M* phosphoric acid (92:8, v/v); flow-rate 2 ml/min (1500 p.s.i.). Retention times: I, 5.4 min; internal standard, 7.5 min; rosoxacin, 12.0 min. Temperature: 20°.

Animal study

Three fasted female beagle hounds *ca.* 6 months old and weighing 7–8 kg were suspended in slings, which allowed them to remain standing, but restrained. Foley catheters were inserted into the bladders of two of the dogs; the third animal was not catheterized. The animals received a 5-mg/kg dose of rosoxacin contained in a capsule, which was administered orally, followed by 50 ml of water.

Blood samples were collected (for periods up to 10 h) through a B-D 21-gauge Longdwel catheter-needle with a Safedwel obturator. The catheter was inserted into the saphenous vein of the right hind leg. After the 10-h samples had been taken, the dogs were placed in metabolism cages. Thereafter, blood was sampled by venipuncture of the left cephalic vein, and urine was collected in pans placed under the cages.

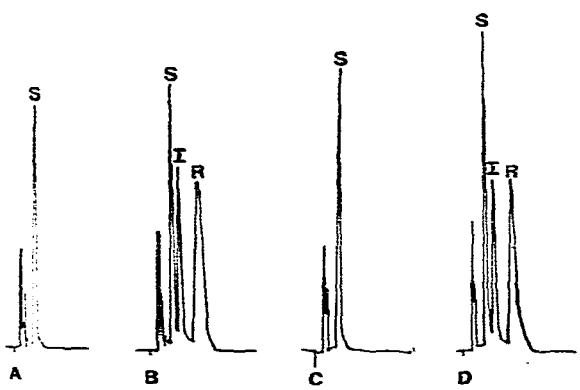

Fig. 2. Chromatograms of: A, processed human plasma containing only the internal standard (S); B, the same sample containing 5 μg each of rosoxacin (R) and its N-oxide (I); C, processed human urine containing only the internal standard (S); D, the same sample containing 10 μg each of rosoxacin (R) and its N-oxide (I).

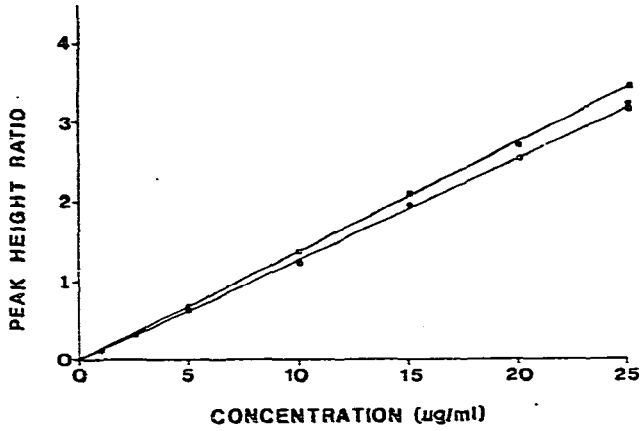

Fig. 3. Extracted standards of control human plasma augmented with rosoxacin (●) and I (■).

TABLE I

SUMMARY OF DATA FOR ROSOXACIN IN HUMAN PLASMA

Concentration level (µg/ml)		*Assayed concentration (µg/ml)* *	*Assayed concentration (µg/ml)* **
0	Mean ($n = 3$)	< MQL***	< MQL
	S.E. (%)§	—	—
	Mean % Diff.§§	—	—
0.52	Mean ($n = 3$)	0.56	0.52
	S.E. (%)	0.59	1.71
	Mean % Diff.	+8.33	−0.64
2.4	Mean ($n = 3$)	2.35	2.32
	S.E. (%)	1.23	0.72
	Mean % Diff.	−2.08	−3.47
8.6	Mean ($n = 3$)	8.37	8.27
	S.E. (%)	0.80	0.40
	Mean % Diff.	−2.71	−3.88
13.0	Mean ($n = 3$)	13.07	12.77
	S.E. (%)	0.67	0.67
	Mean % Diff.	+0.51	−1.79
21.5	Mean ($n = 3$)	20.87	20.73
	S.E. (%)	0.42	0.80
	Mean % Diff.	−2.95	−3.57

* Assayed following sample preparation.

** Samples frozen at −4° for 1 week before analysis.

*** MQL = 0.13 µg/ml.

§ $\text{S.E. (\%)} = \dfrac{\text{S.E.M.}}{\text{Mean}} \times 100.$

§§ $\text{Mean \% Diff.} = \left(\dfrac{\text{Assayed mean}}{\text{Concn. level}} - 1\right) \times 100.$

Blood samples were drawn at intervals up to 28 h after medication. The blood was immediately transferred to a 5-ml tube containing 12.5 mg of potassium oxalate as anticoagulant. The tube contents were mixed gently and centrifuged for 15 min, then the separated plasma was pipetted into a clean glass tube and stored at −4° until analyzed.

Urine was collected at 0, 0–2, 2–4, 4–6, 6–8 and 8–10 h with the catheter, and at 10–24 and 24–48 h after medication in the pans. For the non-catheterized dog, urine samples were obtained at 0, 0–93 min, 93 min–10 h, 10–24 and 24–48 h after medication.

RESULTS AND DISCUSSION

Fig. 2 depicts representative chromatograms derived from extracted control plasma and urine and from plasma and urine samples containing rosoxacin and I. Fig. 3 shows a typical plot of the peak-height ratio of rosoxacin and I to the internal standard *versus* the concentration of rosoxacin or I added to plasma. A regression analysis of the peak-height ratio of the cited compounds to the internal standard *versus* the concentration added to plasma and urine showed that this relationship was linear for both compounds.

Table I summarizes plasma analysis data for rosoxacin. The accuracy, as

TABLE II

SUMMARY OF DATA FOR I IN HUMAN PLASMA

Concentration level (μg/ml)		*Assayed concentration (μg/ml)* *	*Assayed concentration (μg/ml)* **
0	Mean (n = 3)	< MQL***	< MQL
	S.E. (%)§	—	—
	Mean % Diff.§§	—	—
0.55	Mean (n = 3)	0.54	0.57
	S.E. (%)	1.85	1.54
	Mean % Diff.	−1.82	+4.24
2.8	Mean (n = 3)	2.7	2.63
	S.E. (%)	0.00	1.27
	Mean % Diff.	−3.57	−5.95
6.1	Mean (n = 3)	5.93	5.80
	S.E. (%)	1.12	1.00
	Mean % Diff.	−2.73	−4.92
12.0	Mean (n = 3)	11.93	11.5
	S.E. (%)	0.28	1.00
	Mean % Diff.	−0.56	−4.17
23.0	Mean (n = 3)	22.63	22.0
	S.E. (%)	0.74	0.26
	Mean % Diff.	−1.59	−3.48

*,** See corresponding footnotes to Table I.

*** MQL = 0.21 μg/ml.

§,§§ See corresponding footnotes to Table I.

defined by the mean difference (%) from the expected value, ranged from 3.9% low to 8.3% high. The minimum quantifiable level (MQL) was determined by inverse prediction[2] as that concentration whose lower 80% confidence limit just encompassed zero[3]. The mean MQL for the two experimental runs was 0.13 μg/ml. The estimate of assay variance (defining precision) was based on the failure of the concentration levels to be the same over the two experimental runs. The standard deviation of the rosoxacin assay was ± 2.7%.

Table II summarizes plasma analysis data for I. The accuracy ranged from 5.9% low to 4.2% high, and the estimated precision of the assay was ± 3.1%. The average MQL for the two runs was 0.21 ± 0.05 μg/ml.

Tables III and IV summarize urine analysis data for rosoxacin and I, respectively. The accuracy of the rosoxacin assay ranged from 3.1% low to 8.0% high, and that of the assay for I from 5.2% low to 8.5% high. The average MQL was 0.67 ± 0.03 μg/ml for rosoxacin and 0.60 ± 0.00 μg/ml for I. The estimated precision of the assay was ± 1.6% for rosoxacin and ± 2.7% for I.

The data from the analysis of the plasma and urine from the three dogs are presented in Tables V and VI, respectively. Following oral administration of rosoxacin, the maximum plasma concentration in the three dogs ranged from 1.9 to

TABLE III

SUMMARY OF DATA FOR ROSOXACIN IN HUMAN URINE

Concentration level (μg/ml)		*Assayed concentration (μg/ml)* *	*Assayed concentration (μg/ml)* **
0	Mean ($n = 3$)	< MQL***	< MQL§
	S.E. (%)§§	—	—
	Mean % Diff.§§§	—	—
2.8	Mean ($n = 3$)	3.02	3.02
	S.E. (%)	0.69	1.27
	Mean % Diff.	+7.86	+7.98
15.0	Mean ($n = 3$)	14.95	14.73
	S.E. (%)	0.22	0.82
	Mean % Diff.	−0.44	−1.78
22.0	Mean ($n = 3$)	22.2	21.9
	S.E. (%)	0.00	1.05
	Mean % Diff.	+0.91	−0.45
31.5	Mean ($n = 3$)	31.57	31.23
	S.E. (%)	0.28	0.43
	Mean % Diff.	+0.21	−0.85
55.2	Mean ($n = 3$)	56.0	53.5
	S.E. (%)	0.21	1.13
	Mean % Diff.	+1.45	−3.08

*, ** See corresponding footnotes to Table I.

*** MQL = 0.7 μg/ml.

§ MQL = 0.64 μg/ml.

§§, §§§ See footnotes § and §§, respectively, to Table I.

4.6 μg/ml and occurred between 1 and 2 h after administration. Based on linear regression of the post-absorption phase of the plasma *versus* time data, the average elimination-rate constant was 0.36 ± 0.02 h^{-1} and the average first-order elimination half-life ($t_{1/2}$) was 2.0 ± 0.1 h. The average plasma clearance calculated from area under the curve divided by dose was 65 ± 3 ml/min, and the average volume of distribution calculated from clearance divided by rate constant was 11.3 ± 1 l (Table VII).

TABLE IV

SUMMARY OF DATA FOR I IN HUMAN URINE

Concentration level (μg/ml)		*Assayed concentration (μg/ml)**	*Assayed concentration (μg/ml)***
0	Mean (n = 3)	< MQL***	< MQL
	S.E. (%)‡	—	—
	Mean % Diff.‡‡	—	—
2.40	Mean (n = 3)	2.54	2.60
	S.E. (%)	1.12	1.36
	Mean % Diff.	+5.69	+8.47
13.5	Mean (n = 3)	13.17	12.93
	S.E. (%)	0.51	0.26
	Mean % Diff.	−2.47	−4.20
22.0	Mean (n = 3)	22.2	20.87
	S.E. (%)	0.52	2.24
	Mean % Diff.	+0.91	−5.15
38.0	Mean (n = 3)	37.77	36.83
	S.E. (%)	1.16	0.86
	Mean % Diff.	−0.61	−3.07
50.4	Mean (n = 3)	50.17	48.73
	S.E. (%)	0.48	0.72
	Mean % Diff.	−0.46	−3.31

*,** See corresponding footnotes to Table I.

*** MQL = 0.6 μg/ml.

‡,‡‡ See corresponding footnotes to Table I.

In Table VI, urinary excretion data are presented. Unconjugated rosoxacin, which accounted for less than 0.5% of the dose, was detected in the urine of the two catheterized dogs up to 8 h after administration. Conjugated rosoxacin was detected for up to 10 h and accounted for 3.4% and 4.4% of the dose in the two catheterized dogs. Free I was excreted over the 24-h collection period and accounted for 4.2% and 2.9% of dose in the two dogs. Since there was no increase in the concentration of I in acid-hydrolyzed urine samples, there was probably no conjugated I in the dog urine. For the three dogs, the average total (± S.E.M.) urinary excretion was 7.7 ± 0.[illegible]% of the dose during 48 h.

TABLE V

PLASMA CONCENTRATION (μg/ml) OF ROSOXACIN IN BEAGLES FOLLOWING ORAL ADMINISTRATION OF THE DRUG AT 5 mg/kg

Time after administration (h)	*Dog QCQ*	*Dog THQ*	*Dog VLK*
0	< MQL*	< MQL	< MQL
0.25	1.20	0.60	< MQL
0.50	2.78	1.42	1.00
0.75	4.00	1.58	1.60
1.0	4.66	2.32	1.94
1.5	3.90	2.80	2.50
2.0	3.64	2.60	2.68
3.0	2.26	1.68	1.74
4.0	1.50	1.16	1.10
5.0	0.50	0.92	0.72
6.0	< MQL	0.68	0.54
8.0	< MQL	< MQL	< MQL
10.0	< MQL	< MQL	< MQL
24.0	< MQL	< MQL	< MQL
48.0	< MQL	< MQL	< MQL

* MQL = 0.12 μg/ml.

TABLE VI

URINARY EXCRETION OF FREE AND CONJUGATED ROSOXACIN AND FREE I FOLLOWING DOSAGE AT 5 mg/kg

Immediately after administration (0 h), and from 24–28 h after administration, the amounts of free and conjugated rosoxacin and free I were < MQL for all dogs.

Time after administration (h)	*Excretion (mg)*			
	*Free rosoxacin**	*Free I*	*Conjugated rosoxacin***	*Total*
Dog QCQ				
0–2	0.081	0.253	0.457	0.791
2–4	0.071	0.268	0.338	0.677
4–6	< MQL	0.397	0.360	0.757
6–8	0.034	0.251	0.122	0.407
8–10	< MQL	0.148	0.072	0.220
10–24	< MQL	0.341	< MQL	0.341
Percentage of dose	0.5	4.2	3.4	8.1
Dog THQ				
0–2	0.049	0.203	0.503	0.755
2–4	0.034	0.259	0.572	0.865
4–6	< MQL	0.216	0.290	0.506
6–8	< MQL	0.171	0.188	0.259
8–10	< MQL	0.116	0.120	0.236
10–24	< MQL	0.125	< MQL	0.125
Percentage of dose	0.2	2.9	4.4	7.5
Dog VLK (uncatheterized)				
0–1.55	< MQL	0.082	0.109	0.191
1.55–10	0.399	0.966	0.808	2.173
10–24	< MQL	0.354	< MQL	0.254
Percentage of dose	1.1	3.9	2.4	7.4

* Free extractable drug.

** Conjugated drug (difference between hydrolyzable and free).

TABLE VII

PHARMACOKINETIC PARAMETERS OF ROSOXACIN

Dog	*Weight of dog (kg)*	*Elimination rate constant (h^{-1})*	*Half-life (h)*	*Clearance (ml/min)*	*Volume of distribution (l)*
QCQ	8.0	0.38	1.9	59	9.3
THQ	7.6	0.33	2.1	69	12.7
VLK	7.2	0.38	1.9	67	10.6
	Mean ± S.E.M.	0.36 ± 0.002	2.0 ± 0.1	65 ± 3	11.3 ± 1

REFERENCES

1 J. Edelson, C. Davison and D. P. Benziger, *Drug Metab. Rev.*, 6 (1977) 105.
2 R. R. Sokal and F. J. Rohlf, *Biometry*, W. H. Freeman and Co., San Francisco, 1969.
3 R. W. Ross and H. Stander, *Some Statistical Problems in Drug Metabolism*, paper presented at the *Princeton Conference on Applied Statistics, December, 1975.*