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DETERMINATION OF RANITIDINE AND ITS METABOLITES IN HUMAN URINE BY REVERSED-PHASE ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

P.F. CAREY, L.E. MARTIN* and P.E. OWEN

Biochemical Pharmacology Department, Glaxo Group Research Ltd., Ware, Hertfordshire SJ12 ODJ (Great Britain)

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SUMMARY

A method using ion-pair high-performance liquid chromatography is presented for determining ranitidine, ranitidine N-oxide, ranitidine S-oxide and desmethyl ranitidine in the urine from four volunteers, given on separate occasions an intravenous and oral dose of 100 mg ranitidine. This method has been used to study the metabolism and pharmacokinetics of ranitidine by man. It was found that the elimination half-life of ranitidine ranged from 110—246 min. The mean renal clearance of ranitidine in these four volunteers was 512 ml/min.

INTRODUCTION

Ranitidine hydrochloride is a new histamine H_2-receptor antagonist effective in the treatment of peptic ulcers [1]. Metabolic studies in rat and dog using [^{14}C] ranitidine showed that the ranitidine was mainly metabolised by oxidation to give metabolites I, II and III (Fig. 1). The relative amount of each metabolite formed was found to vary with the species [2]. Thin-layer chromatographic analysis of the urine from volunteers given oral and intravenous doses of ranitidine showed that ranitidine was the major component present, compound I was the major metabolite, and small quantities of compounds II and III were also present. As part of the clinical investigations on ranitidine it was necessary to develop a quantitative method for the determination of ranitidine and its metabolites in human urine. A two-step solvent extraction procedure which separates ranitidine from its metabolites has been described for the determination of ranitidine in plasma by high-performance liquid chromatography (HPLC) [3]. The reversed-phase HPLC system used [3] did not com-

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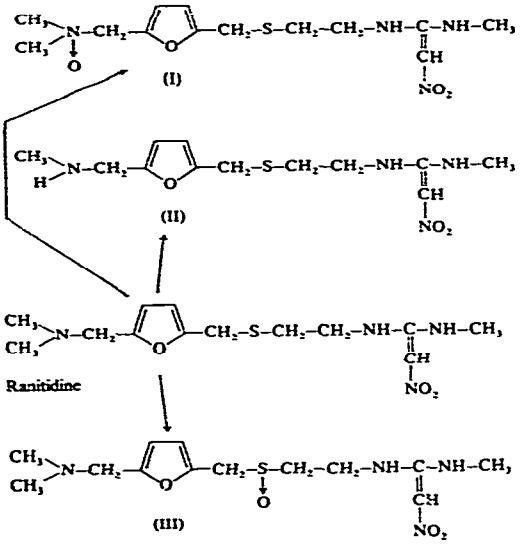

Fig. 1. Formulae of ranitidine and its three metabolites. Metabolite I (ranitidine N-oxide), Metabolite II (desmethyl ranitidine) and Metabolite III (ranitidine S-oxide).

pletely resolve ranitidine from metabolite II and could not be used for the quantitative determination of ranitidine in urine.

The possibility of using ion-pair HPLC for the determination of ranitidine and its metabolites in urine was investigated and an HPLC method using sodium lauryl sulphate as a counter-ion has been developed. This method has been used to study the pharmacokinetic renal clearance of ranitidine and the metabolism of the drug by man.

EXPERIMENTAL

Reagents and materials

Unless otherwise stated all reagents were of analytical grade. The sodium lauryl sulphate was obtained from Cambrian Chemicals (Croydon, Great Britain). Ranitidine hydrochloride, ranitidine N-oxide, (I), ranitidine S-oxide (III) and desmethyl ranitidine (II) hydrochloride were synthesised in the Chemistry Division of Glaxo Group Research Labs. (Ware, Great Britain).

Chromatography

A Spectra Physics 740B dual reciprocating pump (Spectra Physics, St. Albans, Great Britain) was used to deliver eluent to the WISP automatic sample injector (Waters Assoc., Northwich, Great Britain). Stainless steel columns (5 × 100 mm) from Shandon Southern Products (Runcorn, Great Britain) were packed with 5-μm particles of Spherisorb ODS (Phase Separations, Clwyd, Great Britain) and maintained at 45°C in a DuPont 860 air circulating oven [DuPont (U.K.), Hitchin, Great Britain].

The mobile phase was pumped at 1.0 ml/min, and a Pye LC3 variable-wavelength UV detector (Pye Unicam, Cambridge, Great Britain) was set at 320 nm and 0.01 a.u.f.s. to monitor the eluate. Chromatograms were recorded on a

Bryans 28000 pen recorder (Bryans Southern Instruments, Surrey, Great Britain), input 0—10 mV, chart speed 5 mm/min, and also on a Spectra Physics SP4050 printer plotter, ×5 attenuation, chart speed 0.25 cm/min. Peak areas were recorded and integrated using the Spectra Physics SP4000 Chromatography Data System. Data reduction was performed using a pre-programmed procedure resident in the Spectra Physics SP4100 computing integrator. A least squares fit to a quadratic equation was applied to the calibration data, and the coefficients of best fit were stored and used for the calculation of sample concentration.

Calibration and accuracy

Ranitidine hydrochloride (44.7 mg) was weighed and transferred with approximately 30 ml of control human urine into a 100-ml volumetric flask. Fresh aqueous solutions of metabolites I, II and III were prepared containing 1 mg of metabolites I and III and the equivalent of 1 mg base of metabolite II per ml and 2 ml of each of these solutions were added to the volumetric flask. The volume was made up to 100 ml with control human urine, and a series of standards covering the ranges 0—400 μg ranitidine per ml and 0—20 μg of metabolite per ml was prepared by dilution of this solution with an appropriate volume of control human urine. The range of standards was chosen to cover the concentration of ranitidine and its metabolites likely to be present in urine during 24 h after a dose of 100 mg ranitidine. The lowest standard for ranitidine corresponded to 0.8 μg/ml of urine. The accuracy and precision of the method was evaluated by analysing a sample of urine containing 80 μg ranitidine, 3.97 μg metabolite I, 4.01 μg metabolite II and 4.06 μg metabolite III per millilitre.

Human pharmacokinetic and metabolism studies

Doses of 100 mg ranitidine were administered to volunteers either intravenously or orally. Serial samples of blood and urine were collected during the 0—24 h period after giving the drug. The samples were frozen immediately after collection and stored at −20°C until analysed.

Urine analysis

Samples of urine were thawed out at room temperature and 0.25 ml of each was diluted to 2.5 ml with distilled water using a Fisons Model LFA diluter (Fisons Scientific Apparatus, Loughborough, Great Britain). A 15-μl aliquot of a sample was injected on to the chromatography column by means of a WISP automatic sample injector. The precision of the injector of the WISP was better than 1%. Therefore, the urine was analysed by direct injection and no internal standard was used. Standards were prepared containing 0—400 μg ranitidine and 0—20 μg of metabolites I, II and III per ml of urine. Volumes of 15 μl of these standards and the unknown urine samples were used for analysis by HPLC.

Serum analysis

The blood samples were allowed to clot, the serum separated and the ranitidine concentration determined by HPLC [3].

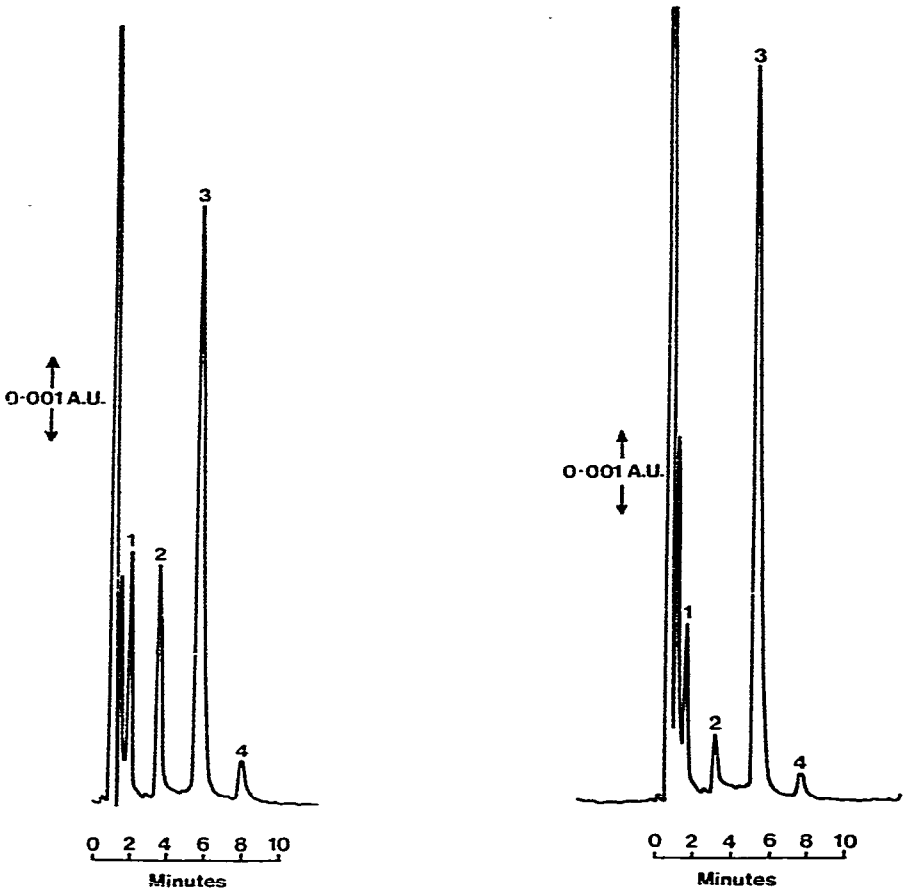

Fig. 2. Chromatogram of urine to which have been added ranitidine and Metabolites I, II and III. Peaks: 1, I 20.8 μg/ml; 2, III 20.4 μg/ml; 3, ranitidine 101 μg/ml; and 4, II 10.0 μg/ml.

Fig. 3. Chromatogram of a urine collected during 2—4 h after an intravenous injection of 100 mg ranitidine. Peaks as given in Fig. 2. The concentration of ranitidine, Metabolites I, II and III in this sample correspond respectively to 113, 11.7, 5.6 and 5.6 μg/ml.

TABLE I

ACCURACY AND PRECISION OF THE DETERMINATION OF RANITIDINE AND ITS METABOLITES BY ION-PAIR HPLC

These data are based on 8 injections of the same standard.

	Ranitidine	Metabolite I	Metabolite II	Metabolite III
Mean concentration (μg/ml)	80.89	3.95	3.96	4.12
S.D.	1.19	0.05	0.54	0.36
C.V.	1.47	1.28	13.54	8.47
Actual concentration (μg/ml)	80.00	3.97	4.01	4.06
Concentration determined expressed as % of standard	101.1	99.5	98.8	101.5

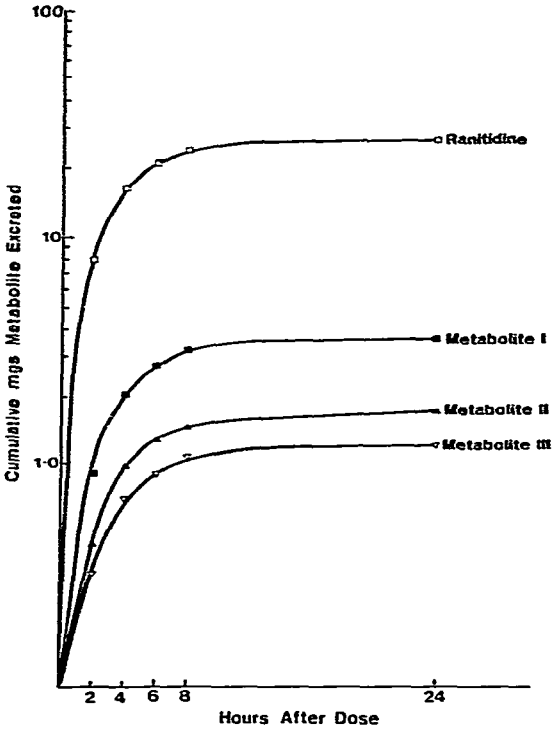

Fig. 4. Plot of mean cumulative excretion of ranitidine and its metabolites in urine from four volunteers given a single oral dose of 100 mg ranitidine.

Elimination half-life

The Sigma minus method [4] was used to calculate the elimination half-life. This is based on plotting on a log scale the amount of drug not yet excreted against the time at the end of each excretion interval in hours. The slope of the line is the elimination half-life in hours.

Renal clearance

The renal clearance values were obtained by plotting the urinary excretion rate against the plasma concentration at the mid-point time of each urine sample. The slope of the line represents the renal clearance [4].

RESULTS AND DISCUSSION

A series of methanol water systems containing 0.005 mol/l concentrations of either sodium sulphate, disodium hydrogen sulphate, sodium dihydrogen phosphate, sulphuric acid or phosphoric acid were investigated as mobile phases in the presence of 0.005 mol/l sodium lauryl sulphate. It was found that the mobile phase consisting of 600 ml methanol, 400 ml of distilled water containing 0.0005 mol/l sodium dihydrogen phosphate and 0.005 mol/l sodium lauryl gave the most efficient and the best chromatographic separation of ranitidine and its three metabolites. This mobile phase has been used over a long period with no evidence of column deterioration. The retention times of raniti-

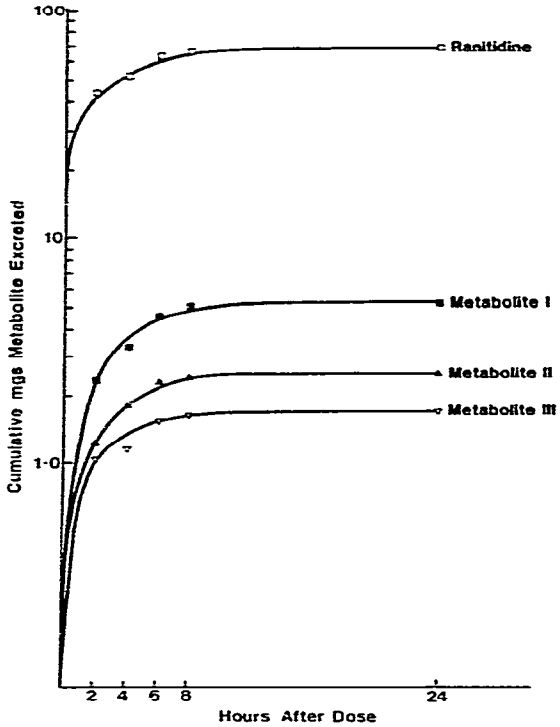

Fig. 5. Plot of mean cumulative excretion of ranitidine and its metabolites in urine from four volunteers given a single intravenous dose of 100 mg ranitidine.

dine and its metabolites in the system chosen were for (I), 1.9 min; (III), 3.9 min; ranitidine, 5.8 min and (II), 7.9 min.

A chromatogram of a urine standard containing 100 μg/ml ranitidine, 20 μg/ml of metabolites I and III, and 10 μg/ml of metabolite II is shown in Fig. 2. The relationships between the concentration of ranitidine, metabolites I, II and III and peak area were found to be linear over the range 0—400 μg/ml for ranitidine and 0—20 μg/ml for each of the three metabolites. The accuracies of determination of ranitidine and the three metabolites are similar. The reproducibility of the method was determined by analysing the results of eight replicate injections of a solution containing 80.89 μg ranitidine, 3.95 μg metabolite I, 3.96 μg metabolite II and 4.12 μg metabolite III per millilitre. Metabolite I which elutes first as a sharp peak was determined with an average coefficient of variation of 1.28%. Metabolite II which has the longest retention time, was determined with a coefficient of variation of 13.54% (Table I). Metabolite III which elutes with a retention time between that of metabolite I and ranitidine was determined with an average coefficient of variation of 8.47%.

Fig. 3. is a chromatogram of a urine sample collected during 2—4 h after an intravenous injection of 100 mg ranitidine. Ranitidine is the major component in the urine, I is the major metabolite and small amounts of II and III are present.

In Figs. 4 and 5 are shown the cumulative urinary excretion of ranitidine and

the three metabolites during 24 h after either an oral or intravenous dose of 100 mg ranitidine. The mean ± standard deviation of the cumulative 0—24 h urinary excretion of ranitidine after intravenous injection was 68.2 ± 7.9% compared with 26.6 ± 6.2% after an oral dose.

The percentage bioavailability, calculated as shown in eqn. 1, was 39% (range 30—51%).

$$\% \text{ bioavailability} = \frac{\text{Amount excreted in 0—24 h urine after 100-mg oral dose}}{\text{Amount excreted in 0—24 h urine after 100-mg intravenous dose}} \times 100 \quad (1)$$

The low bioavailability value found after an oral dose of ranitidine could be due to poor absorption or metabolism of the drug during its passage through the gastrointestinal tract and liver. Ranitidine is a highly water soluble drug with a log P value of 0.2 and pK_a values of 8.2 and 2.7 and it should be well absorbed from the gastrointestinal tract. The percentages (± S.D.) of both the intravenous and the oral dose excreted as metabolites during the first 24 h after administration of ranitidine were respectively for I, 5.1 ± 1.43%, 3.7 ± 0.65; II, 2.4 ± 0.5%, 1.7 ± 0.44%; and III, 1.7 ± 0.69%, 1.2 ± 0.4% of the dose. The similar values for the percentage of the dose of ranitidine excreted in the urine as metabolites after either an oral or intravenous dose of ranitidine would suggest that (a) the oral dose of ranitidine had not been extensively metabolised or (b) that metabolism may have occurred and the metabolites had been preferentially excreted via the bile rather than the urine. The biliary and urinary excretion of [^{14}C]ranitidine and its metabolites have been studied in dogs. Radio thin-layer chromatography of the bile and urine showed that ranitidine N-oxide, the major metabolite, was preferentially excreted via the bile [5]. Man has been shown to metabolise ranitidine in a similar manner to the dog [2] and it could be that in man an oral dose of ranitidine is more extensively metabolised than an intravenous dose, and that there is preferential excretion of the metabolites in the bile.

The elimination half-life of ranitidine after intravenous injection calculated from the urinary excretion data ranged from 110—248 min.

The development of the HPLC assay for urinary ranitidine has been used in conjunction with the plasma ranitidine assay [3] to determine the renal clearance of ranitidine after intravenous administration of the 100-mg dose of ranitidine. The mean renal clearance value obtained was 512 ml/min. This high renal clearance indicated that apart from glomerular filtration there is also extensive tubular excretion of ranitidine.

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