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High-Pressure Liquid Chromatographic Determination of Cimetidine in Plasma and Urine

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Abstract □ An assay is described for the determination of the H_2-receptor antagonist, cimetidine, in human plasma and urine. Alkalinized plasma or urine was extracted with methylene chloride, the organic phase was evaporated, and the reconstituted residue was analysed by high-pressure liquid chromatography (HPLC) using a reversed-phase prepacked plastic column housed in a radial compression module. The metabolite, cimetidine sulfoxide, was identified but could not be quantitated due to interference from the solvent front. The sensitivity limit of the assay was 25 ng/ml. The assay was applied to the measurement of plasma and urine samples in a pilot pharmacokinetic study. Cimetidine was substantially absorbed and rapidly eliminated (plasma elimination half-life of 112–130 min). Plasma cimetidine concentrations could be measured to 12 hr after a 200-mg dose (iv or oral), but they were below the sensitivity of the assay by 24 hr. Urinary excretion of unmetabolized cimetidine accounted for 40–50% of the administered dose in the first 12 hr. This assay is simpler and more sensitive than those previously described, and it is suitable for the measurement of cimetidine in plasma and urine of subjects receiving doses appropriate for clinical use.

Keyphrases □ Cimetidine—high-pressure liquid chromatographic analysis in plasma and urine □ High-pressure liquid chromatography—analysis, cimetidine, human plasma and urine □ H_2-Receptor antagonists—cimetidine, high-pressure liquid chromatographic analysis in plasma and urine

The H_2-receptor antagonist, cimetidine, profoundly inhibits gastric acid secretion and is effective in the treatment of gastric and duodenal ulcers (1). Studies have shown that the degree of inhibition of gastric acid secretion is related to cimetidine plasma level measurements (2).

Chromatographic assays for the measurement of blood, plasma, and urine concentrations of cimetidine have been published. The sample treatment in these methods is complex and involves multiple extraction steps (3, 4). Moreover, samples are chromatographed on silica-based column packing which is likely to limit the column's efficiency and life under alkaline conditions. A simple micromethod for the estimation of plasma cimetidine has also been reported (5). This method exhibited low precision and sensitivity, as well as lengthy chromatography.

A more sensitive liquid chromatographic method for measuring cimetidine in plasma and urine, using a simple, single extraction step in sample treatment, is reported. Chromatography is rapid and conducted on a column system[1,2] which recently has become commercially available. These columns operate under conditions that produce greater column efficiency and allow longer column life. This method has been applied to a pilot study on a patient with a gastric ulcer, in which the influence of a 6-week course of cimetidine (1 g/day) on its own disposition and elimination is investigated.

EXPERIMENTAL

Instrumentation—A constant flow high-pressure liquid chromatograph[3] was used for all assays. This consisted of a solvent delivery system[4], a universal injector[5], and a variable wavelength UV absorbance detector[6] operating at 228 nm. The plastic column was obtained prepacked[1] (100 mm × 8-mm i.d.) and was housed in a radial compression module[2] which maintained external column pressure at ~2500 psi.

Reagents—Pure samples of cimetidine, cimetidine sulfoxide, and the internal standard, burimamide, were obtained[7]. The HPLC mobile phase contained UV grade acetonitrile[8], triethylamine[9], phosphoric acid[10], and glass-distilled water.

Calibration Standards—A pool of drug-free plasma was spiked with pure cimetidine to a concentration of 5000 ng/ml. By a series of quantitative double dilutions with additional drug-free plasma, standards of 2500, 1250, 625, and 312.5 ng/ml were prepared and stored at −20°. Similarly, drug-free urine was spiked with pure cimetidine to a concentration of 200 μg/ml and diluted to prepare standards of 100, 50, and 25 μg/ml. Calibration curves were prepared by plotting the relationship between the peak height ratios of cimetidine–burimamide and the cimetidine concentration in each sample.

Recoveries of cimetidine and burimamide from plasma or urine were estimated by comparing the peak height of cimetidine obtained after extraction, against that obtained when the same amount of cimetidine from an aqueous stock solution was chromatographed.

Extraction of Plasma—Burimamide (internal standard, 10 μg/ml, 200 μl), NaOH (2 *M*, 0.5 ml), and methylene chloride (20 ml) were added to 1.0 ml of plasma in a 30-ml glass tube. After vortex mixing (60 sec) and centrifugation (3000 rpm, 10 min), the organic layer was carefully transferred into a second tube and evaporated under a gentle stream of

[1] Rad Pak A, Waters Associates, Milford, Mass.
[2] RCM-100, Waters Associates, Milford, Mass.
[3] Waters Associates, Carlton, Melbourne, 3053, Australia.
[4] Waters Associates, Model 6000A.
[5] Waters Associates, Model U6K.
[6] Waters Associates, Model 450.
[7] Smith Kline and French Laboratories Ltd. Hertfordshire, England.
[8] Waters Associates, Carlton, Melbourne, 3053, Australia.
[9] BDH Laboratories, Port Fairy, 3284, Australia.
[10] Merck, Darmstadt, West Germany.

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Table I—Pharmacokinetic Parameters and Cumulative Urinary Excretion Data for Cimetidine [a]

Pharmacokinetic Parameters	Study Number I	II	III	IV
Dose, mg	200	200	200	200
Route	iv	oral	oral	iv
$AUC_{0\to\infty}$, ng min/ml × 10^{-3}	350.2	308.0	315.8	487.3
$t_{1/2\beta}$, min	129.7	122.8	114.8	112.6
Cl_s, ml/min	563.6	563.6	406.8	406.8
Vd_β, liters	105.5	99.9	67.4	66.1
F, × 10^2	—	89.3	65.8	—
Cumulative percent of dose excreted in urine:				
4 hr	35.2	29.0	26.7	31.2
8 hr	46.8	37.4	36.0	36.0
12 hr	49.1	41.1	38.8	38.0
24 hr	50.1	44.4	40.4	38.8

[a] Studies I and II were undertaken before a 6-week course of cimetidine (1 g/day), while III and IV were undertaken immediately after the course of therapy. ($AUC_{0\to\infty}$ = area under the plasma concentration–time curve; $t_{1/2\beta}$ = terminal elimination phase half-life; Cl_s = systemic clearance; Vd_β = volume of distribution; F = bioavailability fraction.)

nitrogen at 45°. The residue was reconstituted in 100 μl of the chromatographic mobile phase and 40 μl was injected onto the liquid chromatograph.

Extraction of Urine—Burimamide (1 mg/ml, 25 μl), NaOH (2 *M*, 100 μl), and methylene chloride (5 ml) were added to 250 μl of urine in a 30-ml glass tube, and the sample was treated as described for the plasma. Only 20 μl of the reconstituted residue was injected onto the chromatograph.

Chromatography—The mobile phase was 1% triethylamine and 5% acetonitrile in water, adjusted to pH 3 with phosphoric acid (85% v/v). The flow rate was 3 ml/min at a back pressure of 1500 psi. The retention times of burimamide and cimetidine were 2.4 and 3.8 min, respectively. Under these conditions the peaks were resolved completely to baseline.

Patient Sampling—The effect of a course of cimetidine on drug disposition was assessed in a pilot study of a female patient with a gastric ulcer (age, 49 years; weight, 59 kg). The patient underwent intravenous and oral pharmacokinetic studies before and after a 6-week course of cimetidine. After an overnight fast, the patient received a 200-mg/15-ml dose *iv* of cimetidine over 5 min (Table I, Study I). Venous blood samples (10 ml) were withdrawn before the cimetidine dose and at intervals to 24 hr. Urine was also collected predose and over a 24-hr period. At 48 hr, after an overnight fast, a 200-mg tablet of cimetidine was administered with 150 ml of water (Table I, Study II). Blood and urine were again collected over a 24-hr period. The patient then began a 6-week course of cimetidine (200 mg three times daily and 400 mg at night). Twenty four hours after the final dose, the oral and intravenous pharmacokinetic studies were repeated (Table I, Studies III and IV). In all studies food was withheld for the first 3 hr. Thereafter a standard meal was supplied, and free access to water was allowed.

Calculations—Pharmacokinetic parameters were derived from the plasma cimetidine concentration *versus* time data, using the nonlinear least squares regression program, AUTOAN (6), and standard pharmacokinetic formula (7).

RESULTS AND DISCUSSION

Column effluent was monitored at 228 nm, since this corresponds to the wavelength of maximum UV absorbance of cimetidine. For the chromatographic separation of cimetidine, the radial compression column system offers several advantages over the stainless steel, packed reversed-phase columns. Chromatography is faster and more efficient, enhancing sensitivity. The columns are less expensive and more robust, and the use of an acidified mobile phase (pH 3) containing triethylamine gives longer column life.

Single step extraction with methylene chloride results in simple sample preparation. Figure 1 (A and B) shows the chromatograms of a blank predose plasma extract and a plasma extract obtained after a single oral dose of cimetidine. Plasma from the patient receiving cimetidine showed a distinct peak at 3.8 min, which represented 801 ng/ml of cimetidine. No peaks corresponding to the cimetidine sulfoxide metabolite were present. Figure 1(C) shows the chromatogram of 100 ng of each of the reference compounds, cimetidine sulfoxide, burimamide, and cimetidine, injected as an aqueous solution. There is satisfactory separation of cimetidine from both the internal standard and the sulfoxide metabolite.

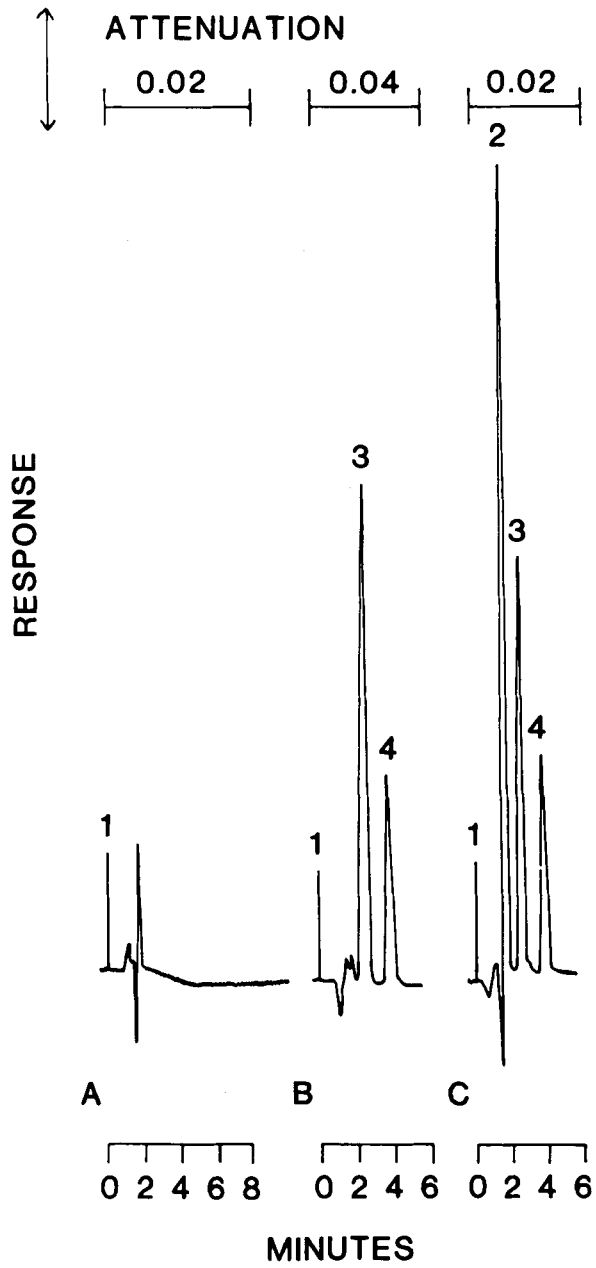

Figure 1—*High-pressure liquid chromatograms for a blank plasma extract (A); an extract of a plasma sample obtained from a patient who received a single dose of cimetidine (cimetidine concentration = 801 ng/ml) (B); and reference compounds showing the injection event (1), cimetidine sulfoxide (2), the internal standard, burimamide (3), and cimetidine (4), for which 100 ng of each compound was injected as a mixed solution in the mobile phase (C).*

In the chromatograms of some patients' plasma samples, a single broad peak eluted at 32 min. This peak was attributed to a cimetidine metabolite because it was absent from predose sample chromatograms. However, it could not be identified, since appropriate pure metabolite standards were unavailable.

Chromatograms of blank urine and a urine specimen collected after a single intravenous dose of cimetidine are shown in Fig. 2. Due to the much higher concentrations of cimetidine in urine, the detector attenuation was reduced. The late-eluting component seen in some plasma chromatograms was not seen in any urine chromatograms, suggesting that this presumed metabolite is not excreted in urine in significant amounts.

Cimetidine sulfoxide, which has been identified as a major metabolite of cimetidine (8), did not interfere with the assay. Although this metabolite appeared in the chromatogram of an aqueous stock solution at a retention time of 1.7 min (Fig. 1C), it was not possible to quantitate it due to interference from the solvent front.

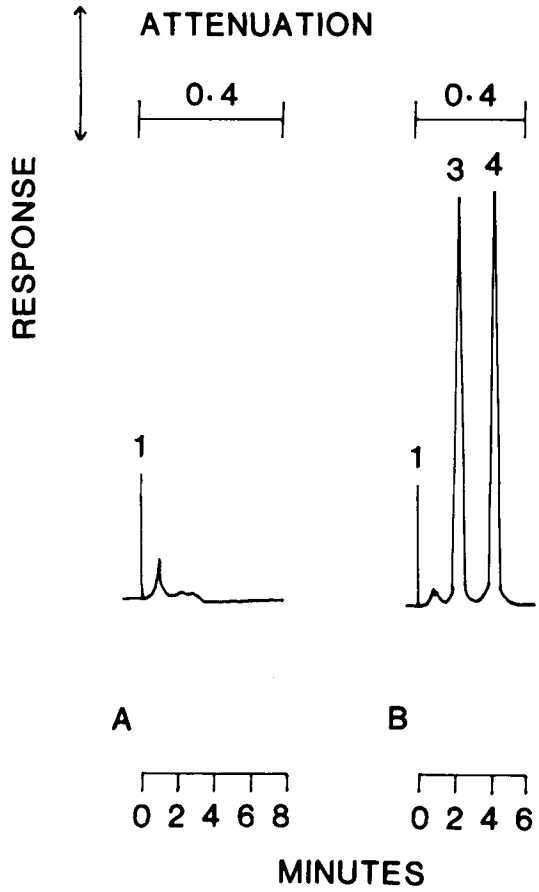

Figure 2—*High-pressure liquid chromatograms for a blank urine extract (A); and an extract of urine sample obtained from a patient who had received a single dose of cimetidine (cimetidine concentration = 156 μg/ml) (B). The peaks are the injection event (1); the internal standard, burimamide (3); and cimetidine (4).*

Methylene chloride was chosen as the extraction solvent because it gave optimal recovery of cimetidine with minimal extraction of endogenous components that might have interfered with quantitation. Analytical recoveries of cimetidine and burimamide were 60 and 31%, respectively. The limit of detection for both cimetidine and burimamide in plasma was 25 ng/ml, which gave peaks four times that of the baseline noise at the highest detector sensitivity. Ranitidine and oxmetidine, two other H_2-receptor antagonists under clinical evaluation, did not interfere with the assay.

The precision of the assay was assessed by replicate assays of aliquots of the same sample spiked with pure cimetidine. The coefficient of variation for same-day assays of plasma cimetidine levels was 4.6% at 1000 ng/ml (n = 6); for urine it was 0.4% at 55 μg/ml (n = 7). The coefficient of variation of day-to-day assays of plasma cimetidine levels over 4 weeks was 6.6% at 960 ng/ml (n = 9). The value for urine over the same period was 3% at 54 μg/ml (n = 5). The accuracy of cimetidine determination was not influenced by dried methylene chloride-extracted residue, stored for up to 5 days at 20°.

This method can be readily extended to the assay of burimamide, although this H_2-receptor antagonist is now only used in animal experiments. For burimamide assays, cimetidine is used as the internal standard. This approach has been successfully applied to the analysis of burimamide in samples obtained from animal studies.

The assay was applied to the measurement of plasma samples obtained from a pilot pharmacokinetic study. Figure 3 shows the semilogarithmic plot of plasma cimetidine concentrations following intravenous and oral doses, which were administered before (Table I, Studies I and II) and after (Table I, Studies III and IV) a 6-week course of cimetidine. The resultant pharmacokinetic and urinary excretion data for this patient are presented in Table I.

In all studies, plasma cimetidine could be estimated only to 12 hr, since by 24 hr, concentrations were below the sensitivity of the assay (25 ng/ml). The pharmacokinetic results agree with earlier studies (9, 10) and show that oral cimetidine is substantially absorbed and that both oral and intravenous cimetidine undergo rapid elimination. The urinary excretion

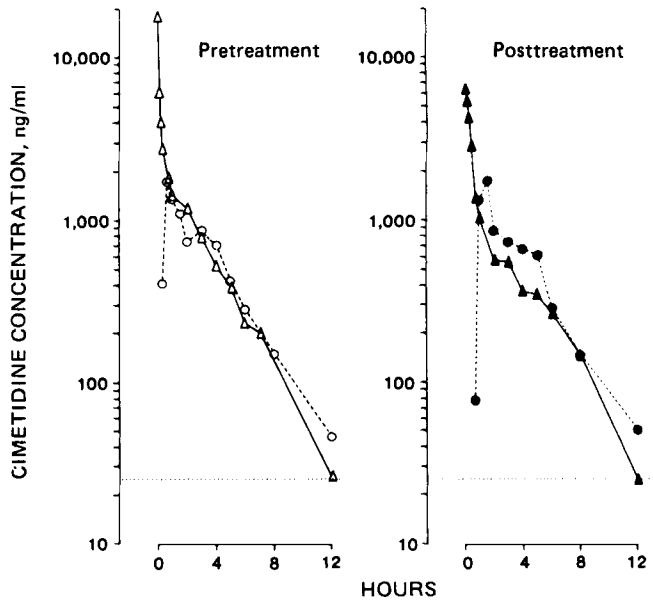

Figure 3—*Semilogarithmic plot of the mean plasma cimetidine concentration* versus *time following intravenous and oral administration of cimetidine to a patient before and after a 6-week course of treatment with cimetidine. Key: Pretreatment: (△) 200 mg, iv; (○) 200 mg, oral. Posttreatment: (▲) 200 mg, iv; (●) 200 mg, oral.*

data (Table I) confirm that renal clearance of unmetabolized drug is an important route of elimination. Approximately 40–50% of an administered dose is excreted unchanged in urine within the first 12 hr.

In contrast to an earlier study (10), the 6-week course of treatment in the patient appeared to alter the disposition of cimetidine (Table I). However, no firm conclusions can be drawn from this pilot study in one patient, and further studies are now under way.

Advantages of this assay over earlier published methods are increased precision and sensitivity (2–4-fold) resulting from improved chromatographic conditions. In addition, sample treatment has been kept simple. From the pilot pharmacokinetic study, it is apparent that the proposed method is sufficiently sensitive and specific for cimetidine quantitation in both plasma and urine, when clinically used doses are administered.

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