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Note

High-performance liquid chromatographic analysis of cimetidine in serum and urine

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Cimetidine {N''-cyano-N-methyl-N'-[2-(5-methyl-1H-imidazol-4-yl)methylthioethyl]guanidine}, a histamine H_2 receptor antagonist, blocks food and pentagastrin-stimulated gastric acid secretion. It has proven to be useful in the treatment of duodenal ulceration, Zollinger-Ellison syndrome, and may be useful in the treatment of gastric ulceration and the prophylaxis of stress ulceration [1—6].

Adverse effects on many organ systems have been reported and quantitated, based on data obtained from over ten million patients treated with the drug since its release for general use in the late 1970's [7—9]. The majority of adverse effects are minor and are gastrointestinal in nature, including nausea, vomiting, anorexia, and diarrhea. However, more serious reactions such as mental confusion and neurotoxicity, impotence and blood dyscrasias have been reported. The central nervous system adverse effects have been reported to be associated with elevated trough serum and cerebrospinal fluid cimetidine concentrations [10,11]. Evidence exists that some instances of cimetidine-associated impotence [12] and blood dyscrasias [13] may also be related to elevated serum or blood cimetidine concentrations. No association of metabolite concentrations with either therapeutic or toxic effects has been noted [10—13].

In selected patients, especially those with multiple organ dysfunction, monitoring of serum cimetidine concentrations may provide a rational basis

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for dosage adjustment in order to ensure optimal therapy. High-performance liquid chromatographic (HPLC) procedures for the analysis of cimetidine in whole blood, serum, and urine have been described [14–18]. The majority of these procedures have required large sample volumes (up to 5 ml of whole blood), expensive mobile phases (up to 91% acetonitrile at flow-rates of up to 3.0 ml/min), and time-consuming, laborious double and triple extraction procedures. Soldin et al. [17] described a rapid, reliable microprocedure for the HPLC analysis of cimetidine in serum, utilizing a single extraction of only 100 μl of serum per sample. However, usefulness of this procedure in the analysis of cimetidine in urine was not demonstrated.

Two assays for the metabolites of cimetidine have been published [18,19]. Lee and Osborne [19] described an HPLC method for the determination of the sulfoxide metabolite in whole blood and urine similar to the cimetidine assay of Randolph et al. [14]. Recently, Ziemniak et al. [18] described an HPLC procedure for the determination of cimetidine as well as its sulfoxide, hydroxymethyl, and guanyl urea metabolites in serum and urine. Both of these assays require large sample volumes, an expensive mobile phase, and incorporate tedious extraction steps.

This paper describes a modification of the procedure of Soldin et al. [17] and assesses its accuracy, sensitivity, and reproducibility in the determination of urine as well as serum cimetidine concentrations.

EXPERIMENTAL

Reagents

Analytical grade acetonitrile, methanol, and methylene chloride were obtained from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Sodium bicarbonate (6.25%) and potassium phosphate buffer (10 m*M*, pH 4.8) were prepared in our laboratory. Cimetidine (Lot 809-CETG-1SC-LZK5) and cimetidine sulfoxide (Lot GAC-9311-23B) powder were kindly supplied by Smith, Kline, and French Labs. (Philadelphia, PA, U.S.A.). Procaine hydrochloride (Lot 39C-0065) was obtained from Sigma (St. Louis, MO, U.S.A.). Stock solutions of cimetidine in methanol and procaine hydrochloride in acetonitrile were prepared and refrigerated until used. Fresh stock solutions were prepared every six months. The mobile phase consisted of 7% acetonitrile in 10 m*M* potassium phosphate buffer, pH 4.8.

Procaine was chosen as the internal standard based on its low cost, ease of procurement, stability over time, good UV absorbance, and rapidity of elution in our solvent system.

Extraction procedure

Two hundred microliters of serum and 100 μl of the internal standard solution (procaine hydrochloride 0.072 μg/μl) were added to a Pyrex culture test-tube (13 × 100 mm). Methylene chloride (5 ml) was then added and the resulting mixture was vortexed for 210 sec. After this single extraction step, the organic phase was transferred to a clean, dry test-tube and evaporated to dryness under nitrogen. The residue was dissolved in 100 μl of mobile phase and 20 μl of this solution were injected. Preparation of urine samples was

similar except that 100 μl of a 6.25% sodium bicarbonate solution was added to each sample. Alkalinization of the sample increased the extraction of cimetidine from urine into the organic phase by two to three fold and also markedly reduced interference from endogenous compounds.

HPLC apparatus

The chromatographic system consisted of a Constametric III solvent delivery pump (Laboratory Data Control, Riviera Beach, FL, U.S.A.) connected to a Model 7125 sample loop injector (Rheodyne, Berkeley, CA, U.S.A.) fitted with a 20-μl sample loop. A Spectromonitor III variable-wavelength UV detector (Laboratory Data Control) monitored the effluent at 228 nm. This wavelength was selected based on the reports of other investigators [16,17] as well as our own spectral analysis findings with respect to UV absorption maxima for cimetidine and procaine. The chromatographic mobile phase of 7% acetonitrile in 10 m*M* potassium phosphate buffer (pH 4.8) was pumped through a Partisil-10 ODS-3 (250 × 4.6 mm, 10 μm particle size) column at 2.0 ml/min. The chromatograms were recorded on a Model 9176 strip chart recorder (Varian Instrument Group, Walnut Creek, CA, U.S.A.). Peak areas for quantitation of cimetidine were integrated with a Model 308 computing integrator (Laboratory Data Control).

Conditions of analysis

Table I illustrates the conditions of analysis. Typical chromatograms for blank serum spiked with internal standard and for a blank serum sample spiked with 1.25 μg/ml of cimetidine and the internal standard are presented in Fig. 1.

Calculations

Serum concentrations were interpolated from standard curves constructed from chromatograms of serum samples containing seven known concentrations of cimetidine (0.2, 1.25, 2.5, 5.0, 10.0, 20.0, and 40.0 μg/ml). Urine concentrations were interpolated from standard curves constructed from chromato-

TABLE I

CONDITIONS OF ANALYSIS

Mobile phase	7% acetonitrile in 10 m*M* potassium phosphate, pH 4.8
Column	Partisil-10 ODS-3
Temperature	Room (21°C)
Pressure	70—100 bar (1000—1500 p.s.i.)
a.u.f.s.	0.05—0.01 (serum) 2.00—0.02 (urine)
Flow-rate	2.0 ml/min
Wavelength	228 nm
Internal standard	procaine hydrochloride
Analysis time	17 min
Serum or urine volume analyzed	0.2 ml

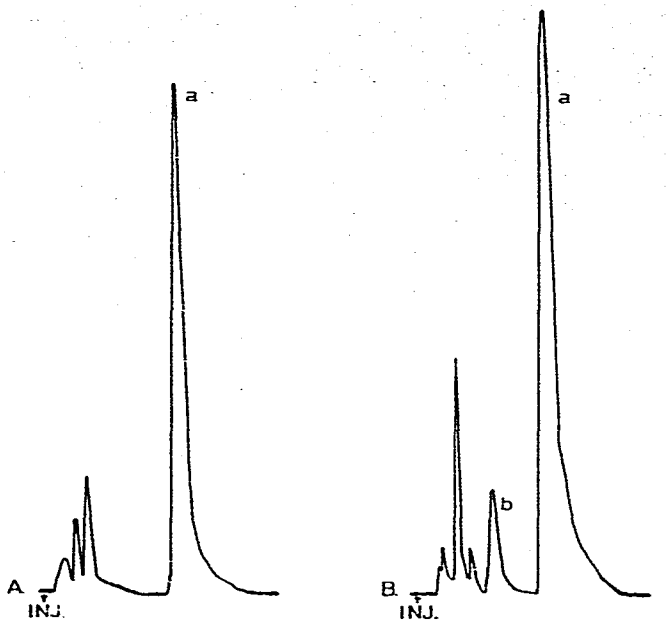

Fig. 1. Chromatograms of extracts from control human serum and internal standard, procaine hydrochloride (A), and serum containing 1.25 μg/ml of cimetidine plus internal standard (B). Peaks: a, procaine hydrochloride; b, cimetidine.

grams of urine samples containing ten known concentrations of cimetidine (0.2, 1.25, 2.5, 5.0, 10.0, 20.0, 40.0, 75.0, 100.0, and 200.0 μg/ml). Standard curves were generated daily by plotting peak area ratios (cimetidine:procaine) against the known cimetidine concentrations.

RESULTS

The retention times of cimetidine and the internal standard were 6.2 and 12.9 min, respectively. The serum standard curves were linear over the range of 0.2—40 μg/ml. The urine standard curves were divided into two concentration ranges, 0.2—40 μg/ml and 40—200 μg/ml, which were also linear. The limit of the method was 0.1 μg/ml when 200 μl of serum or urine were used. The between-day precision of the method ranged from 6.1—13.9% over the concentration range of 0.2—200 μg/ml while the within-day precision ranged from 2.2—8.8% over the same concentration range (Table II). No interference was noted by the following drugs in serum or urine assays: caffeine, minoxidil, furosemide, propranolol, sulfinpyrazone, flurazepam, diazepam, methyldopa, acetaminophen, digoxin, and quinidine. Cimetidine sulfoxide had a short retention time, eluting in the solvent front and did not interfere in the analysis. No attempt was made to quantitate cimetidine metabolites in this procedure. However, procainamide and tolazamide were found to interfere.

Preliminary pharmacokinetic studies in subjects with normal renal function (NRF) and severely-impaired renal function (SIRF) have been completed using this HPLC procedure [20]. Peak serum concentrations following intravenous administration of a 300-mg dose in NRF and SIRF subjects ranged

TABLE II

VARIATION IN THE ASSAY OF SERUM AND URINE CIMETIDINE

Drug concentration (μg/ml)	Between-day				Within-day			
	n	Mean PAR*	S.D.**	C.V.*** (%)	*n*	Mean PAR	S.D.	C.V. (%)
200	6	12.62	0.77	6.1	5	12.54	0.82	6.5
40	15	2.43	0.29	11.9	5	2.32	0.05	2.2
10	20	0.62	0.09	13.9	4	0.57	0.05	8.8
2.5	23	0.14	0.02	13.1	4	0.17	0.01	5.9
1.25	17	0.070	0.006	8.9	4	0.080	0.002	2.3
0.20	9	0.014	0.002	12.3	4	0.015	0.001	7.5

*PAR = Peak area ratio.
**S.D. = Standard deviation.
***C.V. = Coefficient of variation.

from 6—17 and 30—40 μg/ml, respectively. Serum concentrations in NRF subjects were undetectable 6 h after a 300-mg oral dose while the serum concentrations in the SIRF subjects at 24 h ranged from 0.27—0.78 μg/ml. Urine concentrations ranged from 2.8—298 μg/ml in both groups.

DISCUSSION

Monitoring of serum cimetidine concentrations may be useful for selected patients to assure achievement of therapeutic yet non-toxic serum concentrations. Also, the availability of a rapid micro-procedure for the determination of cimetidine in serum and urine would allow detailed pharmacokinetic studies in those subjects in whom sampling of large blood volumes would be deleterious (e.g., neonates, infants, renal/hepatic failure patients). The assay described in this paper has proven sufficiently sensitive and reliable for clinical and research use. Unlike other HPLC methods [14—16,18,19], we have been able to use a low pH mobile phase which has decreased the column degradation rate. The present column has been used in the analysis of over 1000 samples and has shown no signs of deterioration. Because of lower reagent cost, longer column life, and the less time- and labor-consuming sample preparation process, our procedure is more economical than any of the previously described methods, including that of Soldin et al. [17]. With the Ziemniak et al. [18] HPLC procedure one can determine the concentration of cimetidine, its known metabolites, and creatinine in serum and urine. However, the complexity of the extraction process (25 samples per 4 h), the expense of the mobile phase (91% acetonitrile at a flow-rate of 3.0 ml/min), and the long analysis time (26 min) will probably restrict this method to research use only. We did not alter our procedure in order to determine cimetidine metabolites since therapeutic/toxic effects have not been ascribed to these at this time.

We have demonstrated sensitivity and reproducibility over a much wider concentration range than previous HPLC methods, including that of Ziemniak et al. [18]. The concentration range utilized for our standard curves is similar to that which may be seen in actual patient serum and urine. This is espe-

cially important in the analysis of cimetidine in urine where extremely high concentrations may be seen. These advantages may make our procedure the method of choice for routine use in a clinical chemistry laboratory. Additionally, the use of serum has eliminated the possible erroneous results associated with whole blood cimetidine analysis [15].

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