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CIMETIDINE ASSAY IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY

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SUMMARY

An assay for the determination of cimetidine in human plasma is described. Cimetidine was extracted from alkalized plasma with ethyl acetate, washed once over hydrochloric acid, re-extracted into ethyl acetate, and the organic phase was evaporated to dryness. The residue was dissolved in ethanol and injected into a liquid chromatograph.

In vitro sulphoxidation was found to occur in whole blood, for which reason the assay was performed in plasma. The accuracy of the method was found to be within 3% and the lower limit for sensitivity was demonstrated to be 0.1 mg/l using 750 μl plasma.

Five volunteers received 1 g cimetidine perorally per day given in four doses with various intervals. Blood samples were drawn hourly, five dose intervals over two days. The average minimum concentration of plasma cimetidine was found to correlate significantly with the mean value of the area under the time/concentration curve over a period of three dose intervals ($r = 0.96$).

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INTRODUCTION

Cimetidine (CMT) (Tagamet^R SKF), a non-thiourea H_2-receptor antagonist, was recently introduced as a potent inhibitor of gastric acid secretion; for a complete review, see Brogden et al. [1]. It has accordingly proved to be highly efficient in the treatment of patients with peptic ulcer. The degree of inhibition of the acid secretion has been shown to be dose-dependent [2], and measurements of CMT plasma levels may therefore be clinically important, as these may function as a more reliable expression of the dose. However, a prerequisite is that a correlation has been established between the effect (e.g. measured as the reduction of gastric acid secretion) and the amount of CMT in plasma, which again requires a dependable chemical assay of CMT.

Procedures for measurements of the plasma concentration of CMT and its metabolite cimetidine sulphoxide have been published recently [3, 4]. This paper reports a liquid chromatographic method for measuring CMT in plasma using a simplified extraction procedure, as compared to the above mentioned methods [3, 4]. Furthermore, a schedule for blood sampling which provide representative areas under time/concentration curve in plasma is described.

EXPERIMENTAL

Reagents

Ethyl acetate from E. Merck (Darmstadt, G.F.R.) was of analytical-reagent grade. Sodium hydroxide (6 *N*), hydrochloric acid (0.02 *N*) and a saturated sodium chloride solution were all prepared in our laboratory.

Reference substances

Stock solutions (1 g/l) in distilled water of CMT; of the internal standard, metiamide, and of cimetidine sulphoxide, all from Smith, Kline & French (Welwyn Garden City, Great Britain) were prepared. The solutions were kept in a refrigerator and stored in this way, they were stable for at least one year.

Extraction procedure

To a centrifuge tube containing 750 μl plasma, 2 μg of the internal standard were added. To the sample were added 100 μl 6 *N* sodium hydroxide and this was extracted with 6 ml of ethyl acetate by mixing for 5 min in a rotary mixer (20 rpm). After centrifugation for 5 min, the organic phase was transferred into a 10-ml glass-stoppered tube containing 1 ml of 0.02 *N* hydrochloric acid and 1 ml of saturated sodium chloride. The compounds were extracted into the aqueous phase by mixing for 10 min. After centrifugation the organic phase was discarded. The aqueous phase was made alkaline by adding 100 μl 6 *N* sodium hydroxide solution. The compounds were extracted into 3 ml of ethyl acetate by mixing for 5 min. After centrifugation the organic phase was transferred into a tapered tube and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 50 μl of ethanol, and 25 μl of this solution were injected into the chromatograph.

Liquid chromatography

A liquid chromatograph (Pye Unicam, Cambridge, Great Britain) type LC 3 equipped with an ultraviolet detector type LC 3 was used. The column (25 cm × 4.6 mm I.D.) was filled with Partisil^{R} 10-ODS, particle size 10 μm. The mobile phase was acetonitrile—water—ammonia (1000:50:1) with a flow-rate of 2.5 ml/min. The detection was carried out at 228 nm.

Calculations

The plasma concentrations were read from standard curves constructed from chromatograms of plasma samples containing varying, but known amounts of CMT giving concentrations from 0.50 to 4.00 mg/l. The peak height ratios between CMT and metiamide were plotted against the concentrations.

Volunteers

Five subjects (all males, age 18—24 years) consented to participate in a short term CMT study after careful information. They received 1 g CMT daily starting 24 h prior to the study. Blood samples were drawn hourly for two days during a total of five dose intervals (cf. Fig. 3).

RESULTS

Assay

Fig. 1 illustrates chromatograms of two plasma samples containing CMT in a concentration equal to 1.0 mg/l (left) and a blank plasma sample (right). The peak representing CMT appeared 3.2 min after the injection.

Table I gives the ratio (R) between the CMT peak height and the metiamide peak height together with the corresponding concentration of CMT.

Accuracy and recovery tests for CMT were performed on seventy plasma samples with concentrations from 0.5 to 4.0 mg/l. No concentration deviated more than 3% from the mean value (Table II). The calibration graph was constructed from ethanolic solutions containing CMT in known concentrations. The recovery was almost 100%. The lower limit for safe quantitation (sensitiv-

TABLE I

RATIO (R) BETWEEN THE PEAK HEIGHTS OF CIMETIDINE AND METIAMIDE FOR DIFFERENT CIMETIDINE PLASMA CONCENTRATIONS

For each concentration the number of samples was ten.

Concentration added (mg/l)	R*
0.50	0.16 ± 0.01
1.00	0.31 ± 0.02
1.50	0.48 ± 0.01
2.00	0.63 ± 0.02
2.50	0.81 ± 0.01
3.00	0.96 ± 0.01
4.00	1.28 ± 0.02

*Mean ± S.D.

TABLE II

ACCURACY TEST FOR CIMETIDINE FROM PLASMA

The plasma volume extracted was 750 μl. For each concentration the number of samples was ten.

Concentration added (mg/l)	Calculated concentration (mg/l)*
0.50	0.51 ± 0.01
1.00	1.00 ± 0.03
1.50	1.49 ± 0.03
2.00	2.02 ± 0.01
2.50	2.49 ± 0.03
3.00	3.01 ± 0.02
4.00	4.02 ± 0.01

*Mean ± S.D.

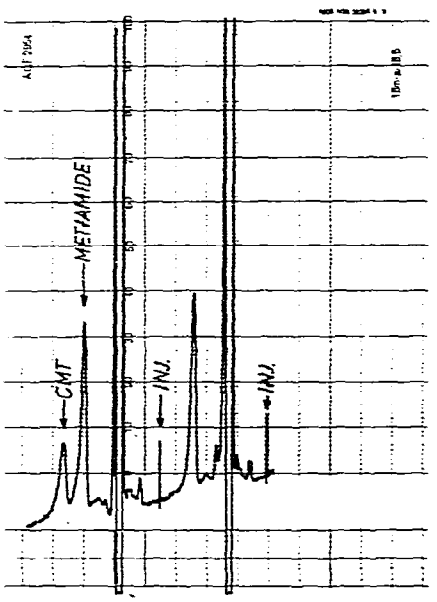

Fig. 1. Chromatograms of two plasma samples. The chromatogram to the right illustrates a blank plasma sample with added internal standard. The left one illustrates a plasma sample with added CMT in a concentration equal to 1.0 mg/l.

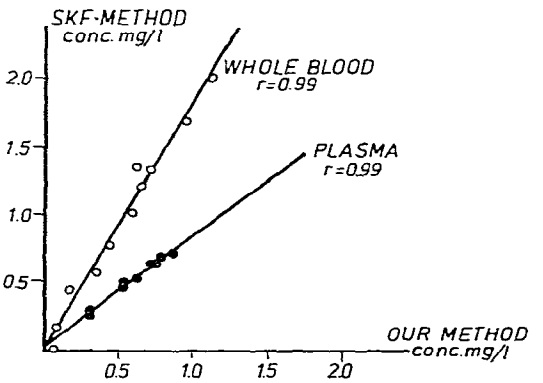

Fig. 2. Correlation of the Smith, Kline & French method and our method, carried out using plasma as well as whole blood samples.

ity) was found to be 0.1 mg/l, with a reproducibility of 10%, when 750 μl plasma was used. Plasma samples from 30 patients not taking CMT did not contain compounds interfering with the assay.

Plasma and whole blood determinations

Fig. 2 demonstrates the correlation between measurements made from iden-

tical samples analyzed at the Smith, Kline & French research laboratory [3] and in our laboratory. Ten whole blood samples were obtained from patients treated in England. The only difference between the handling procedures up to the measurements was that the samples had thawed some time before arrival in Copenhagen, while the samples analyzed in England were thawed immediately before measuring. Our method gave systematically lower results, approximately 40%. Ten plasma samples from Danish patients were also analyzed in both laboratories and the results were identical. Therefore, known amounts of CMT were added to samples of blank whole blood and stored at room temperature for at least four hours. This procedure resulted in two peaks. The first peak represented CMT while the second peak occurring 21 min after injection was found to be identical with that seen after injection of CMT sulphoxide. The ratio we found between the retention times for CMT sulphoxide and CMT was similar to that found in the earlier investigation [4]. No attempt was made to measure the rate with which CMT was metabolized into the sulphoxide.

Plasma levels and areas under the time/concentration curves

The plasma CMT concentration found in the samples drawn in the morning of the first day of the investigation (9 h after a 400-mg dose) was on average 0.34 mg/l with no great difference between the five individuals (Fig. 3).

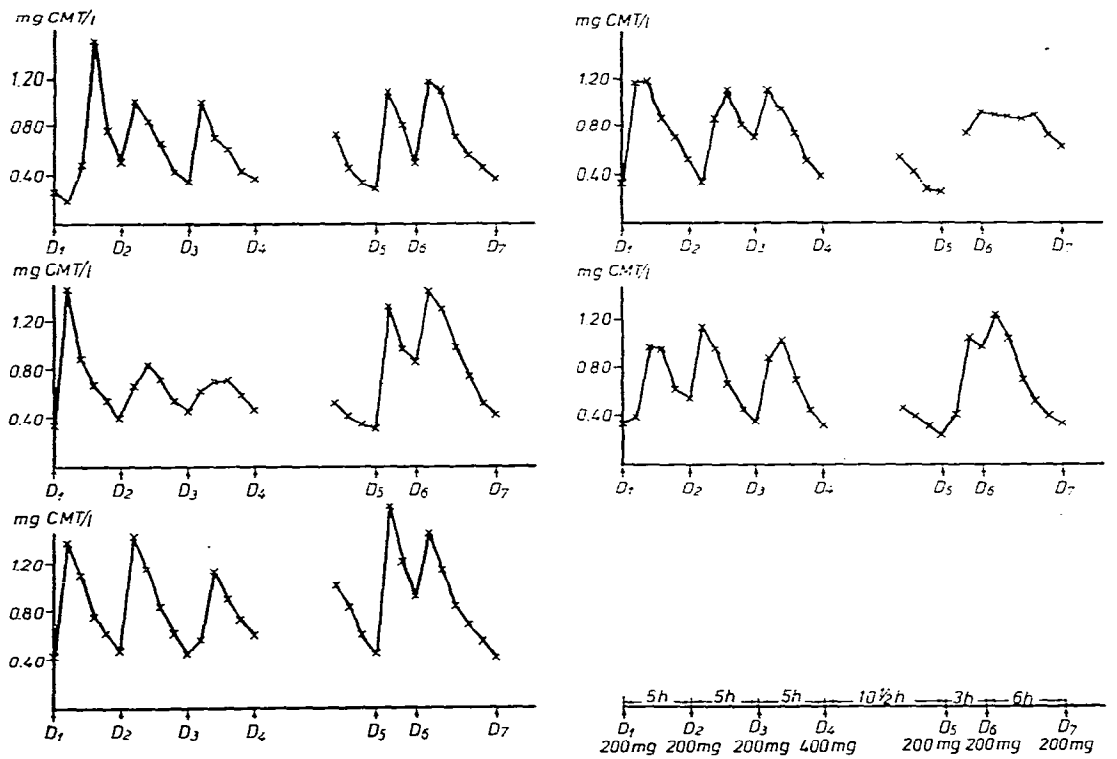

Fig. 3. Individual time/concentration curves for five volunteers after one day of peroral cimetidine ingestion prior to D_1. The dose 9 h before D_1 was 400 mg, the D_4 dose was also 400 mg, the other doses were 200 mg each.

The subsequent minimum concentrations on the first day (always 5 h after a 200-mg dose) came almost invariably very close to the morning value ($C_{D_1} = C_{D_2} = C_{D_3} = C_{D_4}$). This result indicates that steady-state conditions (in the pharmacokinetic meaning of this term) have been obtained. The peak concentrations, in contrast, showed great variations within the same individual and between individuals. Fig. 4 demonstrates that the average minimum concentration [$\overline{C}_{min.}$ (day 1) i.e. ¼ × ($C_{D_1} + C_{D_2} + C_{D_3} + C_{D_4}$)] was significantly correlated to the average area under the time/concentration curve (AUC), i.e. 1/3 × ($AUC_{D_1-D_2} + AUC_{D_2-D_3} + AUC_{D_3-D_4}$).

On the following day the five subjects continued on a total dose of 1 g CMT per day, but the dose fractions (200 mg) were given at non-equidistant intervals. Fig. 3 demonstrates considerable variations in the plasma concentrations, particularly with respect to the minimum concentrations. Consequently, a poor correlation was found between the average minimum concentrations and the average AUC.

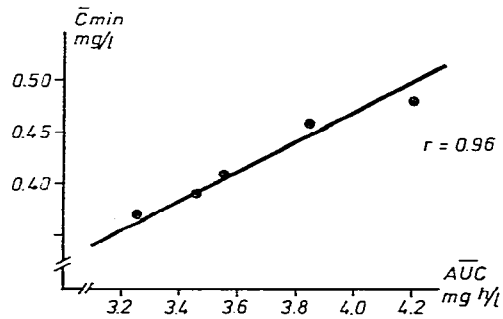

Fig. 4. Correlation between $\overline{C}_{min.}$ and $\overline{AUC}$ for cimetidine, given at equidistant dose intervals during one day.

DISCUSSION

A chemical method for determination of CMT in plasma may be of considerable clinical importance. The assay described in this paper has proved to be sufficiently sensitive and reliable for clinical use.

The problem of whether plasma or whole blood should be used has also been solved in this study. A liquid chromatographic method was early developed in the Smith, Kline & French (SKF) research laboratory [3], and the present assay is similar to the SKF method concerning the chromatographic system and the mode of deteetion. However, the procedures for extraction and isolation of the compound are quite different, but the specificity seems to be the same. Samples analyzed by both assays showed identical results for plasma (Fig. 2), whereas whole blood samples differ systematically probably due to in vitro sulphoxidation at room temperature in the presence of erythrocytes, a phenomenon previously described for other sulphur-containing compounds [5]. In order to avoid erroneous results CMT analysis should therefore be carried out only in plasma.

Marked individual variations in the plasma concentrations between two doses (reflected in AUC) as well as some variations in the minimum values might be foreseen. The resulting individually variable dose/concentration ratio would constitute a good reason for plasma level monitoring. The necessary pharmacokinetic calculations, based on steady-state conditions presume equal dose intervals. This schedule was used during the first day. However, in the calculations applied in Fig. 4 the C_{D_1} value was included, as the night dose (given 9 h earlier) was twice the normal dose.

The amount of drug on receptor site (in the secretory cells of the mucosa) is probably correlated to the amount of drug in the plasma, particularly during steady-state conditions. This amount can be expressed as the area under the time/concentration curve in plasma (AUC) between two doses. Determinations of the AUC during some dose intervals would, however, not be practical in daily clinical work. Another expression must therefore be sought. However, the peak concentration after each dose was not usable, as it was calculated on varying times and poorly reproduced from dose to dose. It is therefore of importance that in further clinical pharmacological investigations with CMT, the AUC covering three 5-h intervals of equal doses during the steady state, can be replaced simply by the average of the four concentrations measured just before each dose (Fig. 4).

On the second day, in which different dose intervals were used, as expected no correlation could be demonstrated between $\overline{C}_{min.}$ and $\overline{AUC}$.

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REFERENCES

1 R.N. Brogden, R.C. Heel, T.M. Speight and G.S. Avery, Drugs, 15 (1978) 93.
2 E. Aadland, A. Berstad and L.S. Semb, in W.L. Burland and M.A. Simkins (Editors), Cimetidine, Excerpta Medica, Amsterdam, 1977, p. 87.
3 W.C. Randolph, V.L. Osborne, S.S. Walkenstein and A.P. Intoccia, J. Pharm. Sci., 66 (1977) 1148.
4 R.M. Lee and P.M. Osborne, J. Chromatogr., 146 (1978) 354.
5 C. Eggert-Hansen, T. Rosted Christensen, J. Elley, L. Bolvig Hansen, P. Kragh-Sørensen, N.-E. Larsen, J. Næstoft and E.F. Hvidberg, Br. J. Clin. Pharmacol., 3 (1976) 915.