PLASMA CONCENTRATIONS OF METHIMAZOLE, A METABOLITE OF CARBIMAZOLE, IN HYPERTHYROID PATIENTS

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- 1 Carbimazole was administered to nine hyperthyroid patients, and blood samples were taken at various time intervals for analysis of carbimazole, and its metabolite methimazole.
- 2 A technique was developed for the measurement of methimazole in serum, using a High-Pressure Liquid Chromatograph, which could detect nanogram quantities of this metabolite.
- 3 After a single oral dose, the patients' blood levels appeared to fall into two groups, either those with a maximum concentration of methimazole between 30 and 60 min, or those whose maximum was 2 to 3 hours.
- 4 The results would suggest that there could be a correlation between the high level of methimazole in serum and the high thyroxine concentration found in some patients.

Introduction

Carbimazole (1-carbethoxy-3-methylthioimidazole, Fig. 1) was initially synthesized as a potentially long-acting antithyroid drug (Lawson, Rimmington & Searle, 1951). Although it has not been shown to be significantly longer acting than other antithyroid drugs, e.g. methimazole and propylthiouracil, it is widely used in the treatment of hyperthyroidism.

Plasma concentrations of carbimazole have been difficult to determine, and to date the presence of carbimazole has not been detected in plasma (Marchant, Alexander, Lazarus, Lees & Clark, 1972). Whilst carbimazole is stable in the solid state or neutral solutions, it is readily hydrolyzed and decarboxylated to methimazole (Fig. 1), in alkaline media. *In vitro* studies with carbimazole in plasma have shown that conversion to methimazole takes place very rapidly (Stenlake, Williams & Skellern, 1970).

Our attention has been focussed on the examination of methods for studying the pharmacokinetics of carbimazole/methimazole. The gas-liquid chromatographic method developed by Stenlake *et al.* (1970) for studying the excretion kinetics of methimazole proved to be unsatisfactory for measuring blood levels because of the large volume of plasma required. Spectrofluorimetry based on derivatization with

either dichlorobenzoquinocechlorimine (Averbach & Angell, 1958) or dansyl chloride (Chen, 1967) was also unsuccessful. We have found, however, that nanogram quantities of methimazole can be measured in plasma, using High-Pressure Liquid Chromatography (HPLC). This paper reports a study by this HPLC method of the plasma concentration of methimazole from hyperthyroid patients receiving carbimazole.

Methods

Nine female patients attending an outpatient thyroid clinic took part in this study. Details of age, weight and medical history are given in Table 1. Hyperthyroidism was diagnosed by clinical examinations and estimation of the total serum thyroxine using commercially available kits (Thyropac-4) supplied by the Radiochemical Centre, Amersham.

A single dose of carbimazole (12 x 5 mg tablets with water) was administered to each patient 1 h after a light breakfast. A blood sample (10 ml) was withdrawn immediately for use as a plasma blank. Five to eight blood samples were taken from each patient at varying time intervals (usually between 15 min and 8 h), after administration of

Fig. 1 Structural formulae of carbimazole, and its metabolite, methimazole.

carbimazole. Blood samples were refrigerated until they could be centrifuged to obtain plasma, which was then kept frozen until required for analysis.

Analytical procedure

Extractions. Plasma (1 ml) was diluted with water (3 ml). Methimazole was extracted with chloroform containing 0.5% v/v *n*-octanol (4 × 10 ml). The extract was transferred to 'evaporating tubes' based on a design of Beckett & Rowland (1965), and evaporated down to 100 µl using nitrogen.

Measurement of methimazole. Methimazole was determined using a Waters Liquid Chromatograph [Model ALC/GPC-501] with a single wavelength detector at 254 nm. Two columns were used:

(A) A column (2 ft × 2.3 mm) packed with Corasil/C₁₈ bonded; solvent, water (flow rate 0.3 ml/minute).

(B) A column (2 ft × 2.3 mm) packed with OPN/Porasil C; solvent, *n*-hexane : tetrahydrofuran 50 : 50 v/v (flow rate 0.75 ml/minute).

Injectons (10 µl) of the concentrated extracts

were made on either of these columns. Calibration curves were obtained by adding appropriate amounts of methimazole (0.3–1.4 µg) to aliquots (1 ml) of blank plasma.

The *n*-hexane was spectroscopic grade, tetrahydrofuran and chloroform were AnalaR, and the *n*-octanol of reagent grade.

Hydrolysis of carbimazole. Carbimazole (20 mg) was dissolved in ethanol (5 ml), and made up with water containing concentrated hydrochloric acid (0.6 ml) to 100 ml (pH 1.8). Appropriate dilutions were made so that the extinction at 290 nm could be measured at given time intervals. Similarly, carbimazole was dissolved in phosphate buffer (pH 7.4), containing pooled plasma (10 ml) and the rate of hydrolysis measured. In both cases the hydrolysis mixture was incubated at 37°C.

Protein-binding. Solutions of human serum albumin (4%) containing methimazole (2.6 µg/ml), were incubated for 1 h at 37°C. Ultrafiltration using either Diaflo membrane cones (Amicon) or an ultrafiltration cell with Pellicon membranes was performed. Blank plasma to which methimazole (2.6 µg/ml) had been added was also ultrafiltered by the above methods.

Results

The HPLC method permits the determination of methimazole (0.1 µg) in plasma (1 ml). Precision of the method was determined by analysing plasma to which methimazole (1 µg) had been added. The mean deviation was 1 ± 0.07 µg. Recovery of methimazole (1 µg/ml plasma) was approximately 80%, compared to water. Column B gave the chromatograms for plasma extracts with and without methimazole and no drug as shown in

Table 1 Details of the hyperthyroid patients who participated in the study

Patient	Age (years)	Weight (kg)	Duration of symptoms (months)	Family history	Serum T ₄ (µg/100 ml)
A	56	50.0	2	Negative	23.2
B	35	51.9	9	Negative	18.2
C	22	50.1	—	Positive	16.9
D	44	49.4	4	Negative	15.5
E	38	48.5	1	Negative	19.5
F	52	35.9	3	Negative	21.8
G	40	45.5	6	Negative	17.5
H	22	53.4	7	Positive	19.6
K	32	53.8	2	Negative	11.3

Total serum thyroxine (Serum T₄) upper limit for non-hyperthyroid patients is 11 µg/100 ml.

Fig. 2 Chromatograms of (a) methimazole recovered from plasma, and (b) plasma blank. Column, OPN/Corasil C; solvent *n*-hexane : tetrahydrofuran 50 : 50 v/v; temperature ambient.

Figs 2a and b, respectively. Of the two columns used, column B was better, since it was found that after approximately fifteen runs column A had to be purged with methanol to retain the efficiency and sensitivity of operation. No retention of carbimazole was detected in any of the samples examined on column A.

Figure 3 is a typical calibration curve obtained from prepared solutions of methimazole in blank plasma from a single patient.

Preliminary protein-binding studies with methimazole demonstrated that about 40% was bound to pure human serum albumin and plasma proteins.

Plasma concentration-time curves for all the subjects in the study are shown in Figures 4 and 5.

Discussion

The results obtained from this group of patients show that carbimazole is rapidly absorbed and metabolized to methimazole after oral administration. From Figs. 4 and 5, it appears that the results fall into two groups. There are those patients who have a maximum concentration of methimazole in the plasma between 30 and 60 min (Fig. 4), and those whose maximum is reached only after 2 to 3 h (Figure 5). Pittman, Beschi & Smitherman (1971), found that the maximum for the concentration of methimazole was at 60 min, after

Fig. 3 A typical calibration curve for the determination of methimazole in plasma.

eleven patients had each received methimazole (60 mg) orally. Our results may fall into two groups because of a difference in the rate of absorption of either carbimazole or methimazole from the gut. Hydrolysis studies of carbimazole in acid media (pH 1.8) have shown that carbimazole is stable for 3 h, and after 3 days less than 20% has been hydrolyzed to methimazole. In the presence of plasma at 37°C however, carbimazole is completely hydrolyzed to methimazole within 2 hours. Thus it would appear that carbimazole, which is more lipid-soluble than methimazole, is absorbed intact and hydrolyzed to the latter in the plasma. It is possible that the rate of enzymatic hydrolysis of carbimazole could be the factor which causes the delay in peak concentration in certain patients if it is assumed that absorption is the same in all patients. This latter assumption may be incorrect, however, as Prescott & Nimmo (1971) have reported that there are 'slow' and 'fast' absorbers of paracetamol, which can result in drug peak concentration being different in these two groups. This effect has been shown not to be related to generic inequivalence. Since this investigation has been conducted over a period of months without rigorous control of tablet batch sources, the importance of this factor cannot be ascertained without further investigation.

For three subjects (A, C and D) the slope of the

Fig. 4 Plasma concentration-time curves of methimazole in patients A (■); B (▲); C (●); and D (○) (see Table 1) after an oral dose of carbimazole (60 mg). (Methimazole was determined for all patients at the 15, 30 and 60 min time interval).

Fig. 5 Plasma concentration-time curves of methimazole in patients E (●); F (△); G (■); H (○); and K (▲) (see Table 1) after an oral dose of carbimazole (60 mg). (Methimazole was determined for all patients at the 15, 30 and 60 min time interval).

concentration-time curve was linear after 90 min, and the biological half-life for methimazole was 4 h, 4 h and 3.5 h respectively.

There would appear to be some correlation between the serum thyroxine level (Table 1), and the fact that carbimazole/methimazole is not so extensively metabolized by some patients (Figures 4 and 5). The patients with high thyroxine levels tend to have high methimazole concentrations in the blood. This would agree with observations by others, (Kato, Takaraka & Takahashi, 1970) that thyroxine depressed the metabolism of drugs in animals. In particular, Juárez-Peñalva & Mitchell (1964) working with guinea pigs pre-treated with thyroxine showed substantial depression of thyroid uptake and metabolism of [^{35}S]-thiourea. Maloof & Soodak (1965) reported similar findings when working with thyroxine-treated rats; though thyroid uptake was more markedly depressed than metabolism. Due to the small number of patients

who took part in this study, we cannot show conclusively a direct correlation.

The non-linearity of the calibration curves is a characteristic of plasma protein binding and we have demonstrated that methimazole is appreciably bound to pure human serum albumin and plasma proteins. This is contrary to other reports (Marchant & Alexander, 1972; Sitar & Thornhill, 1973). Because of interference from protein binding in the assay method, the effect on the linearity of calibration curve of trichloroacetic acid, sodium hydroxide and hydrochloric acid, which destroy binding, was examined. None of these reagents was effective and all resulted in a lowering of the recovery of methimazole. Calibration curves were therefore constructed for each patient to overcome any individual idiosyncrasies.

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