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Note

Measurement of methimazole in human plasma using gas–liquid chromatography

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Three groups[1–3] have independently investigated the metabolic clearance of antipyrine in thyroid disease in man. Each study demonstrates an increased clearance of antipyrine in hyperthyroidism and a reduced clearance in hypothyroidism when compared with the euthyroid (normal) state.

Methimazole (1-methyl-2-mercaptoimidazole; MMI) is the active metabolite of carbimazole (1-methyl-2-mercapto-3-carbethoxyimidazole; CBZ), a drug commonly used to control hyperthyroidism in man (Fig. 1).

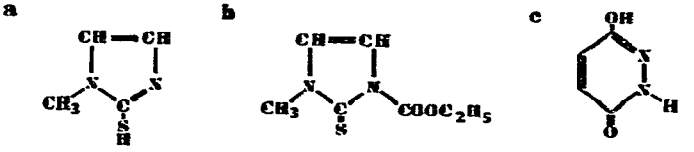

Fig. 1. Structural formulae of (a) methimazole, (b) carbimazole and (c) 6-hydroxypyridazin-3(2H)-one.

To follow the metabolic clearance of methimazole in man, following standard oral therapy of 10 mg of carbimazole, requires an assay with high specificity for methimazole and reproducibility down to 100 ng MMI per ml of plasma. Spectrophotometric[2,4] methods described lack sensitivity below about 2 μg. They also lack specificity due to interference by further metabolites of methimazole. Administration of [^{35}S]- and [^{14}C]methimazole[5–8] also overestimates levels of MMI unless laborious thin-layer separation of metabolites is employed. This further reduces sensitivity. The further metabolites include [^{35}S]methimazole glucuronide and $^{35}SO_4$ (ref. 7).

Gas–liquid chromatography (GLC) has been used to measure methimazole recovered from rat urine[9]. Extracted methimazole was treated with methyl iodide to form the S-methyl methimazole derivative. Reaction time was critical. The instability of the derivative could lead to the retention of free methimazole on the column. These authors do not comment on the applicability of this method to the measurement of methimazole in plasma. High-pressure liquid chromatography[10,11] affords a specific

method with a limit of detection of 0.1 μg MMI per ml of plasma. The relative deviation of peak height ratio was 14% ($n = 7$).

We present a method using GLC with thermionic nitrogen–phosphorus detection. The limit of detection is 30 ng MMI per ml of plasma.

MATERIALS AND METHODS

Standards and reagents

Methimazole and carbimazole (white crystalline solids) were a gift from Nicholas Labs. (Slough, Great Britain). Tetramethyl ammonium hydroxide (TMAH) was obtained as the pentahydrate through Sigma (London, Great Britain). Ethyl iodide was obtained from Aldrich (Milwaukee, Wisc., U.S.A.), methyl iodide, chloroform (AnalaR grade) and methanol (AnalaR grade) from BDH (Poole, Great Britain), and 6-hydroxy pyridazin-3(2H)-one (internal standard, Fig. 1) had been previously synthesised by the authors, in connection with a different project.

Extraction and derivatisation

A portion (1.0 ml) of plasma was whirlimixed for 60 sec with 10 ml of chloroform in a stopped tube. The mixture was centrifuged and the upper (aqueous) layer was discarded. The chloroform layer (9.0 ml) was pipetted into a stoppered tube with a tapered bottom, and was evaporated to dryness under a stream of nitrogen on a water bath at 40°. These extracts were then reconstituted in 50 μl of internal standard solution in methanol, and 10 μl of 0.1 *M* tetramethyl ammonium hydroxide solution in methanol was added as derivatising agent.

Two different internal standards were used: (a) S-ethyl methimazole formed by leaving a solution of methimazole in 20% ethyl iodide in chloroform overnight and then reconstituting in methanol; (b) a solution of 6-hydroxypyridazin-3(2H)-one in methanol (20 μg/ml). Standard curves were prepared by adding known amounts of methimazole to blank plasma. Plasma standards were extracted and assayed with each sample batch.

GLC apparatus and conditions

All measurements were carried out using a Perkin-Elmer F30 gas chromatograph fitted with a specific thermionic nitrogen–phosphorus detector. The column (2 m × 4 mm I.D.) was packed with 10% Apiezon L + 5% KOH on Chromosorb W (100–120 mesh) which had previously been conditioned at 225° for 48 h. The oven temperature was 195°, the injection port temperature 300° and the detector temperature 250°. The carrier gas (helium) flow-rate was 40 ml/min and the detector gas flow-rates (hydrogen) were 2 ml/min and (air) 100 ml/min.

RESULTS AND DISCUSSION

Methimazole was extracted from plasma into chloroform as described. Extraction recovery was 70.6% with a standard deviation of ±2.3% ($n = 5$). A number of different solvents were tried over a pH range of 4–9. None of these manoeuvres improved recovery.

The concentrated plasma extract of methimazole gave no peak on the chro-

matogram. This was shown to be due to retention of methimazole on the column and subsequent elution, as previously described[9]. Three methylating agents were tested.

Reaction between plasma methimazole extract and 20% iodomethane in chloroform at room temperature for 20 min gave a peak corresponding to S-methyl methimazole. However, this peak was only 15% of the S-methyl methimazole peak obtained after identical extraction from water. Interference with this S-methylation was shown to be due to coextractable plasma compounds.

Methylation with diazomethane led to total loss of methimazole peak on the chromatogram. This was interpreted as being due to cleavage of the imidazole ring.

Addition of tetramethyl ammonium hydroxide to the plasma extract containing methimazole was shown to give complete derivatisation. Flash methylation by TMAH occurs at 300° in the injection port.

Two different compounds were tested as internal standards. S-Ethyl methimazole (prepared by reacting methimazole with 20% iodoethane in chloroform for 20 min at room temperature) was initially used. On injecting this compound with TMAH a small peak corresponding to S-methyl methimazole was seen in addition to the large S-ethyl methimazole peak. This was shown to be partly due to methyl iodide contaminating ethyl iodide and partly due to reaction between TMAH and S-ethyl methimazole in the injector port. A standard curve obtained using S-ethyl methimazole as internal standard was linear but gave an intercept on the peak height ratio (y) axis.

Other compounds were tested as possible internal standards. 6-Hydroxypyridazin-3-(2H)-one in the presence of TMAH was found to give a clean narrow peak with suitable retention time (t_R = 3.8 min; t_R of S-methyl methimazole = 3.0 min) (Fig. 2). Standard curves obtained using plasma from a number of patients were compared, as a previous paper suggested that there was individual variation in recovery. A total of ten standard curves prepared over a three-month period showed a standard deviation in slope of 8%. The standard curve is shown in Fig. 3.

Neither of the two internal standards described are suitable for extraction

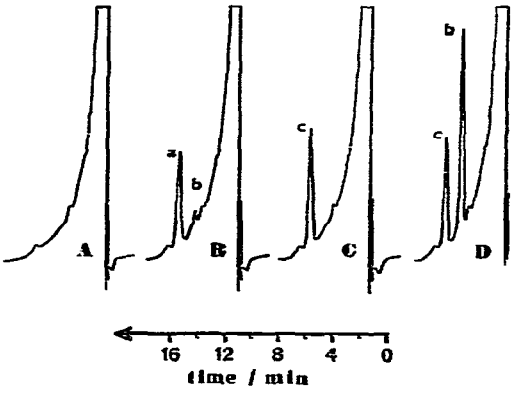

Fig. 2. Gas chromatograms. (A) extract of blank plasma injected with TMAH; (B) extract of plasma injected with S-ethylmethimazole (a) and TMAH, peak b represents S-methyl methimazole formed by reaction between S-ethylmethimazole and TMAH in the injection port; (C) extract of blank plasma injected with 6-hydroxypyridazin-3(2H)-one (c) and TMAH; (D) extract of plasma containing methimazole injected with 6-hydroxypyridazin-3(2H)-one and TMAH.

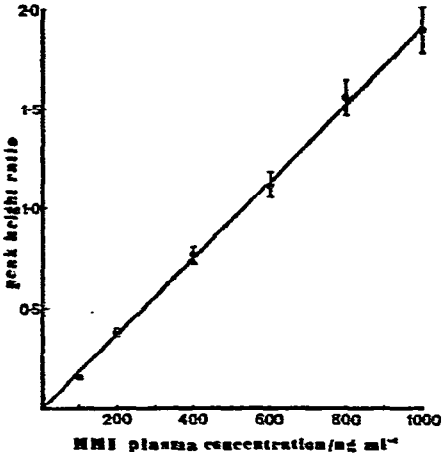

Fig. 3. Standard curve. Plot of peak height ratio (S-methyl methimazole)/[6-hydroxy pyridazin-3(2H)-one] against plasma concentration of methimazole, showing mean (±S.D.) of ten curves.

from plasma by chloroform. S-Ethyl methimazole gave good chromatograms and a linear peak height ration calibration curve, but its instability led to unacceptable error when assaying low levels of S-methyl methimazole.

Injection of methimazole, 6-hydroxypyridazin-3(2H)-one and TMAH gave reproducible results with no problems of retention on the column. This system was, therefore, adopted.

TMAH has the disadvantage of being a nitrogen-containing compound and thus contributing to a tailing solvent front when a nitrogen specific detector is used.

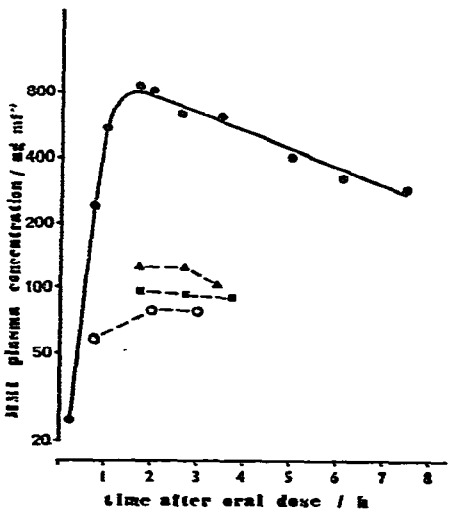

Fig. 4. Logarithm of concentration of methimazole in plasma against time after an oral dose of carbimazole in one patient. See text for meaning of symbols.

However, the advantage of stoichiometric methylation of methimazole to S-methyl methimazole outweighs this.

An example of results obtained using this assay to measure plasma concentractions of methimazole in a patient being treated with carbimazole is shown in Fig. 4. Plasma concentration of methimazole was measured between 0.5 h and 7.5 h after a 60-mg oral dose of carbimazole (●). Steady-state levels of methimazole in the same patient on an oral maintenance dose of carbimazole (10 mg three times a day) are shown one (▲), two (■) and six weeks (○) after commencing therapy.

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