Journal of Chromatography, 564 (1991) 310–314
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5685

Short Communication

Selective liquid chromatographic assay for propylthiouracil in plasma

G. R. CANNELL* and J. P. WILLIAMS

Conjoint Internal Medicine Laboratory, Royal Brisbane Hospital, Herston Road, Brisbane, Queensland 4029 (Australia)

A. S. YAP

Department of Endocrinology, Royal Brisbane Hospital, Herston Road, Brisbane, Queensland 4029 (Australia)

and

R. H. MORTIMER

Conjoint Internal Medicine Laboratory and Department of Endocrinology, Royal Brisbane Hospital, Herston Road, Brisbane, Queensland 4029 (Australia)

(First received June 12th, 1990; revised manuscript received October 16th, 1990)

ABSTRACT

A liquid chromatographic assay was developed to quantitate propylthiouracil in plasma using an internal standard, 5-propyl-2-thiouracil, of similar structure and physical properties. Caffeine, which co-elutes with propylthiouracil, was removed by extraction from serum treated with base. No other compounds were found to interfere in the assay. The drug was extracted from plasma with chloroform with a recovery of 59.4% and the intra- and inter-assay coefficients of variation were 5.7 and 3.3%, respectively. The assay was linear to 3 μg/ml with a lower detection limit of 40 ng/ml for a sample volume of 1 ml.

INTRODUCTION

Propylthiouracil (PTU, 6-propyl-2-thiouracil) blocks thyroid hormone synthesis and is commonly used to treat hyperthyroidism in pregnancy. Its pharmacokinetics have been studied in euthyroid and hyperthyroid subjects [1–4] and in pregnancy [5]. Although it is known that PTU given to a pregnant woman crosses the placenta [6] and can impair fetal thyroid function [7], the kinetics of its transfer are unknown. To study transplacental transfer in an *in vitro* perfusion system [8], an assay was developed capable of measuring low concentrations of the drug in plasma.

0378-4347/91/$03.50

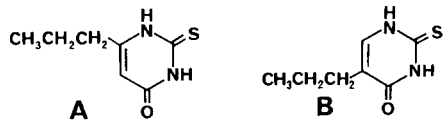

Fig. 1. Structures of propylthiouracil (A) and the internal standard (B).

The concentration of PTU in plasma has previously been measured by high-performance liquid chromatography (HPLC) [9–12], gas chromatography [13] and radioimmunoassay [3]. However, several of these assays did not include an internal standard [9,10] or were not sufficiently sensitive at low concentrations of the drug [9].

This paper describes an HPLC technique that is simple to perform, sensitive for clinical usage and utilizes an internal standard (5-PTU, 5-propyl-2-thiouracil) with a molecular structure closely related to that of PTU (Fig. 1).

EXPERIMENTAL

Chromatography

The HPLC system consisted of an ETP Kortec Model K35D pump and an ETP Kortec Model K95 variable-wavelength detector. Samples were introduced into the system via a Rheodyne Model 7125 manual injector, and the detector response was monitored with an LDC/Milton Roy Model CI-10B integrator and LDC/Milton Roy recorder. Chromatographic separation was achieved using a Newguard RP-18 precolumn (1.5 cm × 3.2 mm I.D.) (Brownlee Labs, Santa Clara, CA, U.S.A.) with a 250 mm × 4.6 mm I.D. column (Spheri-5, RP-18, polyfunctional) and a mobile phase of filtered 30% methanol in 0.05 *M* phosphate buffer (pH 7.4). The column flow-rate was 1.5 ml/min, the temperature 37°C, and the pressure 250 bar. The eluate absorbance was monitored at a wavelength of 214 nm.

Extraction

Sodium hydroxide (0.1 ml, 1 *M*) was added to 1 ml of sample and the mixture was shaken. Chloroform (4 ml) was added, and the contents of the tube were vortex-mixed and then centrifuged for 10 min (300 *g*). The lower chloroform layer was discarded, and hydrochloric acid (0.3 ml, 10%, v/v) was added to the remainder and vortex-mixed thoroughly. A further 2 ml of chloroform were then added, and the contents of the tube were again vortex-mixed, then centrifuged (300 *g*) for 10 min. The lower chloroform layer was separated and evaporated under a stream of nitrogen. The sample was reconstituted in methanol (30 μl), followed by the addition of mobile phase (20 μl), and an aliquot (20 μl) was then analysed.

TABLE I

COMPOUNDS TESTED FOR INTERFERENCE

Atenolol	Diazepam
Bupivicaine	Estriol
Carbamazepine	Hyoscine
Clonazepam	Methimazole
11-Deoxy-17-hydroxycorticosterone	Metoprolol
Cortisol	Paracetamol
Cortisone	Phenobarbital
Progesterone	Prednisolone
Propranolol	Prednisone
Ritodrine	Salbutamol
Verapamil	

Interference studies

Standard solutions of potential interfering substances (Table I) were reconstituted in mobile phase to a concentration of 50 ng/ml. Samples (10 μl) were injected into the column and their relative retention times compared with those of PTU and the internal standard. These studies were repeated using human serum spiked with the same compounds.

Pharmacokinetic studies

Three euthyroid subjects ingested 150 mg of PTU, and heparinized blood samples (5 ml) were taken over an 8-h period. Serum was separated by centrifugation, the internal standard was added and the sample was extracted. Reconstituted samples (50 μl) from the extraction process were analysed.

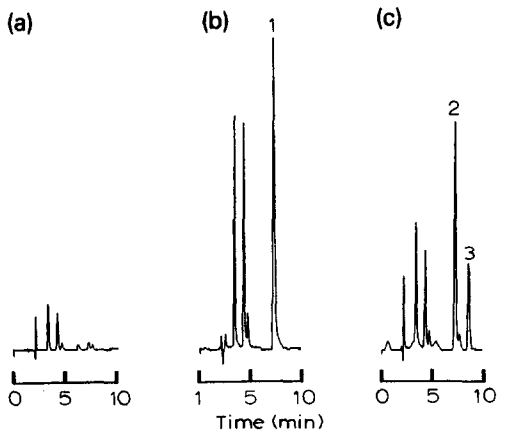

Fig. 2. HPLC analysis of PTU and 5'-PTU in plasma. (A) Blank plasma; (B) plasma containing PTU only (peak 1); (C) plasma containing PTU (peak 2) and 5'-PTU (peak 3), PTU peak corresponds to 1.4 μg/ml.

RESULTS AND DISCUSSION

Under the analysis conditions used, PTU eluted at 7.25 min and 5′-PTU at 8.75 min: the two peaks were completely resolved. A typical chromatogram is shown in Fig. 2, which shows that there is no interference from other compounds with the measurement of either PTU or 5′-PTU. To determine the drug recovery, five replicates of normal human serum were spiked with PTU (500 ng/ml) or 5′-PTU (500 ng/ml). The recovery of PTU was 59.4% and that of 5′-PTU 49.4%. The assay had an intra-assay coefficient of variation (C.V.) of 5.7% (n = 10) and an inter-assay C.V. of 3.3% (n= 10). These are is comparable with those of previous HPLC methods [9–12] and superior to the results of previously reported radioimmunoassays [3].

PTU was added to normal serum to yield concentrations of 50–3000 ng/ml. Concentrations and peak-height ratios were linear over this range (r = 0.999) with each level analysed in duplicate. The lower detection limit was 40 ng/nl, which is superior to those of previous HPLC methods and exceeded only by those of radioimmunoaassays; however, the latter require rigorous extraction methods and prolonged incubation.

Interference studies were performed using normal human serum spiked with common drugs that might be expected to be used clinically in conjunction with PTU (Table I). No interference in the assay was found with a wide range of endogenous and exogenous compounds. However, using chloroform extraction methods similar to those previously published, an interfering substance was present in drug-free human serum. This compound was shown by mass spectroscopy to correspond to caffeine and thus could be a possible source of error in clinical use. This interference was eliminated by initial differential acid–base extraction, based on the different pK_a values of caffeine and PTU, prior to extraction with chloroform. One metabolite of unknown structure was detected, but this compound, eluting at 3.4 min, did not interfere in the assay.

The results of single-dose pharmacokinetic studies performed in three euthy-

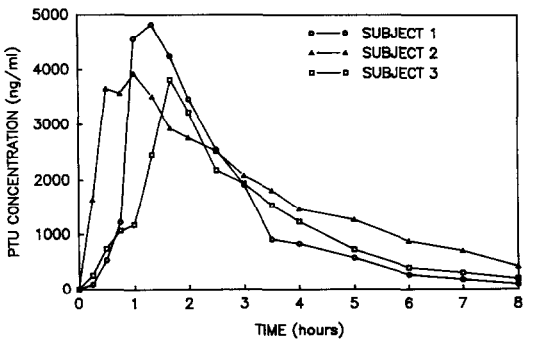

Fig. 3. PTU concentrations following a single dose in three subjects.

roid volunteers are shown in Fig. 3. There was some inter-individual variation in drug absorption, but the average elimination $t_{1/2}$ was 1.6 ± 0.78 h and average time to maximum concentration (1.25 ± 0.47 h) is similar to that previously reported [1,2]. These results demonstrate the clinical applicability of this assay technique.

REFERENCES

1 H. P. Ringhand, W. A. Ritschel, M. C. Meyer, A. B. Straughn and T. Hardt, *Int. J. Clin, Pharmacol. Ther. Toxicol.*, 25 (1982) 57.
2 H. G. Giles, E. A. Roberts, H. Orrego and E. M. Sellers, *J. Clin. Pharmacol.*, 21 (1981) 466.
3 D. S. Cooper, V. C. Saxe, F. Maloof and E. C. Ridgway, *J. Clin. Endocrinol. Metab.*, 52 (1981) 204.
4 K. Sato, H. Mimura, S. Kato, O. Isozaki, T. Tsushima and K. Shizume, *Acta Endocrinol.*, 104 (1983) 189.
5 D. S. Sitar, A. Abu-Bakare and R. J. Gardiner, *Pharmacology*, 25 (1982) 57.
6 B. Marchant, B. E. W. Brownlie, D. M. Hart, P. W. Horton and W. D. Alexander, *J. Clin. Endocrinol. Metab.*, 45 (1977) 1187.
7 R. H. Mortimer, S. A. Tyack, J. P. Galligan, D. A. Perry-Keene and Y. M. Tan, *Clin. Endocrinol.*, 32 (1990) 141.
8 G. R. Cannell, R. M. Kluck, S. E. Hamilton, R. H. Mortimer, W. D. Hooper and R. G. Dickinson, *Clin. Exp. Pharmacol. Physiol.*, 15 (1988) 837.
9 H. P. Ringhand and W. A. Ritschel, *J. Pharm. Sci.*, 68 (1979) 1461.
10 H. G. Giles, R. Miller and E. M. Sellers, *J. Pharm. Sci.*, 68 (1979) 1459.
11 C. Kim, *J. Chromatogr.*, 272 (1983) 376.
12 B. McArthur and J. Miceli, *J. Chromatogr.*, 278 (1983) 464.
13 J. Schulman, S. Riegelman, B. von Lehmann, A. Pilbrandt and C. Becker, *J. Pharmacokin. Biopharm.*, 1 (1973) 307.