Br. J. clin. Pharmac. (1983), **16**, 549–552

SERUM PROTEIN BINDING OF PROPYLTHIOURACIL

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Serum protein binding of propylthiouracil (PTU) was measured by ultrafiltration in healthy and hyperthyroid patients. The serum protein binding in 12 euthyroid subjects was 76.2 ± 1.2% (mean ± s.d.), not significantly different from the values in 10 hyperthyroid patients: 76.6 ± 1.3% Binding was unaffected by incubation time and temperatures between 25 and 37°C, but increased from 76.5 to 79.1% when pH changed from 7.4 to 7.9. PTU is predominantly bound to albumin with two classes of binding groups with different number of binding sites and affinity. Displacement experiments showed interaction with acetylsalicylic acid, warfarin and phenylbutazone, but not with antipyrine and nortriptyline or other basic drugs.

Keywords propylthiouracil protein binding

Introduction

Protein binding of propylthiouracil (PTU) and methimazole in serum seems to be a major factor in the evaluation of drug excretion into human milk (Kampmann *et al.*, 1980; Johansen *et al.*, 1982).

As no detailed information is available regarding serum protein binding characteristics of PTU or other antithyroid compounds we decided to measure the serum protein binding of PTU in euthyroid and hyperthyroid subjects and examine the binding characteristics as influenced by drug concentrations, pH, temperature and the presence of other drugs.

Methods

Serum from blood of human male volunteers aged 20 to 42 years was obtained by centrifugation and used within 30 min. Unlabelled PTU was prepared by dissolving grade pure PTU in distilled water, and labelled PTU with a specific activity of 0.76 mCi/mg was obtained from Amersham.

Serum (2 ml) was incubated for 30 min at room temperature with 0.15 μCi of ^{35}S-PTU (0.2 μg) and unlabelled PTU was added resulting in a final concentration of about 29.4 μmol/l (5 μg/ml). In the experiments studying the displacing effect of other drugs, the drug to be tested was added 15 min after the labelled PTU and incubated for further 30 min. To compensate for an evaporation of carbon dioxide, all samples were perfused for 15 min with a mixture of 5% carbon dioxide and 95% oxygen. This procedure resulted in a constant pH value of 7.4.

The serum protein binding was measured by ultrafiltration according to the method of Borgå *et al.* (1969), using a Visking dialysis tubing and yielding an ultrafiltrate of about 100 μl equivalent to 5% of the original solution.

The data were subjected to statistical analysis using Student's *t*-test. Protein binding characteristics were evaluated by Scatchard plots (Scatchard, 1949) modified according to Rosenthal (1967).

Results

The precision of the method was evaluated by measuring the same serum seven times in the same assay at pH 7.4. The bound fraction varied from 75.6% to 80.0% with a mean value of 77.7 ± 1.7%. Spontaneous variations of the free fraction was evaluated by measuring three subjects four times with intervals of 5 to 7 days. No significant differences were found among the three subjects or among the four values from the same subjects (76.4–79.5%, 75.3–79.9% and 75.6–78.2%, respectively).

Effect of incubation time

A serum sample was measured after 5, 30, 60 and 180 min of incubation. No influence of the incubation time was found as the serum protein binding was 75.7%, 75.1%, 74.9% and 75.3%, respectively.

Influence of temperature and pH

Five sera measured at 25°C and 37°C at two different pH-values (7.4 and 7.9) showed no differences, whereas the binding was significantly lower at pH 7.4 compared to 7.9 (76.5 ± 2.0% and 79.1 ± 1.6%), respectively.

Consequently all following results were obtained at room temperature and pH = 7.4.

Binding in euthyroid subjects

The serum protein binding measured in 12 healthy subjects varied between 74.5% and 78.1% with an average value of 76.2 ± 1.2%.

Binding in hyperthyroid patients

The serum binding in 10 hyperthyroid patients with a mean serum thyroxine of 21.8 μg/100 ml (281 nmol/l) and a mean serum triiodothyronine of 435 ng 100 ml (6.7 nmol/l) varied between 75.2% and 79.0% with a mean value of 76.6 ± 1.3%, not significantly different from euthyroid subjects.

Binding characteristics

Increasing concentrations of PTU up to about 200 μg/ml added to four sera increased the free fraction of PTU from about 20% to about 50%. A Scatchard analysis (Figure 1) showed that PTU has two classes of binding groups, one with a small capacity with a large affinity and another with great capacity and a small affinity. To investigate to which serum proteins PTU was bound a gel-electrophoresis in Tris-buffer (pH 8.4) was performed with concentrations of PTU varying from 14.7–73.5 μmol/l (2.5–12.5 μg/ml). PTU was bound predominantly to albumin as only about 5% was bound to prealbumin.

Displacement of propylthiouracil by other drugs

Table 1 shows the influence of some drugs on the serum protein binding of PTU. No effect was seen after addition of either antipyrine, nortriptyline, phenytoin or propranolol while a displacing effect with a significant increase in the free fraction of PTU was seen after the addition of acetylsalicylic acid, phenylbutazone and warfarin. A weak displacement was also observed after the addition of large amounts of methylthiouracil while no displacing effect was seen when methylthiouracil was added in concentrations twice those of propylthiouracil.

Discussion

The plasma protein binding of PTU has previously been preliminary reported by Giles and coworkers (1981) who in 10 euthyroid males found a free fraction of PTU varying from 16.2% to 20.2% with a median value of 18.2%. This value obtained by equilibrium dialysis was slightly smaller compared to our finding of a free fraction of 23.8 ± 1.2%. In contrast

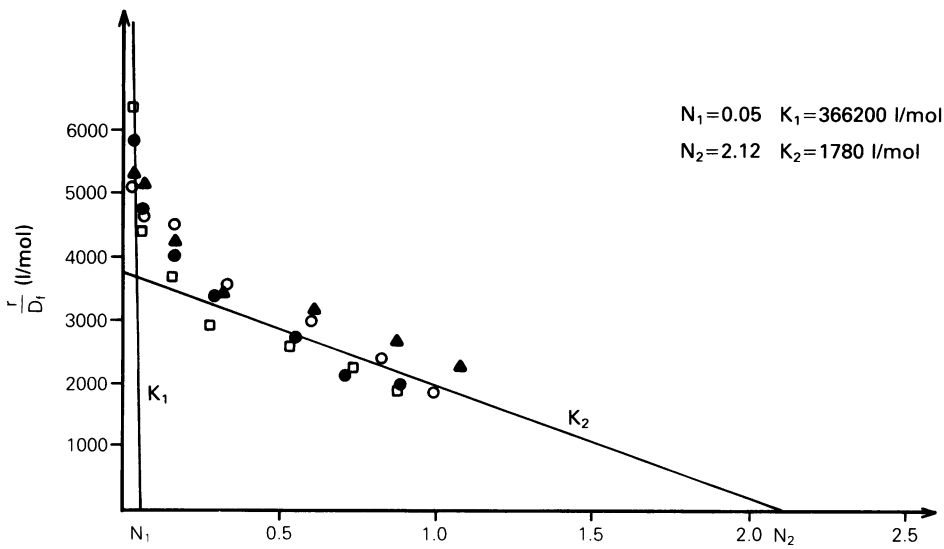

Figure 1 Binding characteristics of propylthiouracil in male human serum analyzed by a modified Scatchard plot (Rosenthal *et al.*, 1967). r denotes moles drug bound per total moles of protein, while D_f is free drug concentration. N is number of binding sites with the different association constants (K). The lines represent the average of the four individual computercalculated values.

Table 1 Displacement of propylthiouracil by other drugs. All values are mean ± s.d. of five measurements and compared with the same serum containing propylthiouracil only.

Drug (concentration)	*Free fraction*	*Significance*
Propylthiouracil (5 mg/l = 29.4 μmol/l)	23.3 ± 2.2%	—
Methylthiouracil (10 mg/l = 70.3 μmol/l)	23.3 ± 2.6%	NS
Methylthiouracil (100 mg/l = 703.2 μmol/l)	28.8 ± 1.5%	$P < 0.01$
Warfarin (100 mg/l = 324.4 μmol/l)	30.5 ± 1.7%	$P < 0.001$
Antipyrine (15 mg/l = 79.7 μmol/l)	23.7 ± 2.3%	NS
Phenylbutazone (50 mg/l = 162.1 μmol/l)	29.8 ± 2.3%	$P < 0.01$
Nortriptyline (100 μg/l = 379.5 nmol/l)	22.7 ± 2.0%	NS
Phenytoin (15 mg/l = 60 μmol/l)	25.0 ± 1.2%	NS
Propranolol (100 μg/l = 385.7 nmol/l)	22.7 ± 2.3%	NS
Acetylsalicylic acid (100 mg/l = 555 μmol/l)	34.2 ± 2.9%	$P < 0.01$

to these rather similar results Cooper *et al.* (1981) summarily reported a bound fraction of 67.2 ± 5.5% (s.e. mean) which gives a higher free fraction of more than 30%. This result was also obtained by equilibrium dialysis. It is difficult to compare the various results due to lack of methodological details, but both methods are normally satisfactory (Kurz *et al.*, 1977). A bound fraction of PTU around 80% is in contrast to methimazole which is completely unbound in serum (Johansen *et al.*, 1982).

Incubation time was unimportant when measuring binding. This is in agreement with previous measurements of rate constants between serum proteins and drugs (Froese *et al.*, 1962; Jansen, 1979).

The effect of pH was in agreement with previous results from studies of PTU binding to bovine serum albumin (Sakurai *et al.*, 1980) and from studies with other drugs such as salicylazo-sulphapyridine which also exhibited an increased binding in the therapeutic range with higher pH-values. No differences were found between hyperthyroid and euthyroid subjects which is in accordance with studies of Storstein (1977) and of Kelly & McDevitt (1978) concerning digitoxin, isoprenaline and propranolol, but in contrast to the study of Feely *et al.* (1981), who found a slight decrease in the protein binding of warfarin and propranolol in hyperthyroid patients.

Displacement interactions seem to assign PTU to binding site I for acidic drugs as warfarin displaced PTU (Sudlow *et al.*, 1976). No interaction was seen with the basic nortriptyline or neutral antipyrine.

Preliminary binding studies (Figure 1) suggested two classes of binding groups with different binding capacity and affinity. Variations in binding, however, is negligible in the levels normally obtained in clinical practice (up to 10 μg/ml).

We are grateful to Dr J.A. Jansen for assistance with the computer calculations of the binding characteristics.

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(Received June 6, 1983, accepted July 1, 1983)