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Bioavailability of propylthiouracil from two formulation tablets

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Bioavailability studies were performed for 50 mg propylthiouracil tablets (Jelfa, Poland) *versus* 50 mg propycil tablets (Solvay, Germany). An open-label, two-phase, crossover study was conducted with 15 healthy subjects. All subjects were randomly assigned a drug assignment number from I to XV, which was used throughout the experimental period. Dosing periods for both formulation tablets: Propylthiouracil, Jelfa vs. Propycil, Solvay were separated by at least 7 days washout period. Following single dose drug administration, venous blood samples were obtained at the required times for 12 h and the drug serum levels were determined by HPLC and used for PK analysis. PK parameters were calculated by the computer program TopFit 2.0. HPLC chromatograms show retention times for propylthiouracil and methylthiouracil (internal standard) of 5.97 and 2.75 min, respectively at 20 °C, providing adequate separation from each other and from endogenous serum components. Pharmacokinetic parameters for both tablets were not significantly different. Serum concentration-time profiles are superimposed for the above tablets according to an open one-compartment body model. EBA for Propythiouracil Jelfa tablets vs. Propycil tablets was 96.8%, and not significantly different. Some authors applied a two-compartment body model for the calculation of propylthiouracil pharmacokinetic parameters, which approach is not rational according to our data.

1. Introduction

Propylthiouracil is the first line antithyroid medication to control hyperthyroidism in patients with Graves'- Basedow disease. Remission is achieved primarily in the patients with mild forms of the disease, small goiters, and low titer of TRAb. The medication is given p.o. three times or twice a day for several weeks to render the patients euthyroid, with following gradual tapering to maintenance doses. Afterwards, propylthiouracil should not be discontinued for at least 12 to 24 months. The patients who develop recurrence despite proper medical management are recommended definitive methods of treatment such as radioactive iodine or surgery. A small amount of patients on antithyroid drugs may develop side effects in the form of allergic rashes (Gilbert 2017).

The total volume of distribution is about 30 l for propylthiouracil, which is about 80% protein-bound. It is excreted into breast milk only in small quantities, because it is less lipid-soluble than methimazole (virtually non-protein bound). Propylthiouracil has a half-life of 1-2 h with a clearance of around 120 ml/min/m^2 (Kampmann and Hansen 1981). The drug blocks the conversion of thyroxine to triiodothyronine within the thyroid and in peripheral tissues (Dokupilová and Payer 2013). A peak therapeutic serum concentration of above 4 mg/l is suggested in the treatment thyrotoxicosis (Kampmann and Hansen 1981). Early pharmacokinetic works found a rapid blood concentration decline followed by a more slow phase and therefore suggested a two-compartment model to be used (Kampmann and Skovsted 1974). Later publications provided arguments that a one-compartments body model should be used, because the slow phase of elimination appears after 5 h, when the serum levels of the drug were found minimal or undetectable and semi-logarithmic plot developed a linear terminal line, which indicates a one-compartment body model (Sitar and Hanninghake 1975). Other authors did not use any compartmental analysis (Ringhand et al. 1982; Alexander et al. 1969). The fraction absorbed of propylthiouracil after p. o. administration was found to be f = 0.77. It is a drug with a rapid absorption and elimination. The absorption rate constant ($k_a = 2.82\ h^{-1}$) is only two times greater than the first-order elimination rate constants ($k_e = 1.14\ h^{-1}$) (Kampmann and Skovsted 1974). It is also possible that the absorption and elimination half-lives are fairly similar for some of the subjects (Schuppan et al. 1973). A test formulation administered with and without food was bioequivalent to reference formulation. Food administration increased T_{max} and decreased bioavailability (C_{max} and AUC) (Mendes et al. 2014).

We conducted bioavailability studies of 50 mg propylthiouracil tablets (Jelfa, Poland) *versus* the reference tablets (50 mg Propycil, Solvay, Germany) in 15 healthy volunteers, in order to get a certificate for generic tablets to be used in medical practice. First, however, it was necessary to decide which compartment model should be used – one or two-compartment one. The TopFit 2.0 computer program was used for distinguishing between the above models (Heinzel et al. 1993).

2. Investigations and results

2.1. Study design

The study was conducted in compliance with the ethical principles of Good Clinical Practice and the latest version of the Declaration of Helsinki. The institutional review board of Human Investigations Ethical Committee at Poznan University of Medical Sciences approved the study protocol. The study was of a non-blinded, open-label, single dose, double way crossover design. The subjects randomly swallowed either a 50 mg propylthiouracil generic tablet or a 50 mg Propycil reference tablet according to a drug assignment number from I to XV (Table 1). Dosing periods were separated by at least a 7 days washout period.

Table 1: Crossover tablets administration

Number of subject	Order of tablet kind administration	
	Propylthiouracil (A)	Propycil (B)
I	A	B
II	A	B
III	A	B
IV	A	B
V	A	B
VI	A	B
VII	A	B
VIII	A	B
IX	B	A
X	B	A
XI	B	A
XII	B	A
XIII	B	A
XIV	B	A
XV	B	A

2.2. Subjects

Fifteen healthy, adult, non-smoking, male and female volunteers between 19 and 33 years (mean 24.5±5.3), weighing on average 66.33±12.91, 174±12 cm tall, were selected for participation in the above investigation. The volunteers were selected after completing a thorough history and physical examination, and after usual laboratory examinations including hematology, serum chemistry and urinalysis. All subjects were presented with full details of the investigation, both verbally and in written form, prior to providing written informed consent. Furthermore, the volunteers did not use any drugs and alcohol within 24 h before the experiments and during their course. The subjects were required to fast for at least 10 h prior to the timing of the next tablet administration.

2.3. Results

Calibration curves of peak area ratio *versus* authentic serum propythiouracil concentration were linear over the concentration range 0.025 to 5 mg/l and the intercept was essentially zero. The correlation coefficient for 40 independent determinations was 0.9989, and their precision lays within 3.4 and 11.9 %. The linear calibration curve equation is

$$\frac{A_{PTU}}{A_{IS}} = (2.79 \pm 0.13) \cdot C,$$

where A_{PTU} and A_{IS} are area under the peaks of propylthiouracil and methylthiouracil (IS), respectively, calculated by the chromatograph integrator, and C is concentration of authentic propylthiouracil. The lower limit of quantitation (LOQ) is 0.025 mg/l and the accuracy of the results is within 92 and 116 %. The limit of detection (LOD = 0.010 mg/l) is the injected amount of propylthiouracil that results in a peak with a height at least 2-3 times as high as the baseline noise level. The recovery of propylthiouracil is 69.3±4.1 % if it is compared for the method *versus* those obtained when the extraction step is omitted. The method used is also specific for the drug and its internal standard (methylthiouracil), because it provides adequate separation from each other and endogenous plasma components.

It was clear that the statistical analysis suggests the one-compartment model as more adequate than the two-compartment one to describe the pharmacokinetic profile of propyithiouracil serum concentration as a function of time (Table 2, Fig. 1).

Therefore, the propythiouracil concentrations (C) following the p. o. administration of a single tablet (Propylthiouracil, Jelfa or

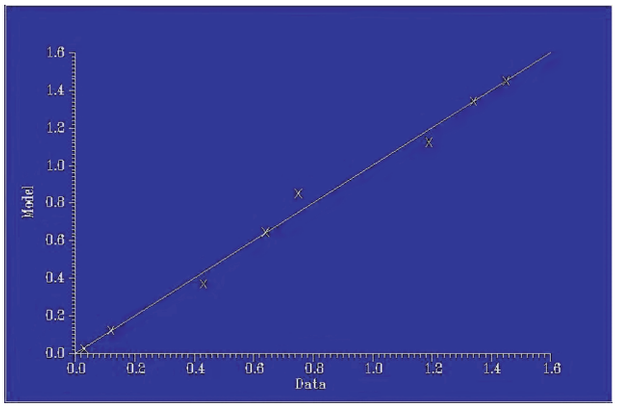

Fig. 1: Plot for the one-compartment body model versus data for the subject XIII after ingestion of a single 50 mg propylthiouracil tablet (Jelfa, Poland) generated by a computer program TopFit 2.0 (Heinzel et al. 1993)

Table 2: Tests for distinguishing between models (TopFit 2.0) for propylthiouracil serum levels as a function of time in subject XIV after per oral ingestion of 50 mg single Propylthiouracil, Jelfa tablet

Tests	0ne-compartment	Two-compartment
Quadratic error SSp	0.0183563	0.0166819
B-value r^2	0.997170	0.997428
Standard deviation SD	0.0677427	0.0913288
Akaike test AIC	-23.9823	-20.7475
Schwarz test SC	-23.6645	-20.2708
Imbimbo test Ib	0.179635	0.456432

Propycil, Solvay) (Table 3), absorbed and eliminated according the first-order process were well characterized by the difference in two exponentials (Fig 2):

$$C = B \cdot e^{-\lambda_2 \cdot t} - A \cdot e^{-\lambda_1 \cdot t}$$

where A and B are the corresponding zero-time intercepts, λ_1 and λ_2 are the apparent first-order fast and slow disposition rate constants, and t is the time. The averaged propylthiouracil serum data and their standard deviations as well the pharmacokinetic parameters are given in Table 3 for both tablets formulations.

Table 3: Mean serum concentrations of propylthiouracil (±SD, mg/l) from 14 subjects after the crossover single dose (50 mg) administration of propythiouracil, Jelfa and propycil, Solvay tablets, and suitable pharmacokinetic parameters calculated for a one compartment body model by the computer program TopFit 2.0

Time [h]	Propythiouracil, Jelfa	Propycil, Solvay	Statistics (ANOVA) α=0.05
0.33	1.12±0.54	1.26±0.80	NS
0.67	1.48±0.38	1.49±0.40	NS
1	1.50±0.59	1.36±0.36	NS
1.5	1.11±0.38	1.09±0.44	NS
2	0.93±0.36	0.85±0.45	NS
3	0.57±0.23	0.60±0.33	NS
5	0.23±0.15	0.24±0.21	NS
8	0.07±0.04	0.08±0.12	NS
12	0.02±0.02	0,01±0,02	NS
Pharmacokinetic	parameters		
AUC [mg (h l)$^{-1}$]	4.19±1.63	4.33±2.10	NS
C_{max} [mg/l]	1.62±0.43	1.77±0.45	NS
t_{max} [h]	0.69±0.30	0.54±0.40	NS
K [h^{-1}]	0.64±0,28	0.62±0,26	NS

Pharmacokinetic	parameters		
$t_{1/2}$[h]	1.22±0.33	1.27±0.40	NS
MRT_{tot}[h]	2,32±0,53	2,31±0,76	NS
T_{lag} [h]	0.16±0,10	0.17±0,14	NS
V_C/f [l]	23.5±8.9	23.2±13.9	NS
Clearance/f [ml/min]	225±80	231±99	NS
EBA [%]	96.8	100	NS

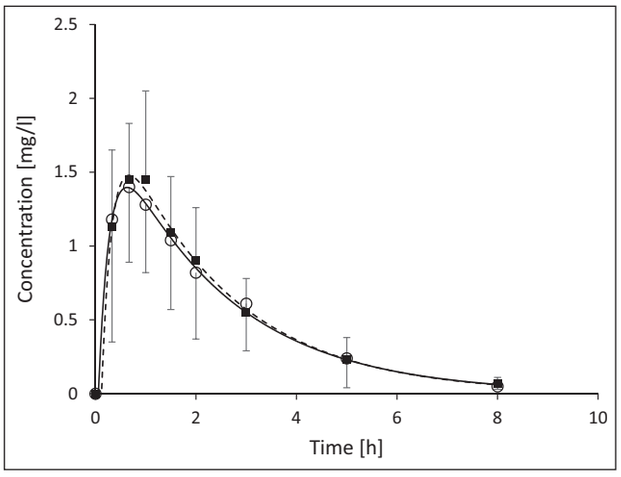

Fig. 2: Mean serum propylthiouracil concentrations (mg/l) as a function of time (t) after the crossover per oral administration of a single 50 mg either propycil (Solvay) or propylthiouracil (Jelfa) tablets to 14 healthy subjects, simulated by a computer program Phoenix WinNonlin 8.0 according to an open one-compartment body model with a lag time where error bars are shown and squares indicate propylthiouracil (Jelfa) and circles open propycil (Solway) observed concentrations

The terminal phase of propylthiouracil pharmacokinetic curve fulfils a monoexponential equation (Fig. 2), because the absorption phase is already negligible:

$$C' = C_{max} \cdot e^{-K \cdot t}$$

Where C` is the propylthiouracil concentration at time when the absorption of the drug can be neglected and the other parameters were provided previously. Semi-logarithmic equation of the above elimination phase is linear.

$$\cdot ln C' = ln C_{max} - K \cdot t$$

The slope of the above equation gives the overall first-order elimination rate constants (Table 2). The biological half-life time (Table 3) is calculated from the equation typical for the first-order processes:

$$t_{1/2} = ln 2 / K$$

Mathematical interpretations of the propylthiouracil fate in human body are assumed in the literature to be correct according to either one- (Kampmann and Hansen 1981; Dokupilová and Payer 2013) or two-compartment (Kampmann and Skovsted 1974) body models. Therefore, we were supposed to prove why we use the one-compartment model. Our decision was substantiated by statistical tools provided by the TopFit 2.0 computer program (Heinzel et al. 1993). The data obtained are provided in Table 2.

3. Discussion

We used a one-compartment body model to describe the fate of propylthiouracil in humans after per oral administration of two tablet formulation (a generic Propylthiouracil, Jelfa and Propycil, Solvay as a reference formulation). The differentiation between one and two-compartment body model has been done by the computer program TopFit 2.0. The statistical tests (Table 2) argue for one-compartment model, because most their values are lower than for two-compartment one. Figure 1 demonstrates also a linear relationship between the one-compartment model and the experimental data and its intercept is essentially zero. However, it should be mentioned that the propylthiouracil concentrations lower than 0.05 mg/l are usually slightly above the data typical for the one-compartment model (Fig. 2). It is a question if such low concentrations have any clinical advantage, since preliminary dose-response studies suggest a peak therapeutic serum concentration of above 4 µg/ml in the treatment of thyreotoxicosis (Kampmann and Hansen 1981). Our assay did not permit a quantitation of the drug below 25 ng/ml, where perhaps the two-compartment body model would operate. Nevertheless, the pharmacokinetic parameters were also calculated from the two-compartment model, but they were not satisfactory and their values were significantly different in the subjects used. The serum levels of propylthiouracil as a function of time in the subject XV were rejected, because they were significantly different if compared to the other subjects data (I –XIV, Table 3). The assay used in the publication where the two-compartment model was used is rather unspecific according to the authors own statement (a color reaction with 2,6-dichloroquinone-chlorimide) and its sensitivity is rather low (>1.5 µg/ml) (Kampmann and Hansen 1981). Our HPLC method is reliable, because the calibration curve is linear over the concentration range of 0.025-5 µg/ml and the intercept is essentially zero. Its lower limit of quantitation (LOQ) is 0,025 µg/ml, and is sufficiently good for bioavailability studies. The precision of the method is <15%, and its recovery 69.3±4.1 %. It provides an adequate separation of propylthiouracil from methylthiouracil (IS) and endogenous serum components and therefore is specific.

The pharmacokinetic data are not significantly different for both tablet formulations (Table 3). There is not a great difference between the first-order rate constants of absorption and elimination (2.84, 1.14, respectively) (Kampmann and Skovsted 1974). Therefore, a flip flop phenomenon could be observed and it was used to calculate some pharmacokinetic parameters according to the TopFit 2.0 program. We did not encourage ourselves to calculate the first-order absorption rate constants, because the propylthiouracil serum data were not available below 0.33 h (Table 3, Fig 2). Our mean elimination half-life time (1.25 h, Tab. 3) meets the data provided in the literature for normal humans 1.28 h (Kampmann and Skovsted 1974), 1.14 h (Sitar and Hanninghake 1975), and 1-2 h (Kampmann and Hansen 1981). Peak plasma concentration after 50 mg p. o. dose was 1.7 mg/l (Fig. 2), whereas in the case of 400 mg p. o. dose it reached 9.2 mg/l (Kampmann and Skovsted 1974) with a maximum time of 42 min versus 77 min in the literature (Kampmann and Skovsted 1974). Total volume of distribution was approximately 24 l which is very close to the literature data of 30 l) (Kampmann and Hansen 1981) or 21.9 l (Kampmann and Skovsted 1974). However, our clearance of 231 ml/min and is greater than provided earlier with 122 ml/min/m2 (Kampann and Skovsted 1974), but there are also higher values determined for five euthyroid subjects (367 ml/min). The hyperthyroid patients have a higher plasma clearance, which may be due to the first-pass effect (Schuppan et al 1973).

4. Experimental

4.1. Procedures

At zero hour the subjects were assigned to a phlebotomist to insert a heparin catheter for the purpose of collecting a 5 ml blood sample. The assigned tablet was swallowed with 200 ml water. All subjects abstained from food until 4-h blood specimen was obtained when a standardized low fat lunch was provided. Regular meals were resumed after 10-h blood samples were obtained. Following drug administration, venous blood samples were obtained (in Serum Gel tubes, S/4.7 ml, Sarstedt Monovette, Germany) from the subject's right or left antecubital fossa catheter at the following times: 0.33, 0.67, 1, 1.5, 2, 3, 5, 8 and 12 h after the administration. Within 30 min following blood withdrawal, the samples were centrifuged. The separated plasma samples were frozen in plastic vials at -20 °C and labeled with the subjects I.D. number, the drug assignment numbers, treatment day and times of sampling. The red blood cells were discarded.

4.2. Drug assay

A Hewlelt-Packard liquid chromatograph (HP 1100, Wien, Austria) with a UV detector, degasser, auto sampler, column thermostat and recorder, directed by a computer program Chem-Station, was coupled to 5 µm Lichrospher RP-18 column (240 x 4.6 mm). The analytical column was protected by a guard RP-18 column (4 x 4 mm, Merck, Darmstadt, Germany). Absorbance of eluent was monitored at 275 nm. A mixture of methanol-0.05 M phosphate buffer pH 7.4 (300:700 v/v) was filtered through 0.45 µm nylon-66 membrane and pumped at a rate 1 ml/min.

To each 0.2 ml subject serum sample 50 µl of 4 mg/l methanol methylthiouracil (internal standard), 100 µl 10% (ortho) phosphoric acid and 1 ml dichloromethane were added. After shaking for 10 min and centrifuging 3 min, the lower dichloromethane layer was aspirated and transferred to a disposable screw cap culture tube and evaporated to dryness under nitrogen flow at 35 °C. The residue was reconstituted in 30 µl of the mobile phase, and 20 µl was injected onto the column. To construct the calibration curve, stock solutions each 1 g/l of propylthiouracil and methylthiouracil were prepared in methanol. Then methanol standard solutions of propylthiouracil containing 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 mg/l, and methylthiouracil containing 4 mg/l were prepared from the above standard solutions. To 0.2 ml subject serum taken before drug administration was added 50 µl each of drug standard solutions and internal standard solution. The resulted serum concentrations of propylthiouracil were following: 0.025, 0.05, 0.125, 0.25, 0.5 and 5 mg/l and the methylthiouracil concentration was 1 mg/l in each standard solution. The further step of extraction was the same as above specified for subjects samples. Peak area ratios of propylthiouracil to methylthiouracil were plotted versus propylthiouracil concentration in mg/l, and the resulting calibration curve was used to calculate the propylthiouracil serum concentration of unknown subjects samples.

Products samples identified as Propylthiouracil 50 mg tablets, lot # 11297p, Pharmaceuticals S.A., Jelfa, Jelenia Góra, Poland as well as Propycil 50 mg tablets, lot # 6320407246, Solvay Arzneimittel Kali Chemie Pharma, Germany were received from Jelfa. Propylthiouracil and methylthiouracil authentic samples were obtained from either Jelfa or purchased from Sigma-Aldrich, Steinheim, Germany, respectively. Methanol (Merck, Darmstadt, Germany), phosphoric(V) acid 85% (P.O.Ch., Gliwice, Poland), methylene chloride (Sigma-Aldrich), potassium dihydrogen phosphate(V) (Xenon, Łódź, Poland) and disodium hydrogen phosphate(V) anhydrous (Fluka ChemieAG, Switzerland) were of HPLC (the first one) or reagent grade, respectively.

4.3. Pharmacokinetic analysis

The plasma propylthiouracil levels as a function of time were simulated according to an open one-compartment body model using TopFit 2.0 software (Heinzel el al. 1993). That computer program let us to calculate the following pharmacokinetic parameters: first-order overall elimination rate constant (K, h^{-1}), biological half-life time ($t_{1/2}$, h), area under concentration-time curve, (AUC, mg (h l)$^{-1}$), time to peak plasma concentration (t_{max}, h), peak plasma concentration (C_{max}, mg l^{-1}), total mean residence time (MRT_{tot}, h), volume of distribution of the central compartment (V_C, l), clearance (mg/min), initial serum drug concentration (C(0), mg/l), and lag time of absorption (T_{lag}, h). However, Fig. 2 was drawn by Phoenix WinNonlin 8.0 software, because a TopFit 2.0 software does not allow to mark error bars for the data point presented. The extent of bioavailability (EBA, %) was also calculated for Propylthiouracil tablets *versus* Propycil tablets. Statistical significance of propylthiouracil serum variations of the above two tablets was tested according to an ANOVA test by an Excel 13 program.

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Conflicts of interest are not declared.

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