Effects of Propylthiouracil on Urinary Metabolites of Cyclophosphamide in Rats

Kazuo Chijiiwa, Willem G. Linscheer, Krishan L. Raheja, and Chaidong Cho

Departments of Medicine [K. C., W. G. L.], Pharmacology [K. L. R.], and Pathology [C. C.], VA and Upstate Medical Centers, State University of New York, Syracuse, New York 13210

ABSTRACT

Our previous studies have shown a protective effect of propylthiouracil (PTU) pretreatment against the toxicity of cyclophosphamide (CP). The present study was undertaken to investigate the mechanism of the PTU protection. CP is metabolized by the cytochrome P-450 drug-metabolizing enzyme system in the liver to alkylating metabolites, to active antineoplastic agents, and to acrolein, the most toxic and least antineoplastic metabolite. Measurements of CP metabolites in blood and urine during a 4-hr i.v. infusion of CP (50 mg/kg body weight/hr) showed urinary acrolein excretion to be 2.5 times higher in control rats as compared to PTU-treated rats. Since it has been reported that urinary acrolein levels are directly related to the frequency and severity of hemorrhagic cystitis, it is concluded from our observations that prevention of hemorrhagic cystitis is probably mediated by the PTU effect on lowering urinary acrolein concentration and excretion. Serum alkylating activity was significantly higher in the PTU-pretreated rats, which may enhance the antineoplastic potential of CP.

INTRODUCTION

CP, widely used as an immunosuppressive agent and as a treatment for neoplastic diseases, has a number of toxic side effects (7, 8, 12, 17). CP is a prodrug and, like many other xenobiotics, is activated by the cytochrome P-450 DME system in the hepatocytes (4, 10, 30). There is general agreement that PPAM and 4-OH-CP are the most active alkylating antineoplastic metabolites (11, 15, 27, 32, 33), and acrolein, with little alkylating activity or antitumor effect, is considered to be the most toxic metabolite (7, 12, 17). Acrolein partly conjugates with GSH and is excreted as mercapturic acid derivative, but free acrolein is also released into the urine (2, 22). Except for hemorrhagic cystitis, it is difficult to determine which side effect is related to acrolein (12, 31). However, 2 precursors of acrolein, 4-OH-CP and aldophosphamide, are present in the blood (20, 33), and acrolein is probably released in multiple organ systems. If the toxicity of acrolein could be reduced, tolerance to CP would be improved, and higher therapeutic doses could be used.

We have studied previously in detail the protective mechanism of PTU against AAP toxicity (25, 28). Like CP, AAP is also activated by the cytochrome P-450 DME system, and a toxic metabolite is conjugated to GSH (26). Pretreatment with PTU leads to increased hepatic and renal GSH levels and prevents GSH depletion by AAP (25). In addition, Yamada et al. (36) showed conjugation of the toxic metabolite to PTU. Control rats developed extensive hepatic necrosis which could be prevented by PTU administration. Since acrolein is a highly reactive metabolite and also conjugates to GSH (2), we have studied the interaction of CP and PTU in order to evaluate the possibility that the protective effect of PTU is mediated by changing the metabolism of acrolein without affecting the most active antineoplastic metabolites, PPAM and 4-OH-CP. Rats were primed for 12 days with PTU incorporated in rat chow. [14C]-CP was then administered i.v. in control and PTU-treated rats for a 4-hr period while bile, blood, and urine samples were taken. CP and its metabolites were analyzed in these samples and in hepatic and renal tissues. In addition, GSH levels were determined in these organs.

MATERIALS AND METHODS

Male Wistar rats (Charles River, Wilmington, Mass.) weighing 225 to 250 g were divided on a body weight basis into 2 groups, a control and a PTU-pretreated group. Rats were housed in individual raised wire-screen cages in an air-conditioned laboratory with a 12-hr-light-12-hr-dark cycle. Both groups were fed ground Purina chow ad libitum for 12 days, but the chow of the PTU group contained 0.15% PTU as described previously (25).

The rats, following an overnight fast, were anesthetized with pentobarbital (50 mg/kg i.p.), and a tracheostomy was performed using PE 240 polyethylene tubing (Clay Adams, Parsippany, N. J.). The abdomen was opened, and cannulation of the bile duct was performed using PE 10 tubing. PE 10 tubing was inserted into the femoral vein for administration of 4-[14C]CP during a 4-hr infusion period. A cystostomy (PE 50 tubing with a purse-string suture) was used to obtain urine collections. NaCl solution (0.9%) was infused i.v. initially at the rate of 2.5 ml/hr for 30 min using a syringe pump (Sage Instruments model 255-1) and, during this period, bile, urine, and serum samples were collected for base-line determinations and background counts prior to CP infusion. The body temperature was maintained at 35 ± 1.0°C (S.E.) using a heating pad.

After 30 min of 0.9% NaCl solution infusion, both groups were then infused with an isotonic [14C]CP solution (1 μCi/10 ml) through the femoral vein catheter at the same rate of 2.5 ml/hr for a period of 4 hr. Each rat received a 200-mg/kg body weight dose of CP during the 4-hr infusion.

During the experiment, 15-min bile and 30-min urine collections were obtained. Blood was drawn from the tail every 30 min. Bile and urine were collected in preweighed glass tubes over ice. Volumes were determined gravimetrically assuming a specific gravity of 1.0. The urine and bile were stored at −70°C.

At the end of the experiment, all rats were bled by cardiac puncture. Liver, kidneys, and spleen were removed and weighed. A part of the
formalin for histological examination. The rest of the liver and kidneys was immediately frozen in acetone dry ice and stored at -70° for later analysis.

Total radioactivity of [14C]CP and of its derived metabolites in the liver, kidneys, serum, urine, and bile were determined. In addition, free CP (parent compound), total alkylating activity, and acrolein were determined in urine, bile, and serum. Creatinine clearance was derived from serum and urine creatinine determinations. Clearance of alkylating activity was measured similarly.

CP was obtained from Mead Johnson and Co., Evansville, Ind.; ring-labeled 4-[14C]CP (specific activity, 52.5 mCi/mmol) was obtained from New England Nuclear, Boston, Mass. The purity of both compounds, as tested by thin-layer chromatography and high-pressure liquid chromatography, was more than 98%.

Analytical Procedures. In order to determine total radioactivity, duplicate aliquots of bile (50 µl), serum (50 µl), and urine (10 µl) were dissolved in 10 ml of ACS scintillation fluid (Amerham-Searle Corp.) and counted in a liquid scintillation counter (Model 3385 Packard Tri-Carb Scintillation Spectrometer). Radioactivity in liver and kidney tissues was measured by homogenization in a known volume of 0.1 M phosphate buffer (pH 7.4) at 0°. An aliquot of the homogenate was then digested in Protosol (New England Nuclear, Boston, Mass.), neutralized by HCl, and counted. Radioactivity was corrected for background counts and for quenching by external standardization method. The rest of the homogenate was used for GSH determination by the method of Ellman (13). 14C-labeled free CP (parent compound) in urine, bile, serum, and tissues were measured by chloroform extraction according to the method of Bagley et al. (3). Free acrolein in urine, bile, and serum was determined by the method of Alarcon (1). Alkylating activity equivalents of nitroso nitrogen mustards in urine, bile, and serum were determined according to the method of Friedman and Boger (14) as modified by Juma et al. (21). Alkylating activity in liver tissue was determined by the method of Sladek (30).

Data and Statistical Analysis. Total radioactivity excreted in bile and urine was calculated as nmol equivalent for CP. Alkylating activity was expressed in terms of equivalents of nonnitrogen mustard. Values were expressed as the mean ± S.E. Data were analyzed using the unpaired Student’s t test or 2-way analysis of variance using Tektronix 4051 statistical program. Values with p < 0.05 were considered to be significant.

RESULTS

Our previous studies using similar PTU treatment schedule in male Wistar rats showed that PTU induced hypothyroidism (25).

No significant difference in bile flow (controls, 368 ± 29.2 µl/100 g body weight/hr versus 354 ± 39.8 µl/100 g body weight/hr in the PTU rats) or in urine production (controls, 109 ± 27.0 µl/100 g body weight/hr versus 86 ± 21.6 µl/100 g body weight/hr for PTU rats) was observed. Flow rates of bile and urine were constant during the 4-hr infusion period.

Effects of PTU on CP Metabolism

Serum Concentrations. CP i.v. infused was removed efficiently from the blood circulation. Serum concentrations of total radioactivity ([14C]CP and its labeled metabolites) reached a plateau with a maximum concentration of 0.46 ± 0.03 µmol/ml in the controls and 0.54 ± 0.06 µmol/ml in the PTU-treated animals (p < 0.05) (Chart 1). The concentration of free CP in serum was 0.33 ± 0.02 µmol/ml in the controls and 0.42 ± 0.04 µmol/ml in the PTU rats. There was no significant difference between the groups. However, total serum-alkylating activity was higher in the PTU rats (158 ± 10.8 nmol/ml in PTU rats versus 89 ± 10.3 nmol/ml in controls; p < 0.001) (Chart 2). Free acrolein concentrations in serum were not measurable by the very sensitive method of Alarcon (1).

Hepatic Metabolism. There is a low retention of CP in the liver (0.21 ± 0.03% in PTU rats and 0.15 ± 0.02% in controls). CP is rapidly metabolized as evidenced by the low percentage of the free parent compound in bile and urine (Charts 3 and 4).

Alkylating activity in liver tissue was not different between the 2 groups (155 ± 19.1 nmol/g liver in controls and 150 ± 12.6 nmol/g liver in PTU rats).

Biliary Excretion of CP and Metabolites. While free CP secretion rate was not affected by PTU, the release of labeled metabolites became significantly lower during the last 2 hr of infusion in the PTU rats (p < 0.01) (Chart 3). Alkylating activity became consequently lower in the PTU rats after 2 hr (p < 0.001). Free acrolein was not detectable in bile since it is conjugated to GSH. Due to high polarity of this compound, it cannot be measured by mass spectrometry (4). High-pressure liquid chromatography is not sensitive enough for measuring the conjugate in bile.

Renal Excretion of CP and Its Metabolites. Urinary excretion of unmetabolized CP was significantly lower in the PTU rats (p < 0.005; Chart 4). Total radioactivity representing the sum of free and metabolized CP was significantly higher in the controls (p < 0.01). Urinary excretion of alkylating activity was lower in PTU rats (p < 0.02; Chart 5). Total urinary excretion of alkylating activity was 12.4 ± 1.54 µmol/100 g body weight/4 hr in the
Effects of PTU Pretreatment on Hepatic and Renal GSH

PTU pretreatment resulted in significantly higher hepatic GSH concentrations compared to controls in the fasted state (3.03 ± 0.052 versus 2.18 ± 0.118 μmol/g in controls, p < 0.001). A significant decrease following CP administration was observed in the control group as well as in the PTU-pretreated group. Hepatic GSH concentrations after CP infusion, however, remained significantly higher in the PTU pretreatment group (1.66 ± 0.391 μmol/g) compared to controls (0.48 ± 0.096 μmol/g, p < 0.001). (Chart 7).

Renal GSH concentrations before CP administration were also significantly higher in the PTU group than in the controls (1.51 ± 0.095 μmol/g for the PTU group versus 1.18 ± 0.037 μmol/g for the controls, p < 0.001). CP administration reduced GSH levels in both groups, but PTU rats maintained higher renal GSH concentrations (0.83 ± 0.083 μmol/g) than did the control rats (0.52 ± 0.039 μmol/g, p < 0.001). (Chart 8).

Charts:

- Chart 3: Excretion rate of total 4-|C|CP and its metabolites and free CP (parent compound) in bile. Points, mean (n = 6); bars, S.E.
- Chart 4: Free CP (parent compound) in 30-min urine samples. Points, mean for 6 observations; numbers 1 to 8, time sequence of 30-min urine collections.
- Chart 5: Total alkylating activity in 30-min urine samples. Points, mean for 6 rats; numbers 1 to 8, time sequence of 30-min urine collections.
- Chart 6: Acrolein excretion rate in 30-min urine samples. Points, mean for 6 rats; numbers 1 to 8, time sequence of 30-min urine collections.
- Chart 7: Effect of PTU treatment on GSH in the fasted rat liver before and after CP administration.
Increased alkylation activity in serum and decreased activity in the urine of PTU-treated rats are probably related to PTU effect on renal clearance as shown by the determination of the creatinine clearance. It is most likely related to induced hypothyroidism (35).

Other sulfhydryl-containing compounds such as N-acetylcycteine, sodium 2-mercaptoethane sulfonate (mesna), and di-sulfiram have been reported to protect against cystitis (5, 6, 18, 23).

Hacker et al. (18) reported that disulfiram decreased mixed-function oxidation pathway and augmented the antitumor activity of CP against L1210 murine leukemia. Our results, showing an increase of alkylation activity in serum, indicate the possibility of potentiating the effectiveness of CP.

It has been concluded from these studies that PTU affects the metabolic pathways of CP resulting in a decreased incidence of hemorrhagic cystitis in a rat model. This is most likely related to its effect on urinary acrolein concentrations. Others have shown similar results with other sulfhydryl-containing compounds. The mechanism of the observations should be further elucidated and compared with the effects of other protective compounds.

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REFERENCES


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