INFLUENCE OF PROPIVERINE ON HEPATIC MICROSOMAL CYTOCHROME P450 ENZYMES IN MALE RATS

R. WALTER, C. ULLMANN, D. THÜMMLER, AND W. SIEGMUND

Department of Clinical Pharmacology of the Peter Holtz Research Center of Pharmacology and Experimental Therapeutics, University of Greifswald, Greifswald (R.W., C.U., W.S.); and Apogepha Arzneimittel GmbH, Dresden, Germany (D.T.)

(Received November 25, 2002; accepted February 19, 2003)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:
The bladder spasmolytics propiverine was shown to induce hepatic cytochrome P450 (P450) and aminopyrine and aniline oxidation in rats. To characterize the type of enzyme induction and its dose dependence, activities of seven hepatic microsomal P450-dependent monoxygenases were measured in 72 male LEW1A albino rats (body weight 236–295 g) after oral treatment with 0.5, 2, 6, and 60 mg/kg of propiverine hydrochloride for 5 days and compared with the effects of 40 mg/kg β-naphthoflavone, 10 mg/kg phenobarbital, and 20 mg/kg dexamethasone (each group, n = 8). CYP2B expression was measured by Western blotting. Furthermore, the inhibitory potency of propiverine on P450 enzymes was evaluated in competition assays with three most specific monoxygenases. Results show that Propiverine induced several monoxygenases and CYP2B expression dose dependently. The effects were well comparable with a phenobarbital-type inducer with 60 mg/kg being equipotent to 10 mg/kg phenobarbital. Furthermore, propiverine in low concentrations inhibited pentyresorufin O-dealkylase (for CYP2B) in vitro. In conclusion, propiverine is a phenobarbital-type inducer on hepatic P450 enzymes in rats in doses about 100-times above the therapeutic doses in man.

The benzilic acid derivative, propiverine \( \left[ \text{2,2-diphenyl-2(1-propoxy) acetic acid (1-methylpiperid-4-yl) ester} \right] \) (MICTONORM, Apogepha, Dresden), has been shown in controlled clinical trials to be effective in the treatment of children, adults, and elderly suffering from detrusor hyperreflexia and symptoms of an overactive bladder. Pharmacodynamic investigations showed anticholinergic and additional effects on calcium influx and calcium homeostasis in urinary bladder preparations, thus proving the dual mode of action of propiverine in relaxing detrusor smooth muscle. The drug is rapidly absorbed from the gastrointestinal tract, widely distributed, and highly bound to plasma proteins. Incomplete oral bioavailability is mainly caused by intensive first-pass metabolism. Propiverine undergoes \( N \)-oxidation of the piperidine moiety and dealkylation of the propyl side chain by enzymes of the hepatic microsomal drug-oxidizing system (for review, Madersbacher and Mürtz, 2001).

There is evidence from former animal studies that propiverine at higher doses increases the content of cytochrome P450 (P450) and the activities of aniline hydroxylase and aminopyrine demethylase in rat liver (Borchert et al., 1986; Wengler et al., 1989; Yamashita et al., 1990). Since most patients suffering from symptoms of overactive bladder are over 60 years and consumers of two and more concomitant drugs, information on potential enzyme-inducing or -inhibiting properties of a drug, which is subjected for chronic treatment, is required from studies in animals and man. Therefore, the influence of repeated oral administration of propiverine on the most important hepatic microsomal P450-dependent monoxygenases was measured in rats to evaluate its influence on drug metabolism and to identify the dose without effect on P450 enzymes. Propiverine hydrochloride was given in doses of 0.6, 2.0, 6.0, and 60 mg/kg. In man, propiverine hydrochloride is used in doses between 0.4 and 0.6 mg/kg.

Materials and Methods

Materials and Equipment. Propiverine hydrochloride was a gift from Apogepha (Dresden, Germany). β-Naphthoflavone was obtained from Sigma Chemicals (Steinheim, Germany), dexamethasone was obtained from Fluka (Buchs, Switzerland), and phenobarbital was purchased from Synopharm (Barsbüttel, Germany). Glucose 6-phosphate, glucose 6-phosphate dehydrogenase, and NADP were purchased from Roche Diagnostics (Mannheim, Germany); dextrorphan tartrate was obtained from ICN Biomedicals (Eschwege, Germany); and formaldehyde was purchased from Riedel-de-Haen (Hannover, Germany); 7-hydroxycoumarin was purchased from Sigma (St. Louis, MO); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris(hydroxymethyl)-aminomethan, and SDS were purchased from Carl Roth (Karlsruhe, Germany); 5-bromo-4-chloro-3-indolyl phosphate, nitroblue tetrazolium salt, and \( N,N' \)-dimethylformamid were obtained from Sigma-Aldrich (Schnelldorf, Germany); TWEEN 20 was purchased from BioRad (München, Germany); and formaldehyde was obtained from Riedel-de-Haen (Hannover, Germany). All other chemicals were obtained from Sigma Chemicals.

Liver tissue was homogenized with the Ultra-Turrax T25 (IKA Labortechnik, Staufen, Germany) and centrifuged with Centriflex H-401B and Centriflex T-1170 (Kontron, Neufahrn, Germany). Gel electrophoresis and Western blot were performed with the electrophoresis apparatus and Trans-Blot SD Semi-
Dry Transfer Cell (BioRad) on cellulose membranes Protran (Schleicher and Schuell, Dassel, Germany). The products of the enzyme assays were measured with the spectrophotometer Uvikon 931, the spectrofluorimeter SFM 25 (Kontron), and the gas chromatograph HP 5890 (Hewlett Packard, Palo Alto, CA), respectively.

Animals and Animal Treatment. Seventy-two male LEW/1A albino rats (body weight 236–295 g) were held under standard laboratory conditions in a life island box A 110, Flufrance (Wissous, France) with mass-air displacement, temperature 25°C, 12 h light/dark cycle with light on at 7:00 AM, with four rats per polycarbonate cage, bedding sniffs (Lage, Germany), and free access to R/M-H diet mattress and acidified water. Four weeks after adaptation to the laboratory conditions, the animals were randomly allocated to the following nine treatment groups (each n = 8): 1) control-1 for oral administration of 5 ml/kg distilled water, 2) control-2 for intraperitoneal injection of 2 ml/kg corn oil, 3–6) for oral treatments with 0.6, 2, 6, and 60 mg/kg of propiverine hydrochloride, 7) for intraperitoneal treatment with 40 mg/kg β-naphthoflavone, 8) for oral treatment with 10 mg/kg phenobarbital, 9) for intraperitoneal treatment with 20 mg/kg dexamethasone. The administrations were done between 7:00 and 8:00 Am of 5 days. Substances for intraperitoneal administration were given in 2 ml/kg corn oil, propiverine hydrochloride was dissolved for administrations in 10 ml/kg distilled water.

Twenty-four hours after the last administration and after overnight fasting, the animals were sacrificed by cervical dislocation and decapitation. After bleeding, a cannula was placed into the portal vein to remove the blood by perfusion with ice-cold saline after the liver had been dissected and weighted. The animal experiment had been approved by the Local Authorities according to the German Animal Protection Act.

Preparation of Microsomes. An adequate amount of the liver was homogenized in phosphate buffer and centrifuged at 9,000 × g for 30 min followed by 100,000 × g for 60 min. The microsomal pellets were re-suspended in phosphate buffer followed by centrifugation again at 100,000 × g for 60 min. Then, the microsomes were stored in aliquots for enzyme assays at least at −80°C (Orishiki et al., 1994).

Enzyme Assays. Microsomal protein content was measured with the biuret method and total microsomal P450 content according to Greim (1970). The enzyme activities of the following monooxygenases have been measured with methods adapted to our laboratory conditions: ethylresorufin O-dealkylase (EROD) and pentoxyresorufin O-dealkylase (PROD; Burke and Mayer, 1983), ethoxyresorufin O-deethylation (EROD; Greenlee and Poland, 1978), diazepam N-demethylation (DNMD; Andersson et al., 1994), dextromethorphan O-demethylation (DXDM; Schmid et al., 1985), nitrophenolhydroxylase (NPH; Reineke and Moyer, 1985), erythromycin N-demethylation (ERDM; Wrighton et al., 1985). In a final volume of 1.0 ml, microsomal protein (0.1 to 2 mg), the respective substrates (5 µM 7-ethylresorufin and 7-pentoxyresorufin, 0.5 mM 7-ethoxyresorufin, 0.2 mM dextromethorphan and diazepam, 0.1 M 4-nitrophenol and erythromycin) were incubated with an NADPH-regenerating system consisting of 0.25 to 1.0 mM NADP, 1.5 mM glucose 6-phosphate, 0.6 to 1.3 units glucose 6-phosphate dehydrogenase (final concentrations) at 37°C for 5 to 20 min. After stopping the reaction with trichloroacetic acid, methanol, or sodium hydroxide, the metabolites were measured with photometric (DNMD, NPH, ERDM, fluorometric (EROD, ECOD, PROD) or gaschromatographic (DXDM) methods. Blank samples with microsomes inactivated by denaturation before starting the reaction and samples for calibration were prepared by the same procedure and measured in one run with the samples obtained from the animal study.

Western Blot of CYP2B. Microsomal CYP2B content was determined by Western blot analysis; in the case of three control rats, animals were treated with 60 mg/kg of propiverine hydrochloride and 10 mg/kg of phenobarbital. Rat CYP2B1 (Daichi Pure Chemicals, Tokyo, Japan) and goat anti-rat IgG (Chemicon International, Temecula, CA) were used as antibodies.

Competition Assay with EROD, PROD, and ERDM. Competition assays with propiverine (0.2, 0.5, 1.0, 2.0 µM) were performed with EROD obtained from rats pretreated with β-naphthoflavone, with PROD from rats after phenobarbital treatment, and with ERDM from rats after dexamethasone treatment. The assays were performed with varying substrate concentrations to assess Km and Vmax of the enzyme kinetics.

Biometrical and Statistical Analysis. Means ± standard deviations (S.D.) are given. The statistical comparison was done with the U test according to Mann and Whitney with P < 0.05 as level of significance. K_m and V_max were assessed by nonlinear fitting of Michaelis-Menten plots using the computer program ORIGIN (OriginLab Corp., Northampton, MA).

Results

As expected, the activity of the following monooxygenases was increased after administration of standard enzyme inducers: PROD (36-fold), EROD (6-fold), ERDM (5-fold), and ECOD (3.4-fold) after phenobarbital; EROD (15-fold), ECOD (7-fold), and PROD (3.5-fold) after β-naphthoflavone; and ERDM (8-fold) after dexamethasone. Propiverine hydrochloride in doses of 0.6 and 2 mg/kg was without any significant influence on the P450 enzymes under investigation. After 6 mg/kg of propiverine hydrochloride, only the activities of EROD and ECOD were found to be marginally but significantly increased. At the highest dose of 60 mg/kg, however, the activities of DNMD, ECOD, EROD, and PROD were increased 1.5-, 4-, 5.4-, and 33-fold, respectively. The patterns of the changes in monooxygenase activities after 60 mg/kg of propiverine hydrochloride were similar to the situation after enzyme induction with 10 mg/kg of phenobarbital (Fig. 1). Furthermore, Western blot analysis showed bands of microsomal CYP2B2, which were comparable in density to the bands after phenobarbital induction (Fig. 2).

In competition assays with selected monooxygenases, it could be shown, that propiverine in concentrations up to 2.0 µM did not influence EROD and ERDM (data are not shown). PROD was inhibited in a mixed competitive/noncompetitive manner as shown in Fig. 3. The apparent K_m values were 6.17 ± 4.02 µM for the controls versus 2.44 ± 1.04 µM (p < 0.028) after incubation with 0.2 µM propiverine. The respective V_max were 1.00 ± 0.49 nmol/min × mg versus 0.19 ± 0.04 nmol/min × mg (p < 0.05).

Discussion

The experimental study to evaluate potential enzyme-inducing effects of propiverine were performed in rats which responded to standard enzyme inducers as expected: rifampicin/glucocorticoid type induction (pregnane X receptor induction) by dexamethasone resulted in manifold increase of the ERDM as described by Cooper et al. (1993). Induction according to the polycyclic aromatic hydrocarbon type (Ah receptor induction) by β-naphthoflavone was associated with marked elevation of EROD and ECOD and phe-
In conclusion, propiverine is a phenobarbital-type enzyme inducer on hepatic P450 enzymes in rats in doses about 100-times above the therapeutic doses of propiverine hydrochloride, significant influence on drugs, which are given together with propiverine and which are subjected to biotransformation and/or active transport, is not necessarily to be expected.

Many inducers are also inhibitors of the enzymes they induce (Fuhr, 2000). We observed that propiverine is a mixed competitive/noncompetitive inhibitor of PROD in vitro in concentrations, which are spasmolytic in isolated human urinary bladder and which are reached in serum after chronic treatment with 15 mg three times daily in man (Madersbacher and Mürtz, 2001). The possible clinical relevance of this observation is still unknown but limited to the small number of patients seems to be low since all effects on drug-metabolizing enzymes in rats were observed with daily doses much higher than the efficient therapeutic dose in man, which is 0.5 to 0.6 mg/kg. In rats, 60 mg/kg of propiverine hydrochloride were equipotent to 10 mg/kg of phenobarbital with regard to CYP2B (PROD) induction; 2 mg/kg had no effect but 6 mg/kg seemed to be borderline for P450 enzyme up-regulation. Furthermore, since enzyme-inducing doses of phenobarbital in man (1–3 mg/kg) are about 2- to 6-times higher than the therapeutic doses of propiverine hydrochloride, significant influence on drugs, which are given together with propiverine and which are subjected to biotransformation and/or active transport, is not necessarily to be expected.
References


