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

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ABSTRACT:
The bladder spasmyotics 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 monooxygenases 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 monooxygenases. Results show that Propiverine induced several monooxygenases 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 [(2,2-diphenyl-2(1-propoxy) acetic acid (1-methylpiperid-4-yl) ester] (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 demethylehase 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 monooxygenases 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); dextrophan tartrate was obtained from ICN Biomedicals (Eschwege, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, Missouri); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)aminomethan, and SDS were purchased from Carl Roth (Barsbüttel, Germany). Glucose 6-phosphate dehydrogenase, and NADP were purchased from Roche Diagnostics (Mannheim, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, Missouri); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)aminomethan, and SDS were purchased from Carl Roth (Barsbüttel, Germany). Glucose 6-phosphate dehydrogenase, and NADP were purchased from Roche Diagnostics (Mannheim, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, Missouri); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)aminomethan, and SDS were purchased from Carl Roth (Barsbüttel, Germany). Glucose 6-phosphate dehydrogenase, and NADP were purchased from Roche Diagnostics (Mannheim, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, Missouri); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)aminomethan, and SDS were purchased from Carl Roth (Barsbüttel, Germany). Glucose 6-phosphate dehydrogenase, and NADP were purchased from Roche Diagnostics (Mannheim, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, Missouri); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)aminomethan, and SDS were purchased from Carl Roth (Barsbüttel, Germany). Glucose 6-phosphate dehydrogenase, and NADP were purchased from Roche Diagnostics (Mannheim, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, Missouri); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)aminomethan, and SDS were purchased from Carl Roth (Barsbüttel, Germany). Glucose 6-phosphate dehydrogenase, and NADP were purchased from Roche Diagnostics (Mannheim, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, Missouri); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)aminomethan, and SDS were purchased from Carl Roth (Barsbüttel, Germany). Glucose 6-phosphate dehydrogenase, and NADP were purchased from Roche Diagnostics (Mannheim, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, Missouri); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)aminomethan, and SDS were purchased from Carl Roth (Barsbüttel, Germany). Glucose 6-phosphate dehydrogenase, and NADP were purchased from Roche Diagnostics (Mannheim, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, Missouri); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)aminomethan, and SDS were purchased from Carl Roth (Barsbüttel, Germany). Glucose 6-phosphate dehydrogenase, and NADP were purchased from Roche Diagnostics (Mannheim, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, Missouri); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)aminomethan, and SDS were purchased from Carl Roth (Barsbüttel, Germany). Glucose 6-phosphate dehydrogenase, and NADP were purchased from Roche Diagnostics (Mannheim, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, Missouri); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)aminomethan, and SDS were purchased from Carl Roth (Barsbüttel, Germany). Glucose 6-phosphate dehydrogenase, and NADP were purchased from Roche Diagnostics (Mannheim, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, Missouri); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)aminomethan, and SDS were purchased from Carl Roth (Barsbüttel, Germany).
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 LEW1A albino rats (body weight 236–295 g) were held under standard laboratory conditions in a life island box A 110, Flurflame (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 sniff (Lage, Germany), and free access to R/M-H diet sniff 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 \( \beta \)-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 9,000g for 30 min followed by 100,000g for 60 min. The microsomal pellets were re-suspended in phosphate buffer followed by centrifugation again at 100,000g 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 monoxygenases have been measured with methods adapted to our laboratory conditions: ethylesorufin O-dealkylase (EROD) and pentyresorufin O-dealkylase (PROD; Burke and Mayer, 1983), ethoxyxocumarin O-deethylalase (ECOD; Greenlee and Poland, 1978), diazepam N-demethylase (DNMD; Andersson et al., 1994), dextromethorphan O-deethylalase (DXDM; Schmid et al., 1985), nitrophenolhydroxylation (NPH; Reinke and Moyer, 1985), ethylbenzylamin N-demethylase (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-pentylresorufin, 0.5 mM 7-ethoxycumarin, 0.2 mM dextromethorphan and diazepam, 0.1 M 4-nitrophenol and ethylbenzylamin) 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), fluorimetric (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 \( \beta \)-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 \( K_m \) and \( V_{max} \) 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 monoxygenases 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 \( \beta \)-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, NPH, ERDM, and PROD were increased 1.5-, 4-, 5.4-, and 33-fold, respectively. The patterns of the changes in monoxygenase 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 monoxygenases, 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 \( \beta \)-naphthoflavone was associated with marked elevation of EROD and ECOD and phe-
Chronic treatment of male rats with propiverine hydrochloride induced dose dependently several microsomal P450-dependent monoxygenases. Since the pattern of changes after administration of the highest dose of 60 mg/kg was very similar to the changes caused by phenobarbital, propiverine belongs obviously to the group of the phenobarbital-type enzyme inducers, which influence about 50 genes (Frueh et al., 1997). With regard to cytochrome P450 enzymes, the most pronounced effect is exerted on CYP2B6. Marked effects were also found for CYP2C8, CYP2C9, CYP3A4, CYP1A2, and some UGTs. Human CYP2C19 or CYP2D6 are not influenced (for review, Fuhr, 2000).

The results of our study are in line with the changes expected after administration of a phenobarbital-type inducer. PROD and EROD, which are dependent after phenobarbital induction mainly on CYP2B activity, were 33-fold, respectively 5.4-fold induced (Alterman et al., 1994; Burke et al., 1994). The erythromycin demethylation (ERDM), which is catalyzed by CYP3A, was 5-fold elevated (Zhang and Thomas, 1996) and ECOD, an N-demethylation and O-depentylase activity of male rats.

The risk to induce enzymes of drug metabolism and/or transport in 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.

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 Mürz, 2001). The possible clinical relevance of this observation is still unknown but limited to the small number of drugs which are metabolized by CYP2B enzymes.

In conclusion, propiverine is a phenobarbital-type enzyme inducer on hepatic P450 enzymes in rats in doses about 100-times above the therapeutic doses in man.

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References


