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

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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 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 pentoxyresorufin 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, propiverine1 [(2,2-diphenyl-2(1-propoxy) acetic acid (1-methylpiperid-4-yl) ester] (MICTONORM, Apogetha, 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 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 Apogetha (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). Acrylamide, tetramethyl ethylendiamine, 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. Furthermore, 0.1 M phosphate buffer (KH2PO4/NaH2PO4) pH 7.4 was used.

Liver tissue was homogenized with the Ultra-Turrax T25 (IKA Labortechnik, Staufen, Germany) and centrifuged with Centrikon H-401B and Centrikon 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 spectrophuorimeter 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, 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 ssniff (Lage, Germany), and free access to R/M-H diet ssniff 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 destilled 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. The statistical comparison was done with the 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).

**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 pentylresorufin O-dealkylase (PROD) (Burke and Mayer, 1983), ethoxyccumarin O-deethylase (ECOD; Greenlee and Poland, 1978), diazepam N-demethylase (DNMD; Andersson et al., 1994), dextromethorphan O-demethylase (DXDM; Schmid et al., 1985), nitrophenolhydroxylase (NPH; Reinke and Moyer, 1985), erythromycin 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-ethoxyccumarin, 0.2 mM dextromethorphan and diazepam, 0.1 M 4-nitrophe-nol 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 (Daiichi 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 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
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 demethylolation (ERDM), which is catalyzed by CYP3A, was 5-fold elevated (Zhang and Thomas, 1996) and ECOD, an monooxygenase dependent on CYP3A, was about 4-times enhanced (Edwards et al., 1984). However, DNDM, which is mainly catalyzed by enzymes of the CYP2C families was only marginally (1.5-fold) changed (Yasumori et al., 1993). Furthermore, as expected for a phenobarbital-type inducer, the demethylolation of dextromethorphan (DXDM), a substrate of CYP2D (Kronbach et al., 1987) and the hydroxylolation of 4-nitrophenol (NPH), a substrate of CYP2E1 (Tassaneeyakul et al., 1993; Amato et al., 1998), were not influenced by propiverine.

Because of that specific influence on microsomal monoxygenases, propiverine is considered to be a ligand of the constitutive androstane receptor (CAR), which is the biochemical mechanism behind phenobarbital induction. Similar to phenobarbital, it might translocate CAR to the nucleus where it heterodimerizes with the 9-cis-retinoic acid receptor α (RXRα). This complex binds to the phenobarbital response element in gene promoter regions and enhances gene transcription, as initially described for CYP2B6 (Zelko and Negishi, 2000). However, recent experiments have shown that there is a cross talk with other nuclear receptors. CAR/RXRα may bind to distinct response elements in gene promoters [e.g., to the binding site of the PXR/RXRα complex, which has been shown to mediate effects of the rifampicin/glucocorticoid type induction (Lehmann et al., 1998)]. Thus, CAR/RXRα can bind to PXR response elements and induce the expression of CYP3A4, which is normally regulated by PXR/RXRα (Xie et al., 2000). This is consistent with the observation that phenobarbital exerts a wide range of effects on enzymes of the drug biotransformation and other processes, most likely also on drug transporter proteins. According to the current knowledge on the function of the nuclear receptors PXR and CAR, inducing effects are expected on multidrug resistance gene 1 (ABCB1; Geick et al., 2001), organic anion transporters 2 (Slc21a5; Guo et al., 2002), multidrug resistance protein 2 (ABCC2; Kast et al., 2002), and multidrug resistance protein 3 (ABCC2; Cherrington et al., 2002). Compared with other types of induction, however, high molar concentrations are required to achieve phenobarbital-type induction (Lehmann et al., 1998). Since propiverine was very similar to phenobarbital with regard to the pattern of monoxygenase induction, the clinical significance of potential interactions with CAR- and/or PXR-regulated pharmacokinetic processes of other drugs has to be carefully evaluated in man.

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 spasmodolytic 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 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


