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 oxidations 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 pentylresorufin 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\(^1\) [(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 Mürzt, 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 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); dextromethorphan tartrate was obtained from ICN Biomedicals (Eschwege, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, MO); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)-aminomethane, and SDS were purchased from Carl Roth (Barsbüttel, Germany); and formaldehyde was obtained from Riedel-de-Haen (Hannover, Germany). Glucose 6-phosphate dehydrogenase was obtained from Fluka (Buchs, Switzerland), and phenobarbital was purchased from Synopharm (Barsbüttel, Germany). Acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)-aminomethane, and SDS were purchased from Carl Roth (Barsbüttel, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, MO); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)-aminomethane, and SDS were purchased from Carl Roth (Barsbüttel, Germany); 7-hydroxycoumarin was purchased from Sigma-Aldrich (St. Louis, MO); acrylamide, tetramethyl ethylenediamine, ammonium persulfate, tris-(hydroxymethyl)-aminomethane, and SDS were purchased from Carl Roth (Barsbüttel, Germany). All other chemicals were obtained from Sigma Chemicals. Furthermore, 0.1 M phosphate buffer (KH\(_2\)PO\(_4\)/Na\(_2\)HPO\(_4\)) 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) and centrifuged with Centrikon H-401B and Centrikon T-1170 (Kontron, Neufahrn, 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 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, Fluffrance (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 snuff (Lage, Germany), and free access to R/M-H diet snuff and acidified water. Four weeks after adaptation to the laboratory conditions, the animals were randomly allocated to the following three 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 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), ethoxyccoumarin O-deethylation (ECOD; Greenlee and Poland, 1978), diazepam N-demethylation (DNMD; Andersson et al., 1994), dextromethorphan O-demethylation (DXDM; Schmid et al., 1985), nitrophenolhydroxylase (NPH; Reinke and Moyer, 1985), erythromycin N-demethylation (ERDM; Wrighton et al., 1985). In a final volume of 1 ml microsomal protein (0.1 to 2 mg), the respective substrates (5 μM 7-ethylresorufin and 7-pentylresorufin, 0.5 mM 7-ethoxyccoumarin, 0.2 mM dextromethorphan and diazepam, 0.1 M 4-nitrophenol and erythromycin) were incubated with an NADPH-regenerating system inside a mixed competitive/noncompetitive manner as shown in 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 Km 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 values 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-
Propiverine, a phenobarbital-type inducer, is known to affect various hepatic P450 enzymes. The effects of propiverine were observed in rats treated with 10 mg/kg of phenobarbital. The risk to induce enzymes of drug metabolism and/or transport in patients seems to be low since all effects on drug-metabolizing enzymes were 33-fold, respectively 5.4-fold induced (Alterman et al., 1994). The erythromycin demethylation (ERDM) and the hydroxylation 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).

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 on phenobarbital induction mainly on CYP2B activity, were 33-fold, respectively 5.4-fold induced (Alterman et al., 1994; Burke et al., 1994; Zhang and Thomas, 1996). The erythromycin demethylation (ERDM), which is catalyzed by CYP3A, was 5-fold elevated (Zhang and Thomas, 1996) and ECOD, an monoxygenase dependent on CYP1A1, CYP2A, CYP2B, CYP2C, CYP3A, was about 4-times enhanced (Edwards et al., 1984). However, DNMD, 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 demethylation of dextromethorphan (DXDM), a substrate of CYP2D (Kronbach et al., 1987) and the hydroxylation of 4-nitrophenol (NPH), a substrate of CYP2E1 (Tassaneeyakul et al., 1993; Amato et al., 1998), were not influenced by propiverine.

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