Short Communication

Rat Cytochrome P450 2C11 in Liver Microsomes Involved in Oxidation of Anesthetic Agent Propofol and Deactivated by Prior Treatment with Propofol

Received June 25, 2006; accepted August 4, 2006

ABSTRACT:

Propofol (2,6-diisopropylphenol) is a widely-used anesthetic agent attributable to its rapid biotransformation. Liver microsomal cytochrome P450 (P450) isoforms involved in the biotransformation of propofol in rats and the effects of propofol in vivo on P450 levels in rats were investigated. Of six cDNA-expressed rat P450 isoforms tested, CYP2B1 and CYP2C11 had high catalytic activities from 5 μM and 20 μM propofol concentrations, respectively. Rates of propofol metabolism, at a substrate concentration of 20 μM based on the reported human blood concentration, were decreased by propofol metabolism, at a substrate concentration of 20 μM based on the reported human blood concentration, were decreased by propofol, has an important role in propofol metabolism in rat liver microsomes. Repeated administration of propofol could markedly decrease the biotransformation of propofol via P450 deactivation.

Materials and Methods

Chemicals. Propofol (Diprivan injectable emulsion) was purchased from AstraZeneca (Osaka, Japan). An in vitro substrate source, propofol (2078-54-8) was also obtained from Sigma-Aldrich (St. Louis, MO). The other chemicals and reagents used were obtained in the highest grade available commercially.

Enzyme Preparations. Male and female Wistar rats (7 weeks old) were obtained from Japan SLC (Hamamatsu, Japan). Male rats were treated intraperitoneally with typical P450 inducers including β-naphthoflavone (50 mg/kg, CYP1A), phenobarbital (80 mg/kg, CYP2B and CYP3A), and dexamethasone (50 mg/kg, CYP3A) daily for 3 days (Yamazaki et al., 2001a,b). Some rats were treated with propofol (10 mg/kg) via intraperitoneal administration three times in a half-day. In separate experiments, propofol (10 mg/kg) was administered intraperitoneally to male and female rats once or twice every 4 h. This interval (of 4 h) was based on 10 times as long as the reported half-life of propofol (~20 min at 10 mg/kg/h) (Hamaoka et al., 1999). Liver microsomes from these rats were prepared 4 h after the final treatment of propofol. These studies were approved by the Committee on the Care and Use of Laboratory Animals for Showa Pharmaceutical University. Recombinant rat P450 isoforms expressed in microsomes of insect cells (Supersomes) were obtained from BD Gentest (Woburn, MA). Catalytic activities by those P450 enzymes are provided in the data sheets by the manufacturer.

Enzyme Assays. Disappearance rates of propofol were determined according to the high-performance liquid chromatography method described previously (Dowrie et al., 1996) with minor modifications. In brief, the typical incubation mixture of a total volume of 0.25 ml contained microsomal protein (0.025 mg) or recombinant P450 (8 pmol), 20 μM propofol, and an NADPH-generating system in 0.1 M potassium phosphate buffer (pH 7.4) unless

This work was supported in part by the Ministry of Education, Science, Sports and Culture of Japan, The Research Foundation for Pharmaceutical Sciences, and Japan Research Foundation for Clinical Pharmacology.

Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.

doi:10.1124/dmd.106.011627.

ABBREVIATIONS: P450, cytochrome P450; ANOVA, analysis of variance.
Specified. The principal propofol concentration of 20 μM was chosen because of the blood concentration in humans (McKillop et al., 1998). Propofol protein binding (>90%) was not considered in our in vitro work. Incubations were carried out for 10 min at 37°C. The reaction was terminated by adding 4.0 ml of pentane containing 0.1 μg of 2-tert-butyl-6-methylphenol/ml. After vortex mixing, the tubes were centrifuged at 1200g for 10 min. The organic phase was transferred to a clean tube and evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was dissolved in 0.1 ml of mobile phase. The liquid chromatography system consisted of a pump and an electrochemical detector (L-ECD-6A; Shimadzu, Kyoto, Japan) using an analytical C18 reversed-phase column (150 × 4.6 mm). The mobile phase was acetonitrile/0.028 M phosphate buffer (pH 2.8) (60:40 v/v), at a flow rate of 1.5 ml/min.

Activities of ethoxyresorufin O-deethylation (CYP1A), pentoxyresorufin O-depentylation (CYP2B), and testosterone 16α-, 16β-, and 6β-hydroxylation (CYP2B, CYP2C, and CYP3A, respectively) were determined as described previously (Yamazaki et al., 2001a,b). The concentrations of total P450 (Omura and Sato, 1964), CYP2C (Shimada et al., 1994), and NADPH-P450 reductase (EC 1.6.4.24) (Parikh et al., 1997) were determined as described previously.

Statistical Analysis. Statistical analysis was carried out using the computer program Instat (GraphPad Software, San Diego, CA). One-way ANOVA with Dunnett’s post hoc test was performed for analysis of repeated intravenous administrations of propofol (p < 0.05).

Results and Discussion

Six recombinant rat P450 isoforms were used to determine which P450 isoforms were active in catalyzing the propofol metabolism (Table 1). Based on the reported plasma concentrations of propofol (20 μM) after treatment in humans (McKillop et al., 1998), substrate concentrations of 5 and 20 μM were used in this study. CYP2C11 and CYP2B1 showed high activities of propofol metabolism at both substrate concentrations. However, CYP1A2, CYP2D1, CYP2E1, and CYP3A2 showed low activities under the present conditions.

Among typical P450 inducers administered intraperitoneally to male rats, phenobarbital caused the most induction of propofol metabolism when 20 μM propofol was used as a substrate (Fig. 1A). In contrast, significantly decreased propofol metabolism by propofol treatment itself (10 mg/kg) was observed. To examine in detail whether propofol decreased propofol biotransformation, rats were treated intravenously once or twice with propofol (10 mg/kg every 4 h). Rates of propofol metabolism in male rats were also decreased by an intravenous propofol treatment in liver microsomes from rats (Fig. 1B). This suppression of the oxidative metabolism was dependent on the repeated intravenous propofol treatments. In contrast, liver microsomes from female rats had low and unaffected propofol metabolism in the propofol treatments. These results suggested that constitutive male-specific CYP2C11 and inducible CYP2B isoforms had important roles for propofol metabolism in rat liver microsomes. Because we used limited rat recombinant P450 isoforms (Table 1), it should be mentioned that other major CYP2B (but not female-specific CYP2C12) or CYP2B isoforms might be expected to contribute to propofol metabolism in rats. Accordingly, inhibitory effects of propofol on CYP2B and CYP2C activities, but not CYP3A, were seen when propofol and testosterone were coincubated with rat liver microsomes (Table 2).

Intravenously administered propofol significantly decreased total P450 and CYP2C contents in male rat liver microsomes (Fig. 2A). Propofol also decreased testosterone 16α-hydroxylation activities (CYP2C) in male rat liver microsomes (Fig. 2B), consistent with the immunochemical results. Repeatedly administered propofol (10 mg/kg twice every 4 h) also significantly decreased testosterone 16β-hydroxylation and pentoxyresorufin O-depentylation activities (CYP2B) in male rat liver microsomes. A similar decrease of ethoxyresorufin O-deethylation activities was seen. However, there were no changes with regard to the NADPH-P450 reductase levels or CYP3A-mediated testosterone 6β-hydroxylation activities in rat liver
and CYP3A, respectively) were determined. Data are mean ± S.D. for three rats untreated or treated once (×1) or twice (×2) with intravenous administration of propofol (10 mg/kg) every 4 h. Significantly different from controls by one-way ANOVA with Dunnett’s post hoc test (×, p < 0.05).

microsomes by intravenously administered propofol. Total P450 contents in liver microsomes from female rats were not affected by the propofol treatments (data not shown).

It has been proposed that propofol is able to exert a protective action against oxidative stress caused by free radicals in the liver (Navapurkar et al., 1998). This could be due to a restoration of the protective effect of glutathione (Aarts et al., 1995). With regard to modulating P450 functions, there is no information for the induction or suppression of individual P450 isoforms by propofol so far. Here we show that intravenously administered propofol efficiently deactivates the CYP2C, followed by CYP2B, as determined. Total P450 contents and drug oxidation activities catalyzed by CYP2C11 and CYP2B1 were also decreased, supporting the other evidence for P450 deactivation.

This is the first report, to our knowledge, of the deactivation of P450, especially CYP2C isoforms, by propofol. With regard to bioactivation via secondary oxidation of hydroxylated metabolites, methylphenol has been reported to be activated as evidenced by its glutathione adducts (Yan et al., 2005). In our preliminary study, effects of glutathione (3 mM) on propofol metabolism by rat liver microsomes were not seen under the present standard conditions. Although the mechanism of rapid decrease of the P450 contents caused by propofol is not clear, suppression of stability of CYP2C protein, down-regulation of CYP2C genes, impairment of oxidative phosphorylation, and/or enhancement of degradation of CYP2C might be the candidate factors involved in the phenomena.

In conclusion, we showed that CYP2C11, deactivated by propofol, has an important role in liver microsomal propofol metabolism in rats. The present results suggest that this anesthetic agent propofol not only suppresses CYP2C11 expression in male rats but also directly inhibits the metabolism of several P450 enzymes. Chronic administration of propofol would affect the pharmacokinetics of propofol itself and other coadministered drugs via P450 deactivation. These findings may be useful for the basic understanding of propofol-induced drug interactions.

Acknowledgments. We thank Drs. Yoshiaki Matsumoto and Takahiko Aoyama for their help.

Laboratory of Drug Metabolism and Pharmacokinetics,
Showa Pharmaceutical University,
Machida, Tokyo, Japan

HIROSHI YAMAZAKI
MAKIKO SHIMIZU
TAKASHI NAGASHIMA
MASAKI MINOSHIMA
NOBIE MURAAMA

References

Address correspondence to: Prof. Dr. Hiroshi Yamazaki, Showa Pharmaceutical University, 3-3165 Higashitamagawa Gakuen, Machida, Tokyo 194-8543, Japan. E-mail: hyamazaki@ac.shoyaku.ac.jp