

Rat cytochrome P450 2C11 in liver microsomes involved in oxidation of anesthetic agent propofol and deactivated by prior treatment with propofol¹

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Running title: **CYP2C11 deactivation by propofol**

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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 μ M and 25 μ M propofol concentrations, respectively. Rates of propofol metabolism, at a substrate concentration of 20 μ M based on the reported human blood concentration, were decreased by intraperitoneal treatment of propofol with male rats, in contrast to a strong induction by phenobarbital. Single intravenously administered propofol (10 mg/kg) caused the decrease of total P450 and CYP2C contents and activities of testosterone 16 α -hydroxylation and propofol metabolism in liver microsomes from male rats. The suppressive effects were caused by administered propofol (10 mg/kg) twice in every 4 h on CYP2B activities such as testosterone 16 β -hydroxylation or pentoxyresorufin *O*-depentylation, in addition to the strong suppression of CYP2C function by the single propofol treatment. These results suggest that CYP2C11, presumably deactivated 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.

Introduction

Propofol (2,6-diisopropylphenol) is administered as a bolus for the induction of anesthesia and as an infusion for maintenance of anesthesia or for sedation (Bryson, et al., 1995). One of the major advantages of this drug over other injectable anesthetic agents is the rapid and complete recovery that occurs even after relatively prolonged intravenous infusions (Mandsager, et al., 1995). This property is attributable to rapid and extensive biotransformation of the parent compound, primarily by the liver. The relative contribution of individual metabolic pathways has been found to vary among species and humans (Simons, et al., 1991; Sneyd, et al., 1994). Although there are several reports of the propofol pharmacokinetics or drug interactions in humans (Court, et al., 2001; Guitton, et al., 1998; Hamaoka, et al., 1999; McKillop, et al., 1998; Oda, et al., 2001; Tanaka, et al., 2004), the precise roles of cytochrome P450 (P450) isoforms in the propofol disposition are still unknown. Moreover, there is no report of the effects of propofol on P450 induction or deactivation as a determinant factor of the pharmacodynamic and/or pharmacokinetics of propofol. There has been reported a significant association between receiving a long-term and high-dose propofol infusion and developing progressive myocardial failure (Bray, 1998).

In the present study, the roles of rat P450 isoforms involved in propofol metabolism were investigated with recombinant rat P450 isoforms and rat liver microsomes mainly at a substrate concentration of 20 μ M, based on the human plasma concentration (McKillop, et al., 1998). The effects of P450 deactivation by propofol on the activities of propofol oxidation in rat liver microsomes were also investigated.

Materials and Methods

Chemicals. Propofol (Diprivan injectable emulsion) was purchased from Astra

Zeneca (Osaka, Japan). As an *in vitro* substrate source, propofol (2078-54-8) was also obtained from Sigma-Aldrich (St. Louis, MO, USA). 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 intraperitoneally treated 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; 2001b). 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 intravenously administered to male and female rats once or twice in every 4 h. This interval (of 4 h) was based on ten-times as long as a 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, USA). 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. Briefly, the typical incubation mixture of a total volume of 0.25 mL contained microsomal protein (0.025 mg) or recombinant P450 (8 pmol), 20 μ M propofol, and an NADPH-generating system in 0.1 M potassium phosphate buffer (pH 7.4) unless specified. Principal propofol concentration of 20 μ M was chosen because of the blood concentration in humans (McKillop, et al., 1998). Propofol protein binding (> 90%)

was not considered in our *in vitro* works. 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 2-*tert*-butyl-6-methylphenol/mL. After vortex mixing, the tubes were centrifuged at 1,200g 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*-deptylation (CYP2B) and testosterone 16β-, 16α-, and 6β-hydroxylation (CYP2B, CYP2C, and CYP3A, respectively) were determined as described previously (Yamazaki, et al., 2001a; 2001b). The concentrations of total P450 (Omura and Sato, 1964), CYP2C (Shimada, et al., 1994), and NADPH-P450 reductase (EC 1.6.2.4) (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 test were 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 25 μ M 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 μ M propofol was used as a substrate (Fig. 1A). In contrast, significantly decreased propofol metabolism by propofol itself treatment (10 mg/kg) was observed. To examine whether propofol decreased propofol-biotransformation in detail, rats were intravenously treated once or twice with propofol (10 mg/kg in 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. Since we used limited rat recombinant P450 isoforms (Table 1), it should be mentioned that other major CYP2C (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 co-incubated 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 16 α -hydroxylation activities (CYP2C) in male rat liver microsomes (Fig 2B), consistent with

the immunochemical results. Repeatedly administered propofol (10 mg/kg twice in every 4 h) also significantly decreased testosterone 16 β -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 6 β -hydroxylation activities in rat liver 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 deactivated 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 of the deactivation of P450, especially CYP2C isoforms, by propofol. With regard to bioactivation via secondary oxidations 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 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.

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Footnote

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Legend for figures

Fig. 1. Propofol metabolism catalyzed by rat liver microsomes.

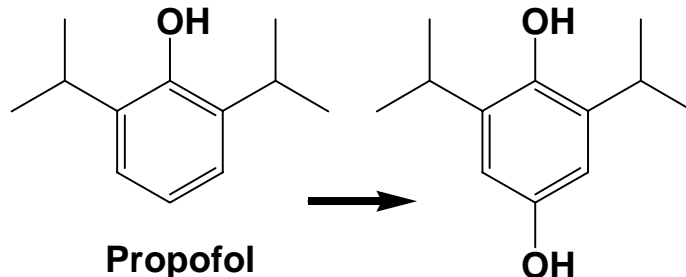
Propofol (20 μ M) was incubated at 37°C for 10 min with rat liver microsomes (0.10 mg of protein/ml) in the presence of an NADPH-generating system. (A), data are mean \pm S.D. for three untreated male rats or male rats after intraperitoneal injection with β -naphthoflavone (CYP1A), phenobarbital (CYP2B and CYP3A), dexamethasone (CYP3A), and propofol. Significantly different from controls ($*p < 0.05$). (B), data are mean \pm S.D. for three rats male (M) and female (F) untreated or treated intravenously once (x 1) or twice (x 2) with propofol (10 mg/kg) in every 4 h. $*P < 0.05$, One-way ANOVA with Dunnett's post test.

Fig. 2. Total P450, CYP2C, and NADPH-P450 reductase contents (A) and typical drug oxidation activities (B) in liver microsomes from male rats untreated and intravenously treated with propofol.

For the immunochemical determination, polyclonal anti-rat CYP2C11 immunoglobulin G fraction (Daiichi Pure Chemicals) was used with recombinant rat CYP2C11 for the standards. Activities of ethoxyresorufin *O*-deethylation (CYP1A), pentoxyresorufin *O*-depentylation (CYP2B) and testosterone 16 β -, 16 α -, and 6 β -hydroxylation (CYP2B, CYP2C, and CYP3A, respectively) were determined. Data are mean \pm S.D. for three rats untreated or treated with once (x 1) or twice (x 2) intravenous administration of propofol (10 mg/kg) in every 4 h. Significantly different from controls by one-way ANOVA with Dunnett's post test ($*p < 0.05$).

Table 1

Propofol metabolism catalyzed by recombinant rat P450 isoforms



Propofol (5 μ M and 20 μ M) was incubated at 37°C for 10 min with each recombinant rat P450 enzymes (0.032 μ M of P450) in the presence of an NADPH-generating system. Results are presented as means of duplicate determinations

P450	Substrate concentration	
	5 μ M	25 μ M
	<i>nmol/min/nmol P450</i>	
CYP1A2	3.0	12
CYP2B1	16	27
CYP2C11	16	46
CYP2D1	2.1	7.1
CYP2E1	1.4	5.0
CYP3A2	3.9	9.1

Table 2

Inhibitory effects of propofol on testosterone 16 α -, 16 β -, and 6 β -hydroxylation (CYP2C, CYP2B, and CYP3A, respectively) in liver microsomes from untreated male rats

Testosterone (50 μ M) was incubated at 37°C for 10 min with male rat liver microsomes in the absence or presence of propofol (20 μ M). Data are means \pm SD from three untreated male rats. * p < 0.01, significant difference from the control (without propofol).

Hydroxylations	Testosterone hydroxylation (%)	
	Without propofol	With propofol
	<i>nmol/min/mg protein</i>	
16 α -	0.99 \pm 0.12 (100)	0.47 \pm 0.07 (47)*
16 β -	0.31 \pm 0.03 (100)	0.08 \pm 0.02 (26)*
6 β -	1.82 \pm 0.18 (100)	1.55 \pm 0.22 (85)

Fig.1

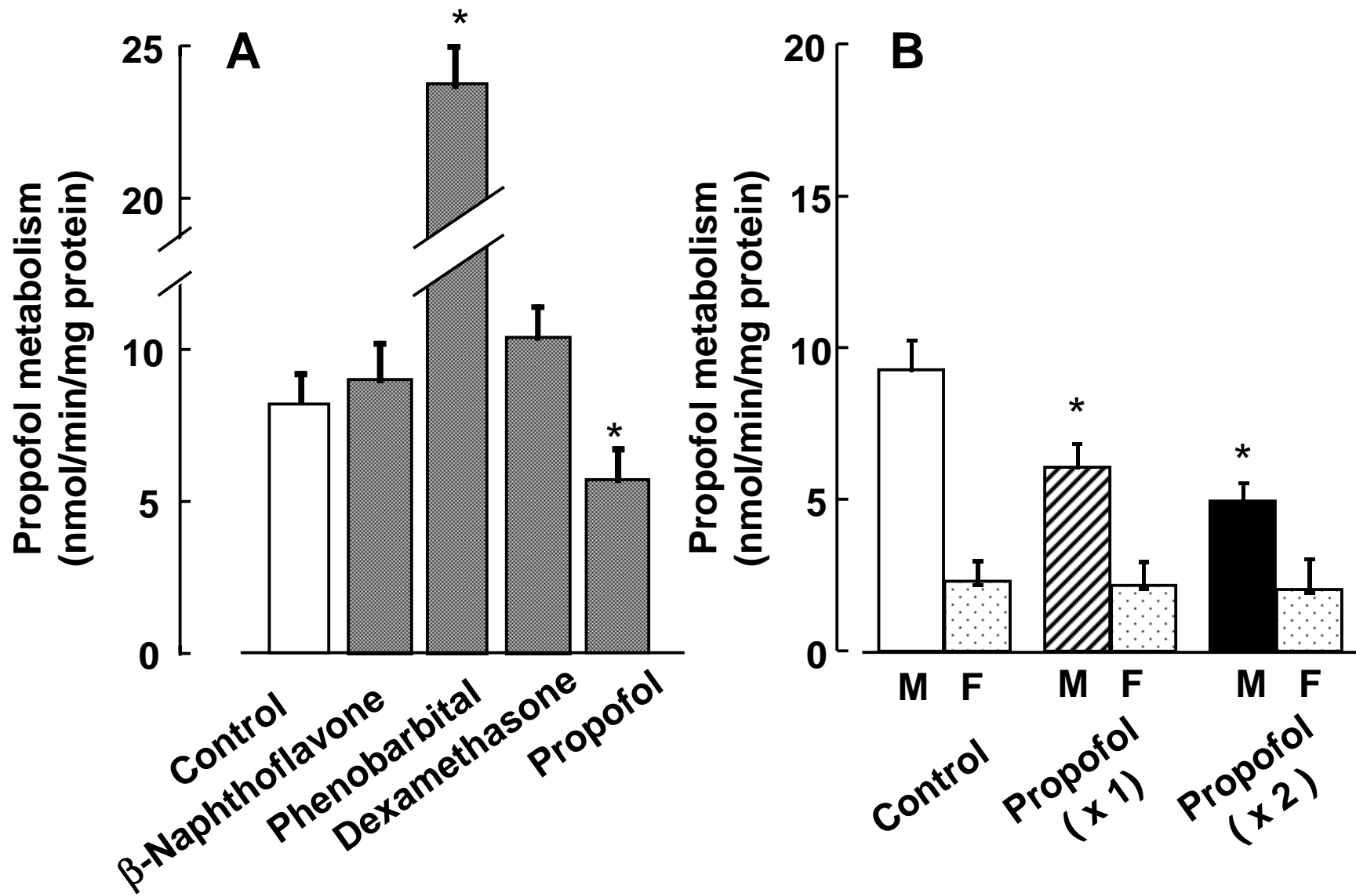


Fig.2

