Propofol inhibits renal cytochrome P450 activity and enflurane defluorination *in vitro* in hamsters

Ta-Liang Chen MD PhD,*
 Tyng-Guey Chen MD,*
 Yu-Ting Tai MD,*
 Huai-Chia Chang MD,*
 Ruei-Ming Chen PhD,*
 Chen-Jung Lin MD,†
 Tzuu-Huei Ueng PhD,‡

Purpose: To determine the effect of propofol on renal cytochrome P450 activity and defluorination of enflurane.

Methods: Renal microsomes were prepared by homogenization and differential centrifugation from pooled hamster kidneys. Defluorination of enflurane was assessed by measuring free fluoride metabolites after reacting enflurane with renal microsomes incubated with various concentrations, 0.05 - 1.0 mmol·L⁻¹ propofol in the NADPH-generating system. Drug metabolizing activities of renal cytochrome P450 mono-oxygenase enzymes were evaluated within microsomes preincubated with propofol and reacted with the specific marker substrates, aniline, benzo(a)pyrene, erythromycin and pentoxiresorufin, for cytochrome P450 2E1, 1A1, 3A4 and 2B1, respectively.

Results: Renal defluorination of enflurane was inhibited by clinical concentrations, 0.05 mmol·L⁻¹ of propofol ($P < 0.05$). Dose-dependent inhibition of defluorination, aniline and benzo(a)pyrene hydroxylase within kidney microsomes was related to propofol concentration. Propofol demonstrated a profound inhibition of renal pentoxiresorufin dealkylase activity even at low concentrations, 0.05 mmol·L⁻¹ ($P < 0.01$). Propofol did not exhibit inhibition of erythromycin N-demethylation of kidney microsomes except at high concentration, 1.0 mmol·L⁻¹. Spectral analyses of key coenzymes of renal cytochrome P450 monooxygenase, cytochrome b₅ and cytochrome c reductase, demonstrated an inhibition when incubated with high concentrations of propofol ($P < 0.05$).

Conclusion: In an *in vitro* study in an NADPH-generating system of hamster kidney microsomes, propofol, in clinical concentrations, exhibited a broad-spectrum of inhibition to renal monooxygenase activities and enflurane defluorination.

Objectif : Déterminer l'effet du propofol sur l'activité du cytochrome P₄₅₀ rénal et sur la défluorination de l'enflurane.

Méthode : Les microsomes rénaux ont été préparés par homogénéisation et centrifugation différentielle de reins de hamsters. La défluorination de l'enflurane a été évaluée en mesurant les métabolites de fluorure libres après la réaction avec les microsomes rénaux incubés dans diverses concentrations, 0,05 - 1,0 mmol·L⁻¹, de propofol dans un système générateur de NADPH. Les activités métabolisantes de médicament des enzymes rénales cytochrome P₄₅₀ mono-oxygénase ont été évaluées dans les microsomes préincubés avec du propofol et qui ont réagi avec des substrats de marqueurs spécifiques, aniline, benzo(a)pyrène, érythromycine et pentoxyrésorufine, pour les cytochromes P₄₅₀, 2E1, 1A1, 3A4 et 2B1, respectivement.

Résultats : La défluorination rénale de l'enflurane a été inhibée par les concentrations cliniques, 0,05 mmol·L⁻¹ de propofol ($P < 0,05$). L'inhibition dose-dépendante de la défluorination, de l'aniline et de la benzo(a)pyrène hydroxylase dans les microsomes rénaux était reliée à la concentration de propofol. Le propofol a démontré une importante inhibition de l'activité de la pentoxyrésorufine dealkylase rénale même avec une faible concentration, 0,05 mmol·L⁻¹ ($P < 0,01$). L'emploi de propofol n'a pas montré d'inhibition de la N-déméthylation de l'érythromycine des microsomes du rein, sauf avec une forte concentration, 1,0 mmol·L⁻¹. Les analyses spectrales des coenzymes clés de la cytochrome P₄₅₀-mono-oxygénase rénale, de la cytochrome b₅ et c-réductase ont démontré une inhibition dans une incubation avec de fortes concentrations de propofol ($P < 0,05$).

Conclusion : Une étude *in vitro* de microsomes rénaux de hamster, réalisée avec un système producteur de NADPH, a montré que le propofol en concentrations cliniques affiche une inhibition à large spectre des activités de la mono-oxygénase rénale et de la défluorination de l'enflurane.

From the Department of Anesthesiology, Taipei Medical College,* Wan-Fang Hospital, and the Department of Anesthesiology,†Graduate Institute of Toxicology,‡ College of Medicine, National Taiwan University, Taipei, Taiwan.

Address correspondence to: Ta-Liang Chen MD PhD, Department of Anesthesiology, Taipei Medical College, Wan-Fang Hospital, No. 111, Sec. 3, Hsing-Lung Rd., Taipei, 116 Taiwan. Phone: 886-2-29307930 Ext. 2150; Fax: 886-2-89315940; E-mail: tlc@tmc.edu.tw

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ALTHOUGH the liver plays the major role in drug and xenobiotic metabolism, drug-metabolizing enzymes are also present at other tissues, such as kidney.¹ The physiological functions of these extrahepatic cytochrome P450 monooxygenase system include the oxidative biotransformation of a large number of endogenous (arachidonic acids, prostaglandins and leukotrienes) and exogenous (polycyclic aromatic hydrocarbons and drugs) compounds.¹⁻⁵ Depending on the specific drugs, substrates and enzymes involved, the extrahepatic cytochrome P450-dependent monooxygenases contribute to the metabolism/elimination of xenobiotics and have considerable importance in drug disposition and toxicity.⁶ For example, nephrotoxicity related to renal biotransformation of the inhalational anesthetics, methoxyflurane or enflurane, is induced by locally produced renal nephrotoxin or metabolites and is contributed to by the renal cytochrome P450 enzyme activity.^{7,8}

Propofol has been used world-wide for intraoperative amnesia as well as long-term sedation for patients in intensive care unit so that its coadministration with other drugs is inevitable.^{9,10} Propofol interacts with drugs such as propranolol, by changing plasma protein binding and affecting its drug disposition.¹¹ The enzymatic degradation of sufentanil and alfentanil in the liver may be inhibited by propofol.¹² Previous investigations in the rat and in our human liver model demonstrated that interference in oxidative metabolism by propofol was mainly through inhibition of the cytochrome P450-dependent monooxygenase system.^{13,14} During intravenous infusion, the extrahepatic uptake and renal clearance of propofol plays an important role in its pharmacokinetic profile.¹⁵⁻¹⁷ The effect of propofol on the renal cytochrome P450 systems and its interactions with renal metabolism of inhalational are unknown. In this study, we applied the NADPH-generating system of hamster kidney microsomes to validate the potential interactions of propofol with renal defluorination of enflurane and the enzymic activities of renal cytochrome P450-dependent monooxygenase system.

Materials and methods

Animals and preparations

The study was approved by the National Science Council of Taiwan, R.O.C. Male Syrian golden hamsters, 12-wk-old, weighing 100-120 g, were purchased from the Animal Center of the College of Medicine (National Taiwan University, Taipei, Taiwan). They were housed and stayed for stabilization in a photo-controlled environment with 12 hr

light period for at least one week. Animals were anesthetized in a glass tank saturated with ether vapour before sacrifice by decapitation. Kidney tissues were removed, rinsed and homogenized in an ice-chilled KCl 1.15% (w/v) solution. After differential centrifugation, washed microsomes were prepared from homogenized tissues as described by Alvares and Mannering.¹⁸ Microsomes from the kidneys were pooled from every four animals and frozen at -70°C until pellets were resuspended in potassium phosphate buffer 0.1 mol·L⁻¹ at pH 7.4 for assay. Microsomal protein was assayed by the method of Lowry using bovine serum albumin as standard.¹⁹

Defluorination assay

Defluorination of enflurane was assessed by adding enflurane into renal microsomes preincubated with various concentrations of propofol in the NADPH-generating system. Pure propofol (ICI, Zeneca, UK) was diluted from 0.05 to 1.0 mmol·L⁻¹ with kidney microsomes from control animals. The microsomal incubation mixture contained 5 mg·mL⁻¹ protein, 2 mmol·L⁻¹ NADPH and 2 μ L enflurane in 2 mL, 100 mmol·L⁻¹ Tris HCl buffer (pH = 7.4) incubated at 37°C for 30 min and then the reaction was stopped by cooling on ice. Defluorination of enflurane was assessed by measuring fluoride metabolites using an Orion fluoride-specific combined electrode (Boston, MA, USA).²⁰ Standard curves of fluoride concentrations were calibrated using freshly prepared free standard fluoride solutions.

Monoxygenase assays

Dose-response effects of various concentrations of propofol (0.05 to 1.0 mmol·L⁻¹) to each monooxygenase was first evaluated in hamster renal microsomes. Pure propofol was diluted from 0.05 to 1.0 mmol·L⁻¹ with kidney microsomes from control animals. Monooxygenases were assayed by reacting with specific marker substrates for each enzyme system. Aniline hydroxylation, indicating the activity of cytochrome P450 2E1, was determined by measuring the formation of the end product, *p*-aminophenol, from aniline.²¹ The incubation system contained 2.8 iu·mL⁻¹ glucose-6-phosphate dehydrogenase, 1 mmol·L⁻¹ glucose-6-phosphate and 0.1 mmol·L⁻¹ NADP in Tris buffer 0.075 mmol·L⁻¹ with 0.5 mmol·L⁻¹ aniline hydrochloride. Microsomes were incubated with various concentrations of propofol in a water bath at 37°C for 20 min. The reaction was stopped by trichloroacetic acid 20% solution. After centrifugation, the supernatant was mixed with Na₂CO₃ and phenol solution to extract the product, which could be measured spectrophotometri-

cally at 630 nm. Erythromycin demethylation, indicating the activity of cytochrome P450 3A4, was determined by measuring the formation of formaldehyde using Nash's reagent.²² Incubation of microsomes contain $7.5 \text{ mmol}\cdot\text{L}^{-1}$ semicarbazide HCl, $4 \text{ mmol}\cdot\text{L}^{-1}$ glucose-6-phosphate, $0.4 \text{ mmol}\cdot\text{L}^{-1}$ NADP, $0.4 \text{ iu}\cdot\text{mL}^{-1}$ glucose-6-phosphate dehydrogenase and $12 \text{ mmol}\cdot\text{L}^{-1}$ erythromycin in $\text{KH}_2\text{PO}_4\text{-K}_2\text{HPO}_4$ buffer $0.02 \text{ mmol}\cdot\text{L}^{-1}$ (pH 7.4). The reagent was incubated in 37°C water bath for 15 min and reaction was stopped with $1.24 \text{ mmol}\cdot\text{L}^{-1}$ $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$. After neutralization and centrifugation, the supernatant reacted with Nash's reagent and the product was detected by UV/VIS spectrophotometry at wavelength of 412 nm using formaldehyde as standard.²²

Pentoxyresorufin dealkylation, indicating the activity of cytochrome 2B, was assayed by the method of Lubet.²³ The microsomal suspension, $0.5 \text{ mg}\cdot\text{mL}^{-1}$, reacted in $0.05 \text{ mmol}\cdot\text{L}^{-1}$ Tris buffer, with $0.025 \text{ mmol}\cdot\text{L}^{-1}$ MgCl_2 , $1.8 \text{ mg}\cdot\text{mL}^{-1}$ bovine serum albumin, $1.45 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$ NADPH, and $10.6 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$ pentoxyresorufin. The system was incubated in a water bath at 37°C for 10 min and stopped by adding methanol. After centrifugation, the metabolite in supernatant was detected by a fluorescence spectrophotometer using

rhodamine B as standard. Benzo(a)pyrene hydroxylation for aryl hydrocarbon hydroxylase activity, indicating the activity of cytochrome P450 1A, was determined by measuring the formation of phenolic metabolites by the method of Nebert and Gelboin.²⁴ In a dark room, $2 \text{ mmol}\cdot\text{L}^{-1}$ microsomes were incubated in phosphate buffer containing $1.05 \text{ mmol}\cdot\text{L}^{-1}$ NADPH, $2.9 \text{ mmol}\cdot\text{L}^{-1}$ MgCl_2 , $0.08 \text{ mmol}\cdot\text{L}^{-1}$ $\text{KH}_2\text{PO}_4\text{-K}_2\text{HPO}_4$ buffer (pH 7.4) and $0.2 \text{ mg}\cdot\text{mL}^{-1}$ bovine serum albumin. The generation system was incubated at 37°C for 10 min with the substrate, benzo(a)pyrene $1 \text{ mmol}\cdot\text{L}^{-1}$, and the reaction was stopped by adding acetone. The fluorescent metabolites were extracted sequentially by n-hexane and NaOH and measured by spectrofluorimetry.²⁴

In order to evaluate interference of the microsomal electron transport system by propofol, renal microsomes were preincubated with various concentrations of propofol (0.5 or $1.0 \text{ mmol}\cdot\text{L}^{-1}$). After incubation, cytochrome b_5 and cytochrome c reductase was determined by the method of carbon monoxide and NADH difference spectral analyses.^{25,26} Unless otherwise stated, all results are presented as mean (SEM). Data were analyzed using one-way and two-way analyses of variance and significant differences between var-

FIGURE 1 Effect of propofol on renal defluorination of enflurane in hamster kidney microsomes. Various concentrations of propofol (0.05 , 0.1 , 0.25 , 0.5 , $1.0 \text{ mmol}\cdot\text{L}^{-1}$) were incubated with $2 \text{ }\mu\text{l}$ enflurane in NADPH-generating systems ($n=8$, each microsome was pooled from four animals). Data are mean (SEM) of duplicate measurements. * $P < 0.05$; † $P < 0.01$ vs control (one-way ANOVA with Student-Newman-Keuls test).

FIGURE 2 Dose-response curves of various concentrations of propofol (0.05 , 0.1 , 0.25 , 0.5 , $1.0 \text{ mmol}\cdot\text{L}^{-1}$) to renal monooxygenase activities. Aniline Hydroxylation (○); Benzo(a)pyrene Hydroxylation (Δ); Erythromycin N-demethylation (●); Pentoxyresorufin O-dealkylation (▲). Data are mean (SEM) of duplicate measurements. * $P < 0.05$; † $P < 0.01$ vs control (one-way ANOVA with Student-Newman-Keuls test).

TABLE I Effects of propofol on extrahepatic P450-dependent monooxygenases in hamster kidney microsomal enzyme systems ($n=8$, each sample of kidney microsome was pooled from four animals). Data are mean (SEM) of duplicate measurements in each enzyme assay. * $P < 0.05$; † $P < 0.01$ when compared with control. C: control.

Assay	Kidney (Incubation with propofol, mmol·L ⁻¹)		
	C	0.05	0.10
Aniline hydroxylation (nmol <i>p</i> -aminophenol·min ⁻¹ ·mg ⁻¹ protein)	0.30 ± 0.06	0.16 ± 0.04*	0.13 ± 0.06*
Benzo(a)pyrene hydroxylation (pmol 3-hydroxybenzo(a)pyrene·min ⁻¹ ·mg ⁻¹ protein)	110.76 ± 4.52	68.52 ± 6.89†	42.32 ± 10.56†
Erythromycin N-demethylation (nmol HCHO formed·min ⁻¹ ·mg ⁻¹ protein)	7.62 ± 0.92	8.25 ± 1.06	8.02 ± 0.88
Pentoxifyresorufin O-dealkylation (pmol resorufin formed·min ⁻¹ ·mg ⁻¹ protein)	23.65 ± 3.44	4.76 ± 1.44†	5.05 ± 1.29†

TABLE II Cytochrome b₅ and cytochrome (cyt.) *c* reductase content in hamster renal microsomes ($n=6$) preincubated with propofol 0.5 and 1.0 mmol·L⁻¹ before assay using NADH difference spectral analysis. Data are mean (SEM). * $P < 0.05$ when compared with control.

	Cytochrome b ₅	Cytochrome <i>c</i> reductase
Incubation	(10 ⁻² × nmol/mg/protein)	(nmol cyt. <i>c</i> reduced/mg protein/min)
Control	15.51 ± 2.46	146.83 ± 20.43
Propofol (0.5 mmol·L ⁻¹)	11.66 ± 1.82	113.18 ± 12.76
Propofol (1.0 mmol·L ⁻¹)	8.77 ± 0.93*	61.18 ± 9.82*

ious concentrations were identified by the Student-Newman-Keuls test or the unpaired *t* test. $P < 0.05$ was considered statistically significant.

Results

At a low concentration of propofol, 0.05 mmol·L⁻¹, defluorination of enflurane in renal microsomes was inhibited compared with that of the control group ($P < 0.05$). The inhibition gradually increased when the concentrations of propofol were elevated (Figure 1). Aniline hydroxylase and benzo(a)pyrene hydroxylase were inhibited in parallel by propofol in a dose-dependent manner (Figure 2). Pentoxifyresorufin O-demethylase was remarkably inhibited by propofol even at low concentration, such as 0.05 mmol·L⁻¹, when compared with control ($P = 0.0017$), while erythromycin N-demethylase exhibited no obvious inhibitory response until incubated with high concentrations of propofol (1.0 mmol·L⁻¹, $P = 0.0046$). Propofol *in vitro*, inhibited renal P450-dependent monooxygenases in the order: pentoxifyresorufin O-dealkylation > benzo(a)pyrene hydroxylation > aniline hydroxylation > erythromycin N-demethylation (Figure 2).

Propofol in therapeutic concentrations of 0.05 - 0.10 mmol·L⁻¹, caused 47 -57% inhibition of aniline hydroxylation in kidney microsomes and inhibition of renal benzo(a)pyrene hydroxylation to 38% of the control, but erythromycin N-demethylation activity in kidney microsomes was similar to control. Renal pentoxifyresorufin O-dealkylation was inhibited to 20 and 21% of control by low concentration of propofol, 0.05 and 0.10 mmol·L⁻¹, respectively (Table I).

Propofol inhibited the binding of NADH with cytochrome b₅ and cytochrome *c* reductase activity (Table II). When incubated with 1.0 mmol·L⁻¹ propofol, the binding of cytochrome b₅ and NADH was inhibited to 57% of control ($P < 0.05$). The cytochrome *c* reductase was also reduced to 42% of control ($P < 0.05$).

Discussion

In addition to the liver, extrahepatic cytochrome P450 in kidney has also been thought to function primarily in the metabolism of endogenous substances, drugs and exogenous compounds.^{1,5,6} The importance of the kidney in the extrahepatic biotransformation of drugs, including volatile anesthetics, has been documented.^{7,8,27} Whether extrahepatic drug metabolism would be affected by propofol through the alteration of pharmacokinetics is yet to be determined. In the present study, we evaluated the *in vitro* effect of propofol on the metabolizing capacities of various enzymes in monooxygenase system of hamster kidney. Metabolism of aniline hydroxylation and erythromycin N-demethylation was used to assess cytochrome P450 2E1 and 3A4 activities.^{21,22} Cytochrome P450 2E1 is associated with the metabolism of small molecular weight compounds and also has been identified as the predominant catalyzing enzyme for the microsomal defluorination of the volatile anesthetics.²⁸ Serum concentrations of propofol in human and animal model for clinical hypnosis have been reported from 0.067 to 0.132 mmol·L⁻¹.^{16,29,30} Under similar concentrations of

propofol *in vitro*, our data clearly demonstrated that the activity of aniline hydroxylase (P450 2E1) was inhibited within renal microsomes by clinical concentrations of propofol in a concentration-dependent manner.

As well as aniline hydroxylation, the capability of renal defluorination was also inhibited by propofol in hamster kidney. Comparing the effect in hepatic tissue, both the extent of inhibition by propofol to the aniline hydroxylase and defluorination of enflurane were greater in the kidney than in the liver (60% *vs* 20%).¹⁴ In a previous study, Conzen *et al.* showed that serum inorganic fluoride metabolites produced by hepatic cytochrome P450 2E1 was not parallel with the post-anesthetic renal toxicity and might not be a good indicator of nephrotoxicity after inhalational anesthesia.³¹ Kharasch *et al.* proposed that enflurane defluorination by renal cytochrome P-450 system instead of hepatic defluorination was the major determinant of clinical nephrotoxicity caused by inhalational agents.⁸ Propofol inhibition of renal defluorination as well as of cytochrome P450 2E1 activity might imply that coadministration of propofol could attenuate the extent of defluorination to the volatile anesthetics as well as imply its potential role in protecting renal toxicity induced by fluoride metabolites. Interestingly, the magnitude of inhibition of renal defluorination by propofol was greater than its inhibition of cytochrome P450 2E1 activity (62% *vs* 47% at 0.05 mmol·L⁻¹ of propofol). This might imply that enzymes other more than P450 2E1 in the kidney were involved in the process of renal defluorination and was also inhibited by propofol.²⁸

Cytochrome P450 3A4 is the main enzyme for dealkylation of anesthetics or drugs including fentanyl, sufentanil, and cyclosporin.^{32,33} Compared with cytochrome P450 2E1, 3A4 exhibited only minor inhibition until high concentration of propofol (1.0 mmol·L⁻¹) in renal microsomes. Propofol eventually blocked the binding site of P450 3A4 in high concentrations but not in clinical concentrations. Thus, coadministration of opioids may not affect drug-to-drug interactions in enzyme level during propofol infusion in extrahepatic tissues. Metabolism of benzo(a)pyrene and pentoxyresorufin was used to assess cytochrome 1A1 (responsible for the metabolism of carcinogens) and 2B1 (phenobarbitone inducible) activities.^{1,6} Both are important isozymes of cytochrome P450 widely distributed in the kidney, lung and other extrahepatic tissues.³⁴ The benzo(a)pyrene hydroxylase and pentoxyresorufin O-dealkylase were markedly inhibited in the kidney even at low concentrations of propofol (0.05 mmol·L⁻¹). Corresponding with our previous data in human liver,¹⁴ the *in vitro* inhibition of P450 2B1 and 1A1 by propo-

fol was more marked than 2E1 in both hepatic and extrahepatic tissue. The difference in enzyme inhibition by propofol revealed P450 selectivity for substrate with the binding of enzyme (isozyme-specificity).¹ The effect of propofol on hamster and human monooxygenase activities also demonstrates tissue variation of inhibition (tissue-specificity).^{5,27} The fact that fewer P450 subfamilies and isozymes are expressed in kidney and other extrahepatic tissues (e.g. heart, intestine) than in the liver markedly enhances the opportunity of identifying isozyme inhibition for these organs.^{5,27}

The mechanism for the inhibition by propofol of extrahepatic cytochrome P450 monooxygenase system might involve many steps including form-specific inactivation including reversible binding of the inhibitor, conversion of the inhibitor to a reactive species, irreversible modification of the enzyme, and loss of enzyme activity.³⁵ The ability of an inactivator, such as propofol, inhibiting more than two different enzymes can result from differential interaction at any of the above steps. The microsomal P450 cytochromes are hemoproteins containing one prosthetic heme (iron-protoporphyrin IX) moiety and inhibition by propofol of the heme group binding site was shown in our previous study.¹⁴ Data in this study also demonstrated dose-dependent inhibition of propofol of cytochrome b₅ and *c* reductase that were the major coenzymes in the chain reaction of oxygenation. Thus, the efficiency of electron transport would be considerably delayed resulting in impairment of electron transport and uptake processes in extrahepatic tissues in the presence of propofol.

Clinically, drug interactions with propofol in extrahepatic tissues such as the kidney and lung, might be caused by: 1) alterations in regional blood flow, 2) competition for the protein binding, 3) inhibition to the regional enzyme activity. With respect to mechanism 1, several studies have shown that propofol decreased regional blood flow by decreasing cardiac output as well as regional perfusion pressure.^{36,37} Thus, tissue uptake of the co-administered drug would be reduced. Secondly, due to the lipophilicity, propofol, will compete with other substrates for the protein binding. This might redistribute the percentage of free fraction of other drugs, such as propranolol, and modify their pharmacodynamic effect.¹¹ Thirdly, data in this study demonstrated that propofol could inhibit the enzyme activity of kidney in a broad-spectrum and dose-dependent manner. Many clinical relevant drug-interactions still need to be rationalized in terms of the specific substrate and inhibitor to individual P450 isoforms.

In conclusion, Propofol exhibited an isozyme-selective inhibition to renal P450 enzymes and enflu-

rane defluorination in a dose-dependent manner. This inhibition reminds clinicians of the potential drug-to-drug interactions and alterations in coadministered drug toxicity during anesthetic practice.

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