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Difference between *in vivo* and *in vitro* effects of propofol on defluorination and metabolic activities of hamster hepatic cytochrome P450-dependent mono-oxygenases

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Summary

We have compared the *in vivo* and *in vitro* effects of propofol on cytochrome P450-dependent mono-oxygenase activities in hamster liver microsomes. Propofol (Diprivan) 10 mg/100 g body weight was injected i.p. twice a day for 2 weeks to induce cytochrome P450 enzymes. Liver microsomes were prepared by differential centrifugation. Metabolism of the cytochrome P450-dependent mono-oxygenase system was evaluated by measuring aniline hydroxylation, benzphetamine demethylation and benzo(a)pyrene hydroxylation. Defluorination of enflurane was assayed by detecting free fluoride metabolites. At similar concentrations as in the *in vivo* group, propofol *in vitro* exhibited concentration-dependent inhibition of metabolism of benzphetamine and benzo(a)pyrene. Aniline hydroxylation and defluorination of enflurane were inhibited to 78 % of control with propofol 0.25 mmol litre^{-1}. In propofol-treated hamsters, there was only minimal inhibitory or inductive effects on either mono-oxygenase activities or capacity for defluorination. This difference between the *in vitro* and *in vivo* effects of propofol on cytochrome P450 mono-oxygenase activities emphasizes the need for care when comparing *in vitro* and clinical data. (*Br. J. Anaesth*. 1995; **75**: 462–466)

Key words

Anaesthetics i.v., propofol. Liver, microsomes. Liver, metabolism. Enzymes, cytochrome P450. Hamster.

Propofol (2,6-diisopropylphenol) is characterized by rapid onset, short duration of action and predictable first-order kinetics [1–3]. It is used for induction and maintenance of anaesthesia, by bolus injection or continuous infusion, and also in the intensive care unit for sedation [4, 5]. During long-term usage, multiple drug interactions should be considered. Indeed, propofol may decrease the intrinsic clearance of propranolol by competing for plasma protein binding [6], and the enzymatic degradation of alfentanil and sufentanil is altered by co-administration of propofol [7]. Metabolism of propofol is mainly hepatic, producing inactive glucuronide conjugates of alkyl phenol and quinol, 2,6-diisopropyl-1,4-quinol, both being produced by the cytochrome P450-dependent mono-oxygenase system [8].

We have previously demonstrated that high concentrations of proprofol inhibited human cytochrome P450 mono-oxygenases [9]. Baker, Chadam and Ronnenberg also reported propofol inhibition using rat liver microsomes [10]. In the present study, we have compared mono-oxygenase activity in propofol-treated hamsters with the enzyme *in vitro*.

Materials and methods

ANIMALS AND TREATMENT

Male Syrian golden hamsters, weighing 100–120 g, were purchased from the Animal Center of the College of Medicine (National Taiwan University, Taipei, Taiwan) and housed, with a controlled photoperiod of 12 h light daily for at least 1 week. In the propofol-treated group, hamsters received propofol 10 mg/100 g body weight i.p. twice daily at 08:00 and 20:00 for 2 weeks for induction of P450 enzymes [11, 12]. Sleeping time, and the interval between righting and co-ordination were recorded. Blood samples were obtained from the retrobulbar venous plexus every other day during the peak of sedation, and serum concentrations of propofol were analysed by the HPLC–fluorescence method of Plummer [13]. The control group received Intralipid. Animals were killed 2 h after the last injection. Livers were removed, rinsed and homogenized in an iced-chilled 1.15 % KCl (w/v) solution. Washed microsomes were prepared by differential centrifugation, as described by Alvares and Mannering [14]. Microsomal pellets were resuspended in potassium phosphate buffer 0.1 mol litre^{-1} at pH 7.4 for assay of mono-oxygenase activities. Microsomal protein was assayed by the method of Lowry and colleagues using bovine serum albumin as standard [15].

MONO-OXYGENASE ASSAYS

Pure propofol was diluted to 0.05, 0.10, 0.15, ,0.20 and 0.25 mmol litre^{-1} with liver microsomes from control animals. Mono-oxygenase activities were as-

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Table 1 Comparisons of microsomal metabolic activities of propofol-treated hamsters ($n = 12$) and *in vitro* microsomal enzyme systems incubated with propofol ($n = 6$). Data are mean (SEM) of duplicate measurements in each enzyme assay. Significant differences ($P < 0.05$) compared with: *control group ($n = 6$); †propofol-treated group

			Incubation with propofol (mmol litre^{-1})	
Assay	Control	Protofol-treated	0.05	0.10
Aniline hydroxylation (nmol *p*-aminophenol/min/mg protein)	1.76 (0.12)	1.82 (0.14)	1.55 (0.06)†	1.46 (0.07)*†
Benzphetamine demethylation (nmol formaldehyde/min/mg protein)	3.44 (0.16)	3.28 (0.12)	3.03 (0.08)	2.75 (0.07)*†
Benzo(a)pyrene hydroxylation (pmol 3-hydroxybenzo(a)pyrene/min/mg protein)	398 (22)	366 (26)	318 (16)*	239 (12)*†
Defluorination of enflurane (nmol fluoride/mg protein)	3.20 (0.08)	3.34 (0.12)	3.07 (0.09) †	2.94 (0.09)*†

sayed by reacting with specific marker substrates for various enzyme systems. Aniline hydroxylation activity was determined by measuring the formation of *p*-aminophenol from aniline [16]. The incubation system contained NADP 0.1 mmol litre^{-1}, glucose-6-phosphate dehydrogenase 2.8 iu ml^{-1} and glucose-6-phosphate 1 mmol litre^{-1} in Tris buffer 0.075 mmol litre^{-1} with aniline hydrochloride 0.5 mmol litre^{-1}. After incubation at 37 °C for 20 min, phenol solution (phenol/0.2 mol litre^{-1} NaOH = 1/40) was added to produce the metabolite, which was measured spectrophotometrically at 630 nm.

Benzphetamine demethylation was assayed by measuring the formation of formaldehyde using Nash's reagent [17] after incubation of microsomes with glucose-6-phosphate 4 mmol litre^{-1}, semicarbazide HCl 8 mmol litre^{-1}, NADP 0.4 mmol litre^{-1}, glucose-6-phosphate dehydrogenase 2 iu and benzphetamine 2 mmol litre^{-1} in KH_2PO_4–K_2HPO_4 buffer 8 mmol litre^{-1} (pH 7.4). The mixture was incubated at 60 °C for 15 min and then cooled to room temperature. The metabolite of formaldehyde was detected spectrophotometrically at 412 nm. Benzo(a)pyrene hydroxylation for aryl hydrocarbon hydroxylase activity was determined by measuring the formation of phenolic metabolites by the method of Nebert and Gelboin [18]. Microsomes were incubated in the dark with protein in phosphate buffer (2 mg ml^{-1}) containing NADPH 1.05 mmol litre^{-1}, $MgCl_2$ 2.9 mmol litre^{-1}, KH_2PO_4–K_2HPO_4 0.08 mmol litre^{-1} (pH = 7.4) and BSA 0.2 mg ml^{-1}. Incubation was performed at 37 °C for 10 min with the substrate, benzo(a)pyrene 1 mmol litre^{-1}, and stopped by adding acetone. The fluorescent metabolite was extracted sequentially by *n*-hexane and NaOH and measured by fluorescence. The defluorination of enflurane was determined by measurement of fluoride metabolites with an Orion fluoride-specific combined electrode (Boston, MA, USA) [19]. The incubation mixture containing microsomal protein 5 mg ml^{-1}, NADPH 2 mmol litre^{-1} and 2 μl of anaesthetic in 2 ml, and Tris HCl buffer 100 mmol litre^{-1} (pH = 7.4) was incubated at 37 °C for 30 min and stopped on ice. Standard curves were calibrated using freshly prepared free standard fluoride solutions.

Microsomal cytochrome P450 content and cytochrome b_5 were determined by the method of carbon monoxide and NADH difference spectral analyses described by Omura and Sato [20]. In addition, microsomes were preincubated with propofol 1.0 mmol litre^{-1} to assess any interference with the binding of the haemeprotein of cytochrome P450 and b_5 to carbon monoxide and NADH, respectively.

Unless otherwise stated, all results are given as mean (SEM). Data were analysed using one-way and two-way analyses of variance and significant differences between groups were identified by the Student–Newman–Keuls test or the unpaired Student's *t* test. $P < 0.05$ was considered statistically significant.

Results

In propofol-treated animals, sleeping time and the interval between righting and co-ordination were 4.6 (1.2) min and 6.8 (1.9) min, respectively. The mean peak serum concentration of propofol was 0.078 (0.012) mmol litre^{-1} in 20 propofol-treated hamsters. Microsomal cytochrome P450 content in the control group was 1.62 (0.15) nmol/mg protein and in propofol-treated animals 1.70 (0.18) nmol/mg protein (ns).

Metabolic activities of microsomal P450 mono-oxygenases in propofol-treated hamsters for aniline hydroxylation, benzphetamine demethylation, benzo(a)pyrene hydroxylation and defluorination of enflurane were similar to controls (table 1). In contrast, propofol *in vitro* exhibited concentration-dependent inhibition of P450 mono-oxygenases in the order of benzo(a)pyrene hydroxylation > benzphetamine demethylation > aniline hydroxylation > defluorination of enflurane (fig. 1).

Metabolic activities of propofol-treated microsomes (serum 0.078 (0.012) mmol litre^{-1}) were compared with microsomes incubated in propofol 0.05 and 0.10 mmol litre^{-1} (table 1). Aniline hydroxylation and defluorination of enflurane were inhibited *in vitro* by propofol 0.05 mmol litre^{-1} and were significantly different from the propofol-treated group ($P < 0.05$). With propofol 0.10 mmol litre^{-1}, benzphetamine demethylation and benzo(a)pyrene hydroxylation showed greater *in vitro* inhibition

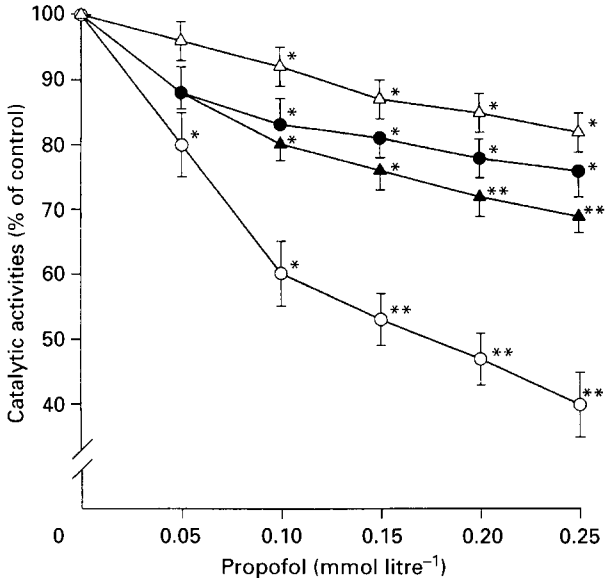

Figure 1 *In vitro* concentration-dependent inhibitory effects of propofol on hamster hepatic cytochrome P450-dependent mono-oxygenase activities: ● = aniline hydroxylation, ▲ = benzphetamine demethylation, ○ = benzo(a)pyrene hydroxylation and △ = defluorination of enflurane. $*P < 0.05$, $**P < 0.01$, compared with the control group.

Table 2 Total cytochrome P450 and cytochrome b_5 content in hamster liver microsomes ($n = 6$) preincubated with propofol 1.0 mmol litre^{-1}, NADPH, or both, before assay using carbon monoxide and NADH difference spectral analyses. Data are mean (SEM). $*P < 0.05$, $**P < 0.01$ compared with control

Incubation	Cytochrome P450 (nmol/mg protein)	Cytochrome b_5 (nmol/mg protein)
Control	1.62 (0.15)	0.45 (0.02)
+Propofol	1.33 (0.12)*	0.19 (0.03)**
+Propofol, NADPH	1.24 (0.08)*	0.10 (0.02)**

compared with the propofol-treated group. All four reactions *in vitro* with propofol 0.10 mmol litre^{-1} were significantly different from the control group ($P < 0.05$).

Propofol 1.0 mmol litre^{-1} inhibited binding between the haemeprotein of cytochrome P450 and carbon monoxide by 18 %, which increased to 24 % when NADPH was added. The binding of cytochrome b_5 and NADH was also inhibited by propofol (42 % and 22 % in the absence and presence of NADPH) (table 2).

Discussion

Metabolism of benzphetamine and benzo(a)pyrene was used to assess cytochrome P450 2B1 (phenobarbitone inducible) and 1A1 (responsible for the metabolism of carcinogens) activities [21]. However P450 2B1 is not the major form of mono-oxygenase in humans [22] and induction by phenobarbitone cannot be assessed easily in human liver. Our previous study in human liver has already shown that the *in vitro* inhibition of P450 2B1 by propofol was more marked than 2E1 [9] and the existence of human P450 2B1 could be detected by immunoblot analysis (data not shown). Aniline hydroxylation characterized the activity of cytochrome P450 2E1, responsible for microsomal defluorination of most inhalation anaesthetics [23]. In this study, we compared P450 activity in microsomes of propofol-treated and control animals. Traditionally, the regimen for induction of P450 enzymes by xenobiotics, such as phenobarbitone, ethanol or isoniazid, usually takes 4–7 days to feed with drug-containing water or via i.p. injections [11, 12, 24–26]. Considering the rapid body clearance of propofol, it seems more rational to use a continuous infusion which mimics the clinical situations. As long-term infusion in hamsters was not possible, we chose i.p. injection to reduce the rate of absorption and prolong the duration of action. We also increased exposure to propofol by twice daily injections for 2 weeks. Our data clearly demonstrated that a difference existed between the *in vitro* and *in vivo* effect of propofol on the hamster mono-oxygenase system. Prolonged treatment with propofol *in vivo* at peak serum concentrations of 0.078 (0.012) mmol litre^{-1} did not produce significant inductive or inhibitory effects on either the quantity or quality of hamster hepatic cytochrome P450-dependent mono-oxygenases.

Our *in vitro* data showed that propofol exhibited significant inhibitory effects on various P450 enzymes at concentrations of 0.05–0.25 mmol litre^{-1}. Similar inhibition of *in vitro* steroidogenesis was demonstrated by Lambert, Mitchell and Robertson with propofol 0.02–0.5 mmol litre^{-1} [27]. These propofol concentrations corresponded with the *in vivo* concentrations measured in different animal models [28]. Plasma concentrations of propofol in humans are variable (0.005–0.03 mmol litre^{-1}) according to the administration regimen [5, 29]. Our previous study demonstrated that the human liver P450 system *in vitro* could be inhibited by propofol, even at concentrations less than 0.05 mmol litre^{-1}, which were close to clinical concentrations [9].

With regard to individual mono-oxygenase activity, benzphetamine demethylation and benzo(a) pyrene hydroxylation showed minor inhibition in propofol-treated microsomes. In the *in vitro* incubation system with similar concentrations as *in vivo*, both reactions showed more profound inhibition with propofol 0.05 and 0.10 mmol litre^{-1}. Concentration-dependent inhibition of mono-oxygenases was demonstrated in another *in vitro* dose–response study. Aniline hydroxylation and defluorination increased in propofol-treated animals compared with control animals, implying a minor *in vivo* inductive effect, but this was not statistically significant. In contrast, propofol inhibited aniline hydroxylation and defluorination in the *in vitro* incubation system in a concentration-dependent manner, even at the lowest concentrations of 0.05 and 0.10 mmol litre^{-1} (table 1).

The reasons for this difference may be multifactorial and complex. Previous work on the inhibitory effects of propofol on drug metabolism were performed *in vitro* with higher concentrations [4, 5, 7, 10]. In our data, with higher concentrations, *in vitro*, propofol could compete with carbon monoxide for binding to cytochrome P450 enzymes analysed by the carbon monoxide difference spectral

study. This inhibitory data obtained *in vitro* may be related to the competitive binding of propofol with P450 haemprotein that forms the main part of cytochrome P450 mono-oxygenase. This competition for binding to the active sites of P450 hinders the efficiency of microsomal electron transport and therefore causes inhibition [20, 30]. In order to exclude this factor, we chose concentrations in the *in vitro* test systems similar to the peak serum concentration *in vivo*.

In addition, haemodynamic changes produced by propofol *in vivo* should be considered. Clinically, systolic and diastolic pressures and systemic vascular resistance decrease after administration of propofol [31, 32]. Moreover, hepatic blood flow also decreases consistently during propofol anaesthesia and these effects probably hinder *in vivo* hepatic uptake of propofol [33]. Both the systemic and regional haemodynamic effects could affect the *in vivo* microsomal concentration of propofol and produce much lower concentrations of propofol than expected.

Propofol is a formulation of emulsion in Intralipid (10 % soybean, 1.2 % phospholipids, 2.25 % glycerin and water). Increased fat or energy uptake may affect microsomal oxidizing activities [34, 35], such as reduction in the metabolism of benzphetamine (P450 2B1), and induction of cytochrome P450 2E1 after long-term exposure to propofol is compatible with our data *in vivo*.

In conclusion, our data emphasize that caution is required when comparing *in vitro* with *in vivo* drug interactions.

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