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LABORATORY INVESTIGATIONS

Effects of propofol on functional activities of hepatic and extrahepatic conjugation enzyme systems

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The effect of propofol on the hepatic and extrahepatic conjugation enzyme systems was assessed *in vitro* using microsomal and cytosolic preparations of human liver, hamster kidney, lung and gut. The functional activities of phase-II enzymes, including uridine diphosphate-glucuronosyltransferase (UDPGT), glutathione *S*-transferase (GST) and *N*-acetyltransferase (NAT) were evaluated in the presence of various concentrations of propofol (0.05–1.0 mmol litre^{-1}), using 1-naphthol, 1-chloro-2,4-dinitrobenzene and *p*-aminobenzoic acid as substrates respectively. Propofol produced concentration-dependent inhibition of UDPGT activity in human liver microsomes. Propofol did not produce significant inhibition of human hepatic GST activity at concentrations below 1.0 mmol litre^{-1}. In contrast, NAT activity was unaffected by propofol 0.05–1.0 mmol litre^{-1} in human liver cytosolic preparations. In extrahepatic tissues, hamster renal and intestinal UDPGT activities were significantly inhibited by propofol at 0.25–1.0 mmol litre^{-1}. In these tissues, GST and NAT were unaffected by propofol at 1.0 mmol litre^{-1}. Propofol produced differential inhibition of human liver and hamster extrahepatic conjugation enzymes as a result of different substrate and tissue specificities. The potential interference of the metabolic profile of phase-II enzymes as a result of inhibition by propofol (especially of UDPGT and GST) should be considered when using propofol with other drugs for anaesthesia.

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The principal metabolic reactions occurring in hepatic and extrahepatic tissues are classified into 'phase I', (including oxidation, reduction and hydrolysis) and 'phase II' (i.e. the conjugation pathways).[1–3] Phase-II drug metabolism is carried out by conjugation of drugs or their metabolites with endogenous compounds such as glucuronic acid, glutathione and acetate to convert hydrophobic compounds to hydrophilic compounds and to facilitate their elimination from the body.[4–6] Glucuronidation, for example, being one of the major conjugation reactions, is the key step involved in the metabolism of anaesthetics such as morphine, meperidine, codeine and the benzodiazepines.[7 8] The functions of these phase-II enzymes are known to be impaired in patients with severe liver disease or acute-phase responses such as inflammation.[9 10] Other factors, including sex, age, fasting and the ingestion of ethanol, may also regulate the function of phase-II enzyme systems.[11–13]

Propofol (2,6-diisopropylphenol) is a rapid-onset, short-acting intravenous anaesthetic agent that is used widely for the induction and maintenance of anaesthesia as well as for long-term sedation in intensive care units.[14 15] It has been noted that there is a pharmacokinetic interaction of propofol with narcotics, such as fentanyl and sufentanil, via inhibition of hepatic microsomal degradation.[16] Previous investigations have demonstrated that propofol interferes with the metabolism of co-administered drugs via animal and human liver phase-I metabolizing enzymes. For example, there is interference with cytochrome P450-dependent monooxygenases via interaction with the haemoprotein, and reduction of the efficiency of electron transport.[17 18] Whether the interactions caused by propofol occur purely via the phase-I enzymes or via phase-I and/or phase-II enzymes has not been examined. There are several lines

of evidence indicating that propofol induces subclinical and reversible disturbance in hepatocellular integrity by affecting the serum level of hepatic transferase (conjugation) enzymes *in vivo* after long-term infusion.[19 20] The exact effect of propofol on the functional activities of specific phase-II conjugation enzymes has not yet been investigated. The aim of this study is to characterize the *in vitro* concentration–response effect of propofol on the metabolic function of various conjugation enzymes in both hepatic and extrahepatic tissues and to demonstrate the potential drug interactions involved in these metabolic pathways and clinical situations.

Materials and methods

Specimens and animal preparations

The study was approved by the National Science Council of Taiwan. After obtaining informed consent and local ethics committee approval, human liver specimens were obtained from six men and two women aged between 32 and 56 yr (mean 39 yr). Two of the specimens were obtained from organ donation for transplantation, and the other six were obtained as wedge biopsies from patients with intestinal pathology, none of whom had a history of liver disease or medication potentially affecting liver function. Exclusion criteria included liver function abnormalities such as abnormal serum levels of bilirubin (normal range 0–1.2 mg litre^{-1}), glutamic oxaloacetic transaminase (normal range 15–37 u litre^{-1}), glutamic pyruvic transaminase (normal range 15–45 u litre^{-1}) and lactate dehydrogenase (normal range 100–190 u litre^{-1}) as well as any histopathological abnormality. All liver tissues were freshly frozen in liquid nitrogen and stored at −80°C within 10 min of resection. Male Syrian hamsters, 10–12 weeks 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 stabilized in a light-controlled environment with a 12-h light period for at least 1 week before being killed by decapitation. Kidneys, lungs and intestinal mucosa were removed, rinsed and homogenized in ice-chilled 1.15% KCl (w/v) solution. After differential centrifugation, cytosolic fractions and washed microsomes were prepared separately from homogenized tissues as described by Alvares and Mannering.[21] Microsomes from the kidneys, lungs and gut were pooled from four animals and frozen at –70°C; the pellets were resuspended in potassium phosphate buffer 0.1 mol litre^{-1} at pH 7.4 for assay. Microsomal and cytosolic proteins were assayed by the method of Lowry using bovine serum albumin as standard.[22]

Phase-II enzyme assays

Pure propofol (ICI Pharmaceutica, Zeneca, Macclesfield, UK) was diluted in microsomal and cytosolic fractions of homogenized tissues. Concentration–response effects of propofol (0.05–1.0 mmol litre^{-1}) for each enzyme assay were evaluated in human liver and hamster renal, lung and gut tissues, and compared with the control.

UDP-glucuronosyltransferase assay

Uridine diphosphate-glucuronosyltransferase (UDPGT) activity was studied by the method of Mackenzie and Hanninen, using 1-naphthol as the substrate.[23] In brief, the enzymic reaction was initiated by mixing various concentrations of propofol and 50% microsomes with 20 mg ml^{-1} sodium cholate (1:1 v:v) and cooling on ice for 30 min. The pretreated microsomal preparation was added to K-PO_4 buffer 50 mmol litre^{-1}, $MgCl_2$ 0.1 mol litre^{-1} and 1-naphthol 4.17 mmol litre^{-1} and incubated in 37°C for 5 min. After adding UDP-glucuronic acid 5 mmol litre^{-1}, microsomal protein was incubated at 37°C for 10 min in the sample group, using K-PO_4 buffer 50 mmol litre^{-1} as blank. The reaction was stopped on ice after 5 min. The fluorescence intensity of the product, 1-naphthol β-D-glucuronide, was measured at excitation and emission wavelengths of 293 and 335 nm respectively.

Glutathione S-transferase assay

In this study, we carried out the glutathione *S*-transferase (GST) assay using the standard substrate 1-chloro-2,4-dinitrobenzene, following the procedure of Habig *et al*.[24] Essentially, the reaction mixture contained 1 mmol litre^{-1} glutathione in 0.1 mmol litre^{-1} K-PO_4 buffer with 1 mmol litre^{-1} EDTA. After addition of 1 mmol litre^{-1} 1-chloro-2,4-dinitrobenzene to the sample cuvette, the absorbance of the mixture was measured at 340 nm for the baseline, with methanol as a reference. Cytosolic protein in 0.1 mmol litre^{-1} K-PO_4 buffer, pH 6.5, preincubated with various concentrations of propofol, was then added to the mixture to start the reaction for 1 min. The rate of reaction was monitored by measuring the absorbance increased at 340 nm.

N-*acetyltransferase assay*

N-acetyltransferase (NAT) activity was measured by quantifying the disappearance of the arylamine substrate, as reflected by decreasing formation of Schiff base with dimethylaminobenzaldehyde.[25] In brief, the incubation system consisted of Tris–HCl 75 mmol litre^{-1}, pH 7.5, at 37°C, dithioerythreitol 1.5 mmol litre^{-1}, EDTA 1.5 mmol litre^{-1}, acetyl phosphate 22.5 mmol litre^{-1}, phosphotransferase 2.5 U ml^{-1}, *p*-aminobenzoic acid 0.4 mmol litre^{-1} in Tris–HCl buffer, and 50 µl of diluted cytosol preincubated with propofol. The reaction was started by the addition of 40 µl of acetyl coenzyme A 1 mmol litre^{-1} to the incubation mixture and was incubated at 37°C for 45 min. The reaction was terminated by the addition of 100 µl of 20% (w/v) tricyclic acid (TCA). The reaction mixture was centrifuged and the supernatant was mixed with 1 ml of 5% dimethylaminobenzaldehyde. The sample

Table 1 Functional activities of various phase-II drug-metabolizing enzymes in human liver, hamster kidney, lung and gut. All values are mean (SD) of *n* observations. Each hamster tissue sample was pooled from four animals

Assay	Human liver	Hamster		
		Kidney	Lung	Gut
n	8	6	6	6
Microsomal protein (mg g tissue^{-1})	35.8 (6.8)	4.62 (0.88)	3.91 (0.74)	3.60 (0.48)
Cytosolic protein (mg g tissue^{-1})	49.7 (4.6)	42.8 (3.9)	37.6 (9.6)	28.4 (3.9)
UDPGT (nmol min^{-1} mg protein $^{-1}$)	3.26 (0.75)	2.07 (0.38)	0.032 (0.004)	0.74 (0.12)
GST (nmol min^{-1} mg protein $^{-1}$)	2.16 (0.86)	1.64 (0.61)	0.64 (0.28)	0.61 (0.20)
NAT (nmol min^{-1} mg protein $^{-1}$)	7.06 (0.67)	2.17 (0.37)	0.87 (0.34)	3.04 (0.98)

was recentrifuged and incubated for at least 10 min at room temperature, and the absorbance at 450 nm was then recorded.

Unless otherwise stated, all results are presented as mean (SD). Data were analysed using one- and two-way analyses of variance and significant differences between concentrations were identified using the Student–Newman–Keuls test or the unpaired *t*-test. $P<0.05$ was considered statistically significant.

Results

The activities of various enzymes were expressed on the basis of protein concentrations in the reaction mixture (Table 1). Protein content in the cytosolic components was consistently greater than in the microsomal fraction. Human liver exhibited the highest rate of catalytic activity. Enzyme activities in hamster lung tissues were lower than in the other tissues studied. Among the extrahepatic tissues, kidney contained the highest activity of UDPGT and GST, whereas the intestine contained the highest NAT activity. NAT showed the highest metabolic activity on a protein basis among all enzymes within all the tissues studied.

The effects of various concentrations of propofol on functional activities of specific phase-II enzymes evaluated within human liver microsomal and cytosolic fractions are shown in Figure 1. In human liver microsomes, propofol 0.05 mmol litre^{-1} produced a small but non-significant decrease in UDPGT activity ($P=0.08$). Between 0.10 and 1.0 mmol litre^{-1}, propofol demonstrated concentration-dependent inhibition of UDPGT activity. The metabolic rate of UDPGT in human microsomes was decreased to 50% of the control by propofol 0.10 mmol litre^{-1} ($P=0.026$) and to 14% of the control by propofol 1.0 mmol litre^{-1} ($P=0.0041$). The activity of GST was not significantly inhibited at low concentrations of propofol (0.10–0.5 mmol litre^{-1}), but was significantly inhibited at a high concentration of propofol (1.0 mmol litre^{-1}) ($P=0.032$). The metabolic rate of NAT in human liver was unaffected by propofol (Fig. 1).

Figure 2 summarizes the effects of propofol on the functional activities of various enzymes in hamster kidney, lung and gut. The specific enzyme activity per unit weight of protein in hamster kidney was about one- to two-thirds of the activity in human liver. Propofol 0.25–1.0 mmol litre^{-1} caused a 40–79% decline in hamster kidney UDPGT activity ($P<0.05$). Unlike the result obtained in human liver, the effect of propofol in hamster kidney was only a minor decline in GST activity, and this did not reach statistical significance ($P=0.12$ at propofol 1.0 mmol litre^{-1}). As in human liver, NAT activity was not influenced by increasing concentrations of propofol in hamster kidney (Fig. 2, top panel). In hamster lung tissues, the activities of GST and NAT were much lower than in human liver. The activity of UDGPT was just detectable in hamster lung. In contrast with kidney and gut, neither of the above enzymes in lung was affected by propofol (Fig. 2, middle panel). In gut, propofol 0.25–1.0 mmol litre^{-1} produced 35–70% inhibition of UDPGT activity ($P<0.05$). However, GST and NAT activities were unaffected (Fig. 2, bottom panel).

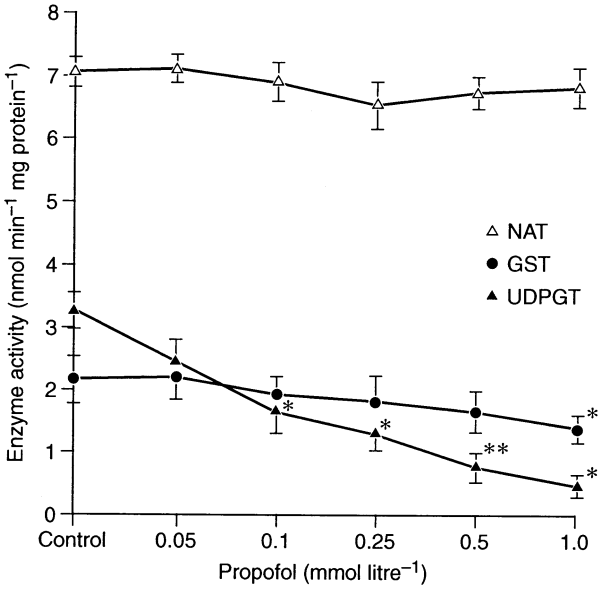

Fig 1 Effect of propofol concentration on the activities of UDPGT, GST and NAT in human liver microsomal and cytosolic preparations. Values are mean (±SEM) of three measurements in each enzyme assay ($n=8$). *$P<0.05$, **$P<0.01$ *vs* control (one-way ANOVA with *post hoc* Student–Newman–Keuls test).

Discussion

Traditionally, plasma levels of conjugation enzymes, such as GST, are important parameters for the assessment of

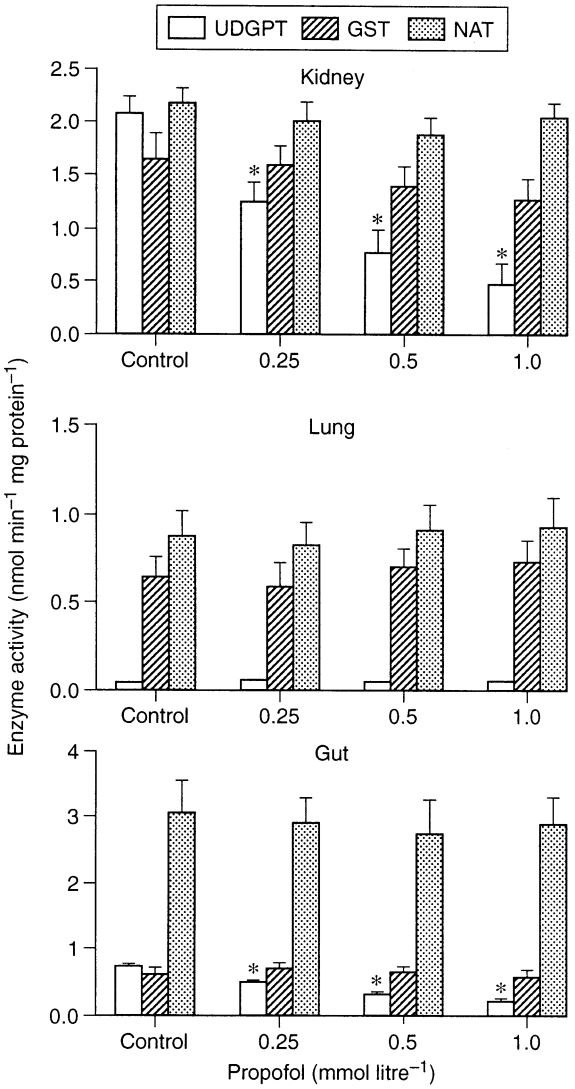

Fig 2 Effect of propofol concentration on the activities of UDPGT, GST and NAT in microsomal and cytosolic preparations of hamster kidney, lung and gut. Values are mean (SEM) of three measurements in each enzyme assay ($n=6$, each pooled from four animals). *$P<0.05$ *vs* control group (one-way ANOVA with *post hoc* Student–Newman–Keuls test).

hepatocellular injury. After exposure to general anaesthesia or long-term infusion of propofol, the plasma level of GST increases significantly, indicating the presence of hepatocellular damage and the leakage of cytosolic enzymes into the extracellular space.[5 19 20 26] UDPGTs, the most important phase-II enzymes we studied in the microsomal fraction, have a wide distribution in hepatic and extra-hepatic tissues.[3 4] Clinically, the plasma concentrations of propofol in humans and the hamster have been reported to reach 0.067–0.10 mmol litre^{-1},[27–29] although this would be reduced by a high level of protein binding. Under similar concentrations *in vitro*, our data showed that propofol produced significant inhibition of the conjugation activities of human liver and hamster extra-hepatic UDPGT. Concentration–response curves demonstrated concentration-dependent inhibition of UDPGT when we increased the concentration of propofol from 0.10 to 1.0 mmol litre^{-1}.[18 27–29] Many endogenous and exogenous amines, steroids and opioid compounds are metabolized through glucuronidation reactions by UDPGTs.[7] A previous investigation by Janicki *et al*. showed that the microsomal degradation of narcotics, such as alfentanil and sufentanil, was hindered by the presence of propofol.[16] Besides propofol's inhibition of microsomal monooxygenases, the inhibition of glucuronidation by propofol provides another possible mechanism for the interference of pharmacokinetics and drug interactions with opioids.[17 18 29]

Many factors have been studied and identified as modulators or inhibitors of UDPGT activity that might provide explanations of the above findings. UDPGT proteins are membrane proteins with a hydrophobic membrane-spanning domain at their carboxyl terminus in addition to other hydrophobic domains throughout the molecule, which probably function to bind hydrophobic molecules.[30 31] Propofol, a molecule with a high octanol/water partition coefficient, is suitable for hydrophobic binding with UDPGT proteins.[14] Also, phenol activity was shown to be dependent on the binding of phospholipids, and phospholipid binding leads to conformational changes in the UDPGT enzyme.[32] As an alkylated phenol, propofol might exhibit its potent inhibition of UDPGT through binding to the membrane protein and induce alterations in enzyme conformation as well as a reduction of its reactivity.[14] The present analysis of extrahepatic tissues in the hamster also showed that the gut and the kidneys are another two important sites for glucuronidation; only modest activity was observed in the lungs. Propofol at 0.25–1.0 mmol litre^{-1} exhibited similar, but lower, inhibition of UPDGT activity in hamster kidney and gut than in human liver. The difference between the hepatic and extrahepatic effects of propofol on UDPGT might be attributable to the species- and tissue-specificity of the enzyme.[3 4] The tissue distribution of the enzyme and the functional heterogeneity of the UDPGT enzyme family within different tissues might explain the differences in the inhibition induced by propofol.[4]

The GSTs are also a complex multigene family of enzymes that are widely distributed in various tissues.[33] These enzymes are primarily located in the cytosol and are important in detoxification by conjugating reduced glutathione with a large number of electrophiles.[34] The plasma level GST has been used as a sensitive indicator of hepatocellular integrity in liver diseases or liver damage induced by drug intoxication.[5 35 36] Our data showed that propofol did not exhibit significant inhibition of GST activity at concentrations less than 1.0 mmol litre^{-1}. There are several possible explanations. First, the present study showed that GST activity was relatively low among these phase-II enzymes in human liver, hamster kidney and gut tissues, making significant inhibition more difficult to detect. Secondly, GST may bind a number of anions, such as bile salts, which might inhibit enzymic activity.[37] Being a phenolic substitute, propofol might participate in the

conjugation reactions as a hydrogen provider (functional anion) and bind with GST to reduce its conjugating ability.[14] This might explain why propofol produced inhibition of GST only at high concentrations. GST in hamster extrahepatic tissues was essentially unaffected by propofol. The discrepancy in propofol's inhibiting effect between hepatic and extrahepatic tissues might result from functional heterogeneity of the distribution of various GST isoenzymes within different species and tissues.[38] It is possible that GSTs serve different functions in different tissues, sometimes acting as carrier proteins rather than being metabolically active.[5]

N-acetylation is another important phase-II conjugation reaction, and NATs are responsible for the metabolism of a large number of drugs and compounds, including isoniazid, hydralazine, procainamide, caffeine and arylamine carcinogens.[39] Previous studies have shown that the effects of liver diseases on acetylation seem to be modest and of lesser magnitude than their effects on oxidative pathways.[6 9] In our data, NAT exhibited the highest activity among these phase-II enzymes. NAT activity in the intestine is about one-half that in liver, 50% higher than that in kidney and three times that in lung, so that the gut is also an important site of acetylation. In contrast to other phase-II enzymes, propofol produced no significant inhibition of NAT activity in either hepatic or extrahepatic tissues. Compared with UDPGT and GST, NAT is less polymorphic in terms of isoenzymes and tissue-specificity.[6] The substrate for the measurement of NAT activity, *p*-aminobenzoic acid, is also different from propofol in physicochemical properties, indicating that interaction of propofol with NAT is clinically irrelevant.[3]

Clinically, the mechanisms involved in the interactions of drugs with propofol might be multifactorial. First, the haemodynamic alterations caused by propofol might reduce the regional blood flow as well as the regional delivery of agents to the sites of metabolism.[40 41] Therefore, it is likely that the tissue uptake and elimination of the coadministered drugs would be modified.[40 41] Secondly, propofol might compete for serum protein binding, which might increase the free fraction and probably potentiate the pharmacodynamic effects of other drugs, such as propranolol.[42] Thirdly, in our previous studies propofol exhibited broad-spectrum, concentration-dependent inhibition of cytochrome P450-dependent monooxygenases (phase-I enzymes). Propofol 0.05–0.10 mmol litre^{-1} showed significant inhibition of cytochrome P450 2E1, 1A1 and 2B1, which might hinder the metabolism of coadministered drugs.[17 18] The present study demonstrated that propofol was also able to inhibit the conjugation enzymes to various degrees at similar concentrations. The interference with these phase-II enzymes by propofol complicated the pharmacokinetics, and clinicians should be aware of potential propofol-related drug interactions during anaesthetic practice.

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