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Abstract

The effect of propofol on the hepatic and extrahepatic conjugation enzyme systems was assessed *in vitro* within microsomal and cytosolic preparations of the human liver, hamster kidney, lung and gut tissues. The functional activities of phase II enzymes including uridine diphosphate-glucuronosyltransferase (UDPGT), glutathione S-transferase (GST), and N-acetyltransferase (NAT) were evaluated under various concentrations, 0.05-1.0 mmol litre^{-1} of propofol, using 1-naphthol, 1-chloro-2,4-dinitrobenzene and *p*-aminobenzoic acid as substrates, respectively. From clinical plasma concentration, 0.05-0.10 mmol litre^{-1}, to high concentration, 1.0 mmol litre^{-1}, propofol demonstrated a dose-dependent inhibition to UDPGT activity in human liver microsomes. Propofol did not exhibit its significant *in vitro* inhibition to human hepatic GST activity until it reached high concentration, 1.0 mmol litre^{-1}. In contrast, NAT activity was basically unaffected by various concentrations of 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 0.25-1.0 mmol litre^{-1} of propofol. While GST and NAT in hamster extrahepatic tissues were unaffected even in high concentration, 1.0 mmol litre^{-1} of propofol. Propofol in various concentrations showed its differential inhibition to human liver and hamster extrahepatic conjugation enzymes due to different substrate- and tissue-specificities. The potential interference to metabolic profile of phase II enzymes due to propofol's inhibition, esp. to UDPGT and GST, should be considered clinically significant in drug interactions when using propofol with other drugs for anaesthesia.

Keywords: anaesthetics i.v., propofol; enzymes, phase II, conjugation; liver, microsomes, cytosol.

Introduction

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

Propofol (2,6-diisopropylphenol), being a rapid onset short-acting intravenous anaesthetic agent, which is used widely to induce and maintain anaesthesia as well as for long-term sedation in intensive care unit.[14 15] It has been noted that propofol interacted pharmacokinetically with narcotics, such as fentanyl and sufentanil, by inhibiting their hepatic microsomal degradation.[16] Previous investigations have demonstrated that propofol interfered the metabolism of co-administered drugs through animal and human liver phase I metabolizing enzymes, cytochrome P450-dependent monooxygenases by interacting with the haemoprotein and reducing the efficiency of electron transport.[17 18] While whether the drug-interactions caused by propofol was purely through the phase I enzymes or phase I and/or phase II enzymes have not been validated. There are several evidences indicating that propofol might induce 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 to the functional activities of specific phase II enzymes, conjugation enzymes, has not yet been investigated. The aim of this study is to characterize the *in vitro* dose-response effect of propofol to 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 animals 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 6 men and 2 women (aged between 32 and 56 years, mean ± S.D. 39 ± 8). Among the specimens, 2 were obtained from the organ donors for the transplantation, the other 6 were obtained from the wedge biopsies from the patients with the pathology of the intestines neither having history of liver diseases nor medications potentially affecting the liver functions. Exclusion criteriae 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 abnormality within histopathological analysis. All liver tissues were freshly frozen in liquid nitrogen and stored at –80°C within 10 min after resection. Male Syrian golden 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 stayed for stabilization in a photo-controlled environment with 12-h light period for at least 1 week before sacrifice by decapitation. Kidneys, lungs and mucosa of intestine was removed, rinsed and homogenized in an 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 guts were pooled from every 4 animals and frozen at -70°C until pellets were resuspended in potassium phosphate buffer 0.1 mol liter^{-1} at pH 7.4 for assay. Microsomal and cytosolic protein was assayed by the method of Lowry using bovine serum albumin as standard.[22]

Phase II enzyme assays

Pure compound of propofol (ICI Pharmaceutica, Zeneca, UK) was diluted in microsomal and cytosolic fractions of homogenized tissues. Dose-response effects of various concentrations of propofol (0.05 to 1.0 mmol litre^{-1}) to each enzyme assay was evaluated within human liver, hamster renal, lung and gut tissues, and compared with the

control group.

UDP-glucuronosyltransferase assay (UDPGT)

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

Glutathione S-transferase assay (GST)

In this study, we carried out GST assay using the standard substrate 1-chloro-2,4-dinitrobenzene following the procedures 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 adding 1 mmol litre^{-1} 1-chloro-2,4-dinitrobenzene to sample cuvette and using methanol as reference, the mixture was scanned at 340 nm for baseline. 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 (NAT)

NAT activity was measured by quantifying the disappearance of the arylamine substrate as reflected by decreasing Schiff's base formation with dimethylamino-benzaldehyde.[25] In brief, the incubation system consists of 75 mmol litre^{-1} Tris-HCl, pH 7.5, in 37℃, 1.5 mmol litre^{-1} dithioerythritol, 1.5 mmol litre^{-1} EDTA, 22.5 mmol litre^{-1} acetyl phosphate, 2.5 U ml^{-1} phosphotransferase, 0.4 mmol litre^{-1} *p*-aminobenzoic acid in Tris-HCl buffer, and 50 μl of properly diluted cytosol preincubated with propofol. The reaction was started by the addition of 40 μl of acetyl CoA, 1 mmol litre^{-1}, into the incubation mixture and incubated at 37℃ for 45 min. The reaction will be terminated by addition of 100 μl of 20 % (w/v) TCA. The reaction mixture was centrifuged and the supernatant was mixed with 1 ml of 5 % dimethylaminobenzaldehyde. The sample was recentrifuged and incubated for at least 10 min at room temperature. Absorbance at 450 nm was recorded.

Unless otherwise stated, all results are presented as mean (SD). Data were analyzed using one-way and two-way analyses of variance and significant differences between various concentrations were identified by 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 cytosol was consistently more abundant than in the microsomal fractions. Human liver exhibited the highest rate of catalytic activity in all reactions within various tissues. While the enzyme activities in hamster lung tissues were relatively lower than the other tissues. Among the extrahepatic tissues, kidney contained the highest activity of UDPGT and GST, whereas the intestine contained the highest NAT activity. As to individual enzymes, NAT showed the highest metabolic activity in protein basis among the enzymes within all the tissues.

Effects of various concentrations of propofol on functional activities of specific phase II enzymes evaluated within human liver microsomal and cytosolic fractions were shown in Figure 1. In human liver microsomes, propofol at 0.05 mmol litre^{-1}, showed a minor but insignificant decrease in UDPGT activity (P=0.08). At 0.10–1.0 mmol litre^{-1}, propofol demonstrated a dose-dependent inhibition to UDPGT activity. Metabolic rate of UDPGT in human microsomes was decreased to 50% activity of the control by 0.10 mmol litre^{-1} of propofol (P=0.026) and to 14% activity of the control by 1.0 mmol litre^{-1} of propofol (P=0.0041). The activity of GST was minimally inhibited at low concentrations, 0.10-0.5 mmol litre^{-1} of propofol, and was significantly inhibited by high concentration, 1.0 mmol litre^{-1}, of propofol (P=0.032). The metabolic rate of NAT in human liver was relatively stable under the influence of various concentrations of propofol (Fig. 1).

As to the extrahepatic tissues, Figure 2 summarized the effects of propofol to 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-third to two-third of activity in human liver. Propofol, in 0.25–1.0 mmol litre^{-1}, caused 40-79% decline of hamster renal UDPGT activity (P<0.05). Unlike human liver, propofol only caused a minor decline in hamster renal GST activity without statistical significance (P=0.12, at 1.0 mmol litre^{-1} of propofol). Similarly to human liver, NAT activity was not influenced by increasing concentrations of propofol in hamster kidney (Fig. 2, upper). In hamster lung tissues, the activities of GST and NAT was much less than in human liver. The activity of UDGPT was minimally detected in hamster lung tissues. In contrast with kidney and gut, neither of above enzymes in lung was affected by various concentrations of propofol (Fig. 2, middle). In gut tissues, propofol in 0.25–1.0 mmol litre^{-1} exhibited 35-70% inhibition to UDPGT activity (P<0.05). However, GST and NAT activities were unaffected by propofol in hamster gut tissues (Fig. 2, lower).

Discussion

Traditionally, plasma level of these conjugation enzymes, GST for example, is an important parameter for the assessment of hepatocellular injury. After exposure to general anaesthesia or long-term infusion of propofol, plasma level of GST increased significantly representing the existence of hepatocellular damage with the leakage of cytosolic enzymes into extracellular space.[5 19 20 26] While the functional impact of anaesthetics, such as propofol, to the conjugation ability of phase II enzymes has been first described in the present study. UDPGTs, the most important phase II enzymes we studied in the microsomal fraction, have a wide tissue distribution in liver and extrahepatic tissues.[3 4] Clinically, the plasma concentrations of propofol in human and hamster have been reported to reach 0.067-0.10 mmol litre^{-1}.[27-29] Under the similar concentrations *in vitro*, our data showed that propofol demonstrated a significant inhibition to human liver and hamster extrahepatic UDPGT conjugation activities. Dose-response curve of propofol-UDPGT activity exhibited a dose-dependent inhibition to UDPGT when we increased the concentration of propofol from 0.10 mmol litre^{-1} (which was clinically relevant) up to 1.0 mmol litre^{-1} (which was kinetically relevant).[18 27-29] Many endogenous and exogenous amines, steroids as well as opioid compounds are catalyzed through the glucuronidation reactions by UDPGTs.[7] Previous investigation by Janicki et al. showed that the microsomal degradation of narcotics, such as alfentanil and sufentanil, was hindered by the existence of propofol.[16] Besides propofol's inhibition to the microsomal monooxygenases, the inhibition to the glucuronidation by propofol provided another possible mechanism for the interference of propofol-related drug-interactions with opioids.[17 18 29]

Many factors have bee studied and

identified as modulator or inhibitors of UDPGT activity, which might be postulated to explain 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 that most likely function to bind hydrophobic molecules.[30] [31] Propofol, the molecule with high octanol/water partition coefficient, is suitable for the 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 of the UDPGT enzyme.[32] As an alkylated phenol, propofol might exhibit its potent inhibition to UDPGT through the binding to the membrane protein and induce the alterations in enzyme conformation as well as reduction of its reactivity.[14] The present analysis of extrahepatic tissues in hamster also showed that the gut and the kidneys are another two important sites for glucuronidation and only modest activity was observed in lung. Propofol, from 0.25-1.0 mmol litre^{-1}, exhibited the similar but less inhibition to the UPDGT activity in hamster kidney and gut than human liver. The difference between the hepatic and extrahepatic effect of propofol to UDPGT might be coming from the species and tissue-specificity of the enzyme.[3] [4] Enzyme tissue distribution as well as functional heterogeneity in UDPGT enzyme family within different tissues might explain the differences for 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 located majorly in cytosol are important in detoxification by conjugating reduced glutathione with a large number of electrophiles.[34] The plasma level GST has been used to be a sensitive indicator of hepatocellular integrity in liver diseases or drug intoxication-induced liver damage.[5] [35] [36] Our data showed that propofol did not exhibit its significant inhibition to GST activity until reaching the highest concentration, 1.0 mmol litre^{-1}. There are several factors might be associated with this phenomenon of inhibition. Firstly, the present study showed that GST activity was relatively low among these phase II enzymes in human liver, hamster kidney and gut tissues that made the significant inhibition more difficult to be achieved. Secondly, the GST could bind a number of anions such as bile salts that would inhibit enzymic activity.[37] Being a phenolic substitute, propofol might participate into the conjugation reactions as a hydrogen provider (functional anion) and bind with GST to reduce its conjugation ability.[14] This might explain why propofol could exhibit its inhibition to GST only in high concentration. The GST in hamster extrahepatic tissues were basically unaffected by various concentrations of propofol. The discrepancy of propofol's inhibition between liver and extrahepatic tissues might be the functional heterogeneity of distribution of various GST isoenzymes within different species and tissues.[38] A possible postulation is that GSTs might 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 showed that the effect of liver diseases on acetylation seemed to be modest and of a lesser magnitude than their effect on oxidative pathways.[6] [9] In our data, NAT exhibited the highest activity among these phase II enzymes. Intestinal NAT is about one half that of liver, 50% higher than that of kidney and triple that of lung, so that gut is also an important site for acetylation. In contrast with other phase II enzymes, propofol exerted no significant inhibition to NAT activity in both hepatic and extrahepatic tissues. Comparing with UDPGT and GST, NAT is relatively less polymorphic in isoenzymes and tissue-specificity.[6] The

substrate for the measurement of NAT activity, *p*-aminobenzoic acid, is also different in physico-chemical properties from propofol that makes propofol seem to be clinically irrelevant to the NAT reactivity.[3]

Clinically, the mechanisms involved in the drug interactions 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 interfered.[40 41] Secondly, propofol could compete for the serum protein binding that 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 a broad spectrum, concentration-dependent inhibition to cytochrome P450-dependent monooxygenases (phase I enzymes). Within therapeutic range of concentrations, 0.05-0.10 mmol litre^{-1}, propofol showed significant inhibition to cytochrome P450 2E1, 1A1 and 2B1 that might hinder the drug-metabolism for the other coadministered drugs.[17 18] Finally, the present study demonstrated that propofol also could inhibit the conjugation enzymes to various extent under the similar concentrations. The interference to these phase II enzymes by propofol made the pharmacokinetics more complex and its clinical significance should be sincerely concerned.

In conclusion, from therapeutic concentrations to high concentrations, propofol could inhibit the *in vitro* conjugation activities of hepatic and extrahepatic phase II enzymes, such as UDPGT, in a dose-dependent manner. In contrast, propofol exerts minimal effect on GST and no effect to NAT activities. The inhibition of propofol to these metabolic enzymes reminds clinicians to be aware of the potential propofol-related drug interactions during anaesthetic practice.

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