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Propofol inhibits lidocaine metabolism in human and rat liver microsomes

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Abstract
Purpose. When two drugs are metabolized by similar P450 isoforms, one drug inhibits the metabolism of the other when both the present. The metabolism of lidocaine and propofol can be mediated by similar P450 isoforms. Therefore, we investigated the relationship in the metabolism between lidocaine and propofol in both rat and human liver microsomal P450 (CYP) systems in vitro.
Methods. (1) Propofol, 4 μg·ml^{-1}, as the substrate and lidocaine (between 0.5 and 8 μg·ml^{-1}) and (2) lidocaine, 4.7 μg·ml^{-1}, as the substrate and propofol (between 0.5 and 40 μg·ml^{-1}) were reacted separately with human and rat microsomes. The concentrations of lidocaine, its major metabolite (monoethylglycinexylidide, MEGX) and propofol were measured using high-pressure liquid chromatography. The metabolism of lidocaine was presented as a reaction activity (MEGX/lidocaine).
Results. The dose-dependent inhibitory effects of propofol on lidocaine metabolism were observed in both the human and rat groups. The IC50 (the concentration producing 50% maximal inhibition) of propofol was 5.0 μg·ml^{-1} and 0.70 μg·ml^{-1} in the human and the rat groups, respectively. The propofol concentration of 5.0 μg·ml^{-1} is within the range of clinical doses for humans. On the other hand, lidocaine did not change propofol metabolism.
Conclusion. Propofol possesses a dose-dependent inhibitory effect on the metabolism of lidocaine in both human and rat CYP systems in vitro.

Key words Cytochrome P450 · Drug interaction · Propofol · Lidocaine · In vitro

Introduction

Importantly, inhibition of the metabolism of coadministered drugs is often observed when both agents are metabolized by the same kind of cytochrome P450 (CYP) isoforms [1]. The CYP enzymatic system is essential for the biotransformation of drugs known to affect the CYP isoform [2]. Propofol (2,6-diisopropylphenol) is frequently used in combination with other agents, such as local anaesthetics used for continuous epidural anesthesia, for the induction or maintenance of anesthesia. Drug metabolism generally occurs through hydroxylation and glucuronidation in the liver CYP system [3,4]. It was recently shown that the metabolism of propofol can be mediated by multiple hepatic CYP isoenzymes including CYP1A2 [2] and CYP3A4 [5]. The local anesthetic lidocaine can also be metabolized by CYP1A2 and CYP3A4 [6]. Additionally, propofol has been shown to inhibit the metabolism of other drugs [5,7]. If a large dose of lidocaine is used for epidural anesthesia in combination with propofol for general anesthesia, local anesthetic toxicity and delayed emergence from anesthesia may result. These disadvantages could be expected to ameliorate the rapid emergence property of propofol, and may give rise to the consideration of a propofol infusion program such as target control infusion (TCI). Accordingly, it is important to determine the drug interaction between lidocaine and propofol. Additionally, in 1997 the US Food and Drug Administration recommended that drug metabolism be assessed by using human tissues. The aim of the present study was to evaluate the metabolic relationship between lidocaine and propofol in human and rat CYP in vitro systems.

Methods

Human microsomes from 11 donors were purchased from Gentest [Pooled human liver microsomes, H161 (Lot number 16, Protein 20 mg/ml in 250 mM sucrose), Woburn, MA, USA], and stored at −80°C. After thawing, human microsomes were stored on ice. Male

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Sprague-Dawley rats ($n = 5$, weighing 280–350 g, 8–10 weeks of age) were obtained from Nihon Clea Co. (Tokyo, Japan). The rats were kept in an air-conditioned room (25 ± 1°C, 50%–60% humidity) with a 12-h light–dark cycle (8:00 A.M.–8:00 P.M.) and given free access to commercial rat chow (Oriental-MF, Tokyo, Japan) and water. Untreated rats were fasted overnight and decapitated under ether anesthesia, and then the liver was immediately removed. The livers obtained from five rats were homogenized in 4 vols (w/v) ice-cold 1.15% KCl, the homogenates were centrifuged at 10000*g* for 20 min, and the supernatants were then ultracentrifuged at 105000*g* for 60 min. The resulting microsomal pellets were resuspended in 50 mM potassium phosphate buffer (pH 7.4), containing 0.1 mM ethylenediaminetetraacetate, and stored at −80°C.

Enzyme assay

Lidocaine and monoethylglycinexylidide (MEGX) were kindly provided by Astra Japan (Osaka, Japan). Propofol and trimethoprim were purchased from Sigma (St. Louis, MO, USA). The incubation mixture contained 0.125 mg liver microsomal protein, 0.1 M potassium phosphate buffer (pH 7.4), nicotinamide adenine dinucleotide phosphate, reduced (NADPH), and either (1) lidocaine, 4.7 μg·ml^{-1}, as the substrate and propofol (0, 0.5, 1.0, 2.5, 5.0, 7.5, 10, 15, 20, and 40 μg·ml^{-1}) or (2) propofol, 4 μg·ml^{-1}, as the substrate and lidocaine (0, 0.5, 1.0, 2.0, 4.0 and 8.0 μg·ml^{-1}), with dimethyl sulfoxide as a solvent in a total volume of 0.5 ml. Additionally, we selected lidocaine (4.7 μg·ml^{-1}; approximately the maximum clinical concentration), and performed an in vitro study to assess the effect of propofol (0, 0.5, 1.0, 2.5, 5.0, 7.5, 10, 15, 20 and 40 μg·ml^{-1}) on lidocaine metabolism. Incubations were initiated following a 5-min preincubation period at 37°C by the addition of NADPH and generally carried out for 15 min in a shaking water bath at 37°C. The reaction was terminated by adding 50 μl 1 N NaOH and 1.5 ml ethyl acetate containing an internal standard. After vortex mixing for 5 min, the tubes were centrifuged at 1200*g* for 5 min and the aqueous phase was removed by aspiration. The organic phase was transferred to a clean conical tube and evaporated in a water bath at about 40°C under a gentle stream of nitrogen. The residue was dissolved in 200 μl mobile phase, and 50 μl was injected into the high-pressure liquid chromatography (HPLC) apparatus.

Determination of lidocaine, MEGX, and propofol concentrations

We used the assay reported by Tanaka et al. [8] to determine lidocaine and MEGX concentrations. The HPLC equipment consisted of a pump (Model CCPS, Tosoh, Tokyo, Japan), a variable-wavelength ultraviolet (UV) detector (Model UV-8000, Tosoh), and a C_{18} reversed-phase column (ODS80-TM: Tosoh). The mobile phase was composed of 0.05 M KH_2PO_4-acetonitril (86:14, v/v; pH 4.0). The absorbance of the eluate was monitored at 205 nm. With this assay method, the maximum coefficient of variation for within-run or between-run precision was 3.3%, and the detection limit for both lidocaine and MEGX was 10 ng·ml^{-1}. Propofol concentrations were determined using HPLC with fluorescence detection at 276 nm (CTO-10A, RF550, and CR7A, Shimadzu, Kyoto, Japan) [9]. A standard curve was computed by using pure propofol liquid to prepare concentrations of 0, 1.0, 2.5, 5.0, and 10.0 μg·ml^{-1}. Propofol concentrations in this study were calculated using the obtained regression equation ($r = 0.999$, method of least squares). The lower limit of detection was 17 ng·ml^{-1}, and the coefficient of variation was 8.4%.

Additionally, we performed an in vitro study to assess the interference between propofol (0.1, 1, 10, 100 μg·ml^{-1}) and lidocaine 4.7 μg·ml^{-1}. All instruments were operated at ambient laboratory temperature (23°C).

Data analysis

Data were expressed at the mean ± SD. Statistical comparisons within each group were made by one-way analysis of variance for repeated measures coupled with Student *t*-test. Two-way analysis of variance was used for between group comparisons. In all cases, *P* values less than 0.05 were considered the minimum level of statistical significance.

Results

The effects of propofol on lidocaine deethylation (MEGX formation) in human and rat liver microsomes are shown in Fig. 1. Lidocaine metabolism was markedly reduced as the dose of propofol increased, and curvilinear relationships were observed in both human and rat groups. The inhibition of lidocaine metabolism was 50% in the human group and 90% in the rat group, at a propofol dose of 5 μg·ml^{-1}. The IC50 (the concentration producing 50% maximal inhibition) of propofol was 5.0 μg·ml^{-1} and 0.70 μg·ml^{-1} in the human and rat groups, respectively. The propofol value of 5 μg·ml^{-1} is within the range of clinical doses in human. On the other hand, the concentrations of propofol were 1.1 ± 0.4, 1.1 ± 0.1, 0.8 ± 0.32, 1.3 ± 0.1, 1.2 ± 0.1, and 1.1 ± 0.3 μg·ml^{-1} in the human group, and 1.1 ± 0.3, 1.1 ± 0.1, 1.2 ± 0.1, 1.2 ± 0.1, 1.1 ± 0.1, and 1.3 ± 0.1 μg·ml^{-1} in the rat group at lidocaine concentrations of 0, 0.5, 1.0,

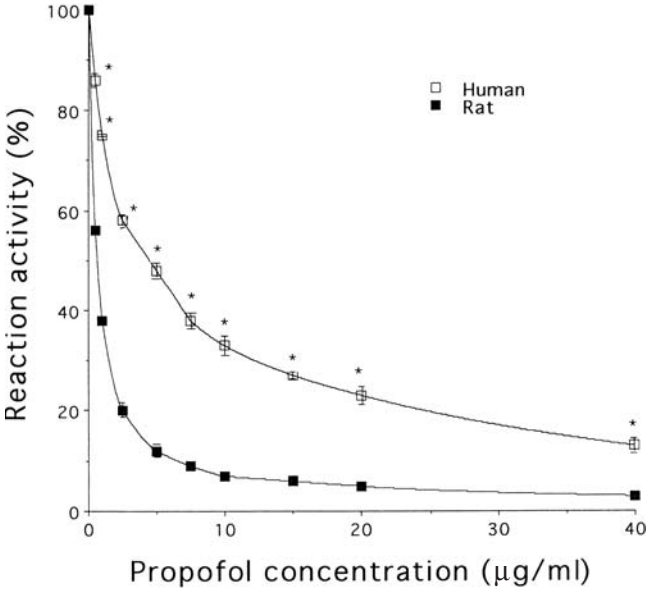

Fig. 1. The effect of propofol concentration (0.5, 1.0, 2.5, 5.0, 7.5, 10, 15, 20, and 40 μg·ml^{-1}) on lidocaine (4.7 μg·ml^{-1}) metabolism [monoethylglycinexylidide (MEGX) formation; reaction activity] in both human and rat liver microsomes in vitro. Values shown are the means ± SD of the data (n = 3). Lidocaine metabolism was inhibited as the dose of propofol increased. *$P < 0.05$, compared with rat microsome

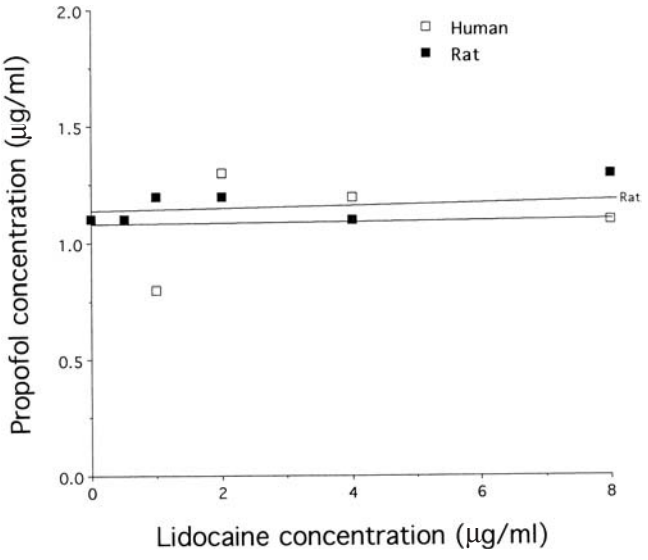

Fig. 2. The effect of lidocaine concentration (0, 0.5, 1.0, 2.0, 4.0, and 8.0 μg·ml^{-1}) on propofol (4 μg·ml^{-1}) in both human and rat liver microsomes in vitro. Values shown are the means ± SD of the data (n = 3). The propofol concentration was not changed as the dose of lidocaine increased

2.0, 4.0, and 8.0 μg·ml^{-1}, respectively (Fig. 2). There was no significant dose-dependent effect of lidocaine on propofol metabolism.

The additional in vitro study to assess interference between propofol (0.1, 1, 10, 100 μg·ml^{-1}) and lidocaine (4.7 μg·ml^{-1}) without microsomes, found no significant difference in the MEGX/lidocaine ratio (Fig. 3). No interference was observed between propofol and lidocaine.

The P450 contents of the microsomes were 0.556 and 0.553 nmol·mg^{-1} protein in the human and rat groups, respectively. The mean activity of lidocaine deethylation (MEGX formation) in three liver microsomal samples was 0.446 and 0.897 nmol·mg^{-1} protein·min^{-1} in the human and rat groups, respectively, when 4.7 μg·ml^{-1} lidocaine was used as the substrate concentration.

Discussion

The dose-dependent inhibitory effects of propofol on lidocaine metabolism (reaction activity measured by MEGX formation, Fig. 1) were observed on both the human and rat groups. Understanding these drug–drug interactions may be important for the prevention of anesthetic complications such as toxicity and delayed emergence from anesthesia.

In this study, the mode of inhibition was different between propofol and lidocaine. Propofol is likely to have more potent affinity to P450 in comparison with lidocaine. Additionally, the mean activities of lidocaine deethylation (MEGX formation) were 0.446 and 0.897 nmol·mg^{-1} protein·min^{-1} in the human and rat groups, respectively, and the difference in enzyme activity may have caused the different inhibitory effects of propofol on lidocaine metabolism between the human and rat groups.

Sharp reductions were observed in both rat and human groups. Lidocaine metabolism was inhibited steeply, by 50% at a dose of only 5 μg·ml^{-1} propofol, and the rat P450 had a more potent reaction activity than doses of human P450 when the same dose of lidocaine was used as the substrate concentration. It has recently been shown that lidocaine is metabolized by multiple hepatic CYP isoenzymes; CYP1A2 and 3A4 in humans [6], and 3A2 and 2B1 in the rats [10]. Propofol is also metabolized by 3A4 and 2B6 in humans [3,5] and by CYP1A2 and 2B1 in rats [2]. The rats possess CYP 3A2 as a homolog of 3A4 [9], instead of genuine 3A4 [11]. Additionally, rat 2B1 is reported to be a homolog of human 2B6 [11]. Generally speaking, lidocaine and propofol share similar CYP isoforms. Leung et al. [12] reported that propofol possibly inhibits the metabolism of midazolam, which is selectively metabolized by CYP3A4 in human liver microsomes. However, the

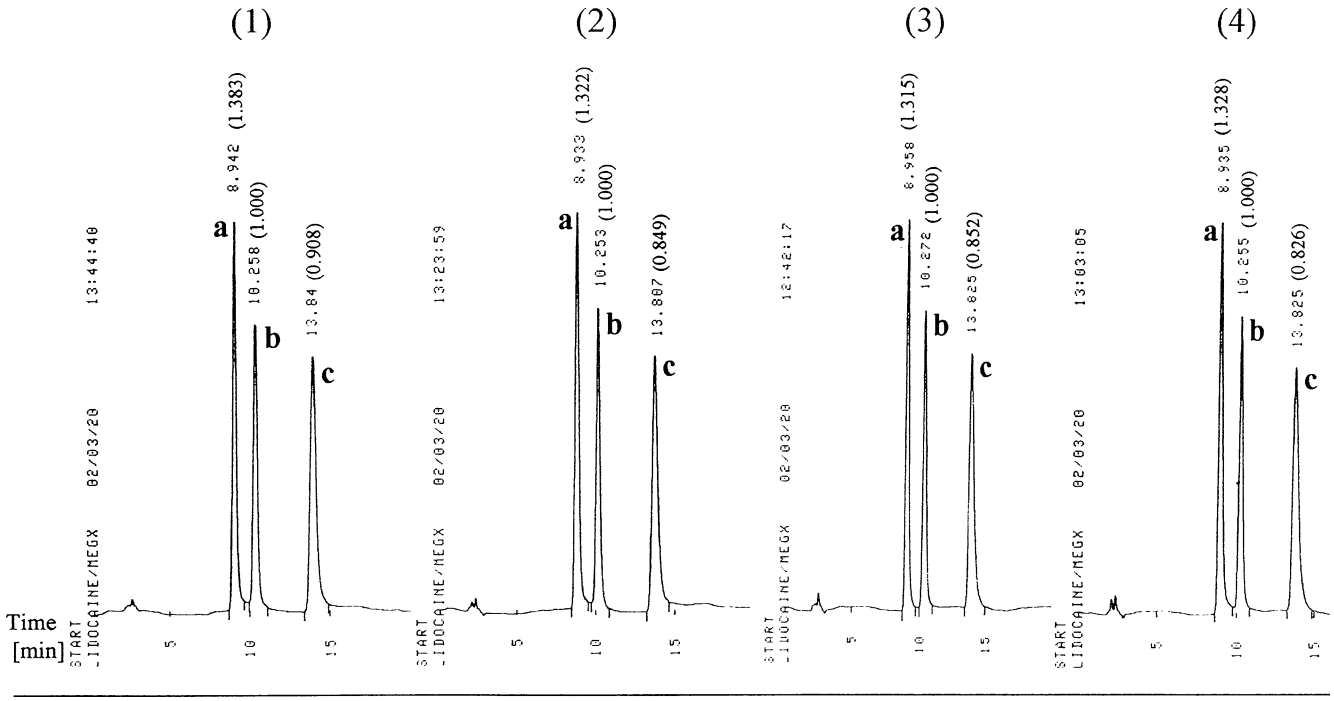

Fig. 3. In vitro study to assess interference between propofol (0.1, 1, 10, and 100 μg·ml^{-1}) and lidocaine (4.7 μg·ml^{-1}) without microsomes. Lidocaine, MEGX (3 μg·ml^{-1}), an internal standard, trimethoprim (1 μg·ml^{-1}), and (*1*) propofol, 0.1, (*2*) propofol, 1, (*3*) propofol, 10, and (*4*) propofol, 100 μg·ml^{-1}. **a** MEGX, **b** internal standard, **c** lidocaine. The number in *parentheses* above the retention time represents the internal standard ratio. There was no significant difference in MEGX/lidocaine ratios

concentration of propofol required to inhibit the metabolism of midazolam is 1 mmol·l^{-1}, which is much higher than the clinical dosage. However, in the current study we observed that lidocaine metabolism is inhibited by 50% at a dose of only 5 μg·ml^{-1} propofol. Based on the findings of Leung et al. [12] and those of this study, propofol is possibly metabolized more predominantly by CYP1A2 and 2B1/6 than by 3A4. Hence, further study is necessary to clarify this aspect.

When two drugs are metabolized by similar P450 isoforms, one drug inhibits the metabolism of the other drug upon simultaneous administration [13]. Our results suggest that the metabolism of lidocaine is dose-dependently inhibited by propofol, probably as a result of inhibition of CYP1A2, 3A4, and/or 2B activities.

In conclusion, propofol inhibited the metabolism of lidocaine in vitro in both human and rat CYP systems.

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