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Effect of propofol on ropivacaine metabolism in human liver microsomes

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Abstract
A combination of the general anesthestic propofol and epidural anesthesia with a local anesthetic is widely used. The metabolism of ropivacaine and that of lidocaine are mediated by similar P450 isoforms. Previously, propofol was found to inhibit the metabolism of lidocaine in vitro. Here we investigated whether propofol inhibits the metabolism of ropivacaine using human liver microsomes in vitro. Ropivacaine (6.0 μmol·l^{-1}) as the substrate and propofol (1–100 μmol·l^{-1}) were reacted together using human microsomes. The concentrations of ropivacaine and its major metabolite 2′,6′-pipecoloxylidide (PPX) were measured using high-performance liquid chromatography. The metabolic activity of ropivacaine was reflected in the production of PPX. The inhibitory effects of propofol on ropivacaine metabolism were observed to be dose-dependent. The IC_{50} of propofol was 34.9 μmol·l^{-1}. Propofol shows a competitive inhibitory effect on the metabolism of ropivacaine (i.e., PPX production mediated by CYP3A4) in human CYP systems in vitro.

Key words Propofol · Ropivacaine · Metabolism · Human · Microsome

Many drugs, including some used during anesthesia, are metabolized by cytochrome P450 (CYP) enzyme. Some drugs are known to interact during metabolism. CYP is inhibited by some drugs and may cause inhibition of other drug metabolism.

Propofol is widely used as a general anesthetic and in combination with ropivacaine as a local anesthetic. It was recently reported that propofol modified the activity of multiple hepatic CYP isozymes [1,2] including CYP1A2 [3] and CYP3A4 [4]. Additionally, propofol has been shown to inhibit the metabolism of such drugs [4,5].

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Ropivacaine, a relatively new long-acting local anesthetic, has lower central nervous and cardiotoxic potential than bupivacaine and is widely used in large doses without opioid co-administration for postoperative epidural analgesia [6]. However, despite its relative innocuousness, ropivacaine does produce a toxic reaction [7]. In previous studies it was reported that lidocaine and ropivacaine are metabolized by CYP1A2 and CYP3A4 [8] and that propofol inhibits lidocaine metabolism in vitro [9]. On the other hand, the metabolic interaction of propofol and ropivacaine has not previously been reported. If propofol inhibits ropivacaine metabolism, local anesthetic toxicity such as cardiovascular and central nervous toxicity may occur when a large dose of ropivacaine is used with propofol. The aim of this study was to examine whether propofol has an inhibitory effect on the metabolism of ropivacaine in in vitro systems using human CYP.

Propofol and tetracaine (internal standard, IS) were purchased from Sigma (Tokyo, Japan). Ropivacaine and 2′,6′-pipecoloxylidide (PPX) were kindly provided by Astrazeneca Japan (Osaka, Japan). Human liver microsomes (pooled multiple human microsomes from 57 donors, lot no. 24) were purchased from Gentest (Woburn, MA, USA) and stored at –80°C until use.

First, propofol in methanol was added until the propofol concentrations in the reaction mixtures were 0, 1, 5, 10, 25, 50, and 100 μmol·l^{-1}. Methanol was evaporated in a waterbath at about 30°C under a gentle stream of nitrogen. The residue was then dissolved in a total volume of 0.5 ml of the reaction mixture, which contained potassium phosphate buffer (pH 7.4), 0.2 mg human liver microsomal protein, and ropivacaine 6 μmol·l^{-1}. The mixture was preincubated for 3 min, after which the reaction was started by additing reduced nicotinamide adenine dinucleotide phosphate (NADPH), followed by incubation for 20 min in a shaking waterbath at 37°C. The reaction was terminated by add-

ing acetonitrile 100 µl, followed by vortex mixing and placing the tube on ice. Tetracaine and *t*-butylmethylether were added to the tube and then mixed by vortex for 5 min. After the tube was centrifuged, the organic phase was aspirated, transferred to a new tube, and evaporated in a waterbath under a gentle stream of nitrogen. The residue was dissolved in the mobile phase and was injected into the high-performance liquid chromatography (HPLC) apparatus. Incubations were performed five times at each propofol concentration.

To determine the enzyme kinetic parameters, the maximum rate of metabolism (Vmax) and the Michaelis-Menten constant (Km), and the manner of inhibition of PPX formation from ropivacaine, another reaction study was performed. The incubation mixture consisted of human liver microsomes, ropivacaine (5, 6.8, 10, and 20 µmol·l^{-1}), potassium phosphate buffer (pH 7.4), propofol as inhibitor (0, 50, 100 µmol·l^{-1}), and NADPH in a final volume 0.5 ml. Incubation was performed at 37°C for 30 min using a shaking waterbath (n = 3). Ropivacaine and PPX were extracted and measured by HPLC.

The HPLC was performed at 25°C using the Inertsil ODS-3 column (4.5 × 150 mm, 5 µm; GL Sciences, Tokyo, Japan). The HPLC apparatus consisted of a pump (model CCPS; Tosoh Tokyo, Japan) and a variable-wavelength ultraviolet (UV) detector (model UV-8000; Tosoh). The mobile phase was composed of NaH_2PO_4 (30 mmol·l^{-1}, pH 5.6)/acetonitrile/methanol 300:100:100 (v/v/v). The flow rate was 1.0 ml·min^{-1}, and the absorbance of the sample was monitored at 210 nm. The linearity of this method for the determination of ropivacaine and PPX was studied in the ranges 0.5–25 µmol·l^{-1} and 0.1–4 µmol·l^{-1}, respectively. With this assay method, the coefficients of variation (CVs) for ropivacaine and PPX were 5.0% and 7.88%, respectively.

Data were expressed as the mean or the mean ± SD. Activity of ropivacaine metabolism was expressed as a reaction activity, rate (%) of PPX formation (concentration of PPX/concentration of PPX without propofol × 100). The IC_{50} value for propofol (propofol concentration corresponding to a 50% decrease in reaction activity) was determined graphically by nonlinear regression analysis of the plot of the propofol concentration versus the percentage of activity remaining after inhibition. Statistical analysis was performed by one-way analysis of variance (ANOVA) using the Boneferroni follow-up test to compare the various propofol concentrations with the control group. $P < 0.05$ was considered statistically significant.

The enzyme kinetic parameters, Vmax and Km, of PPX formation from ropivacaine were calculated according to the Lineweaver-Burk plot. The inhibition manner was determined by visual inspection of the Lineweaver-Burk plot.

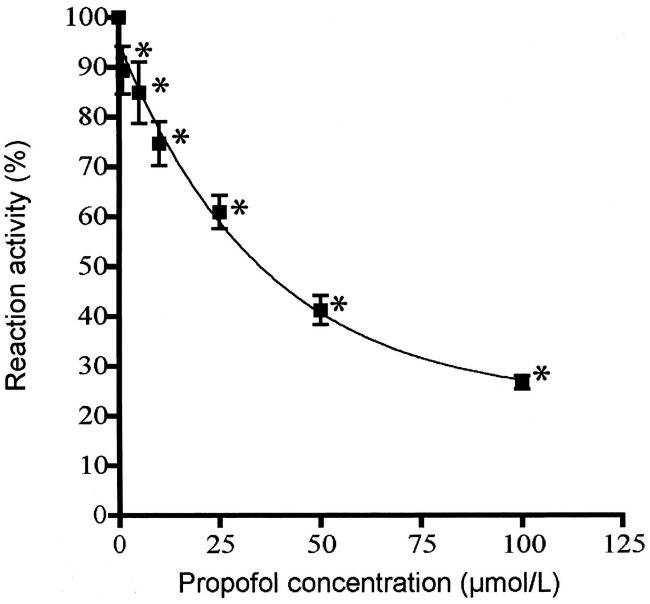

Fig. 1. Effect of propofol concentration on ropivacaine (6 µmol·l^{-1}) metabolism [2′,6′-pipecoloxylidide (PPX) formation] in a human liver microsome system. Reaction activity (%) = concentration of PPX/concentration of PPX without propofol × 100. Ropivacaine metabolism was inhibited as the propofol concentration increased (n = 5). *$P < 0.05$, compared with control

Peaks of ropivacaine, PPX, and IS were separated and detected by HPLC. Retention times for ropivacaine, PPX, and IS were 8.28, 3.06, and 12.91·min, respectively.

At propofol concentrations of 0, 1, 5, 10, 25, 50, and 100 µmol·l^{-1}, the mean ropivacaine concentrations after 20 min of incubation were 3.89 ± 0.15, 4.13 ± 0.07, 4.74 ± 0.11, 5.08 ± 0.12, 5.43 ± 0.13, 5.03 ± 0.49, and 5.62 ± 0.37 µmol·l^{-1}, respectively, and those of PPX were 0.545 ± 0.044, 0.487 ± 0.026, 0.462 ± 0.034, 0.407 ± 0.024, 0.332 ± 0.016, 0.225 ± 0.04 and 0.146 ± 0.013 µmol·l^{-1}.

The effects of propofol on PPX formation using human liver microsomes are shown in Fig. 1. Ropivacaine metabolism was dose-dependently reduced by propofol. The IC_{50} for inhibition of ropivacaine by propofol was 34.9 µmol·l^{-1}.

The Lineweaver-Burk plot for the PPX formation in human liver microsomes is shown in Fig. 2. The Km and Vmax values of PPX formation from ropivacaine were 74.13 µmol·l^{-1} and 1.034 nmol·mg^{-1} protein·min^{-1}, respectively. The formation of PPX from ropivacaine was inhibited in a competitive manner (Fig. 2).

The investigation of drug–drug interactions during anesthesia is important to prevent anesthetic complications such as toxicity and delayed emergence of anes-

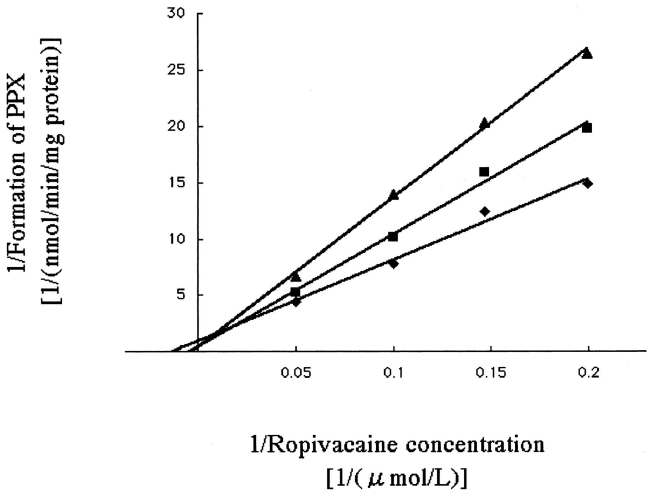

Fig. 2. Lineweaver-Burk plots of PPX formation and the inhibitory effect of propofol on PPX formation by human liver microsomes. The incubation conditions were as follows: microsomal protein content 200 μg·500 μl^{-1}; incubation time 30 min. Ropivacaine concentration was 5–20 μmol·l^{-1}. Propofol concentrations were 0 μmol·l^{-1} (*diamonds*), 50 μmol·l^{-1} (*squares*), and 100 μmol·l^{-1} (*triangles*). Values represent means from three separate experiments

thesia. There are many CYP isozymes, and the isozymes have different substrate selectivity. If drugs are metabolized by the same CYP isozymes, drug–drug interaction may occur.

In this study, we assessed the inhibitory effect of propofol on ropivacaine metabolism. We found that PPX production was also inhibited by propofol. The IC_{50} was 34.9 μmol·l^{-1}. Both the propofol value of 34.9 μmol·l^{-1} and the ropivacaine value of 6 μmol·l^{-1} are within the range of clinical doses [10,11].

Propofol is predominantly eliminated by extensive metabolism in the liver [12]. Recently, it was shown that the isozymes of CYP, which predominantly metabolizes propofol, are CYP2B6 and CYP2C9 [13,14]. Unlike other anesthetic drugs such as fentanyl and midazolam, propofol mostly undergoes phase II metabolism directly. Therefore, the CYP enzyme does not appear to play a major role in propofol metabolism compared to its role in that of other intravenously administered anesthetic drugs. The inhibitory effects of propofol on CYP isozymes [1,2] and the metabolism of drugs such as lidocaine [9] and midazolam [4] have been presented elsewhere. Midazolam is metabolized mainly by CYP3A4 and lidocaine mainly by CYP1A2 and CYP3A4. Importantly, ropivacaine is also metabolized mainly by CYP1A2 and CYP3A4 [15]. Next, we assessed the inhibitory effect of propofol on ropivacaine metabolism.

Ropivacaine is mainly metabolized to PPX by CYP3A4 and to 3-hydroxyropivacaine (3-OH ropivacaine) by CYP1A2 in the liver. During ropivacaine metabolism CYP3A4 has a large capacity and CYP1A2 has high affinity [16]. Therefore, inhibitors of CYP1A2 or CYP3A4 affect ropivacaine metabolism. Ropivacaine clearance in healthy volunteers was found to be decreased by CYP1A2 inhibitor (68% and 31% by fluvoxamine and ciprofoloxacin, respectively) and by CYP3A4 inhibitor (15% by ketoconazole) in vivo [8, 17–19].

In this study, propofol was found to inhibit ropivacaine metabolism of PPX production mediated by CYP3A4. CYP3A4 is one of the major CYP isozymes and metabolizes many substrates. This suggests the possibility that metabolism of a drug going through CYP3A4 is restrained under anesthesia using propofol and ropivacaine in combination. The IC_{50} of propofol in lidocaine metabolism in the previous study, 5.0 μg·ml^{-1} (28.0 μmol·l^{-1}), is smaller than the present result [9]. With regard to this point, ropivacaine seems to be less inhibited by propofol than lidocaine is. However, 3-OH ropivacaine, another major metabolite of ropivacaine, could not be measured in this study. Therefore, it is not clear whether 3-OH ropivacaine formation was inhibited by propofol. For this reason, we could not conclude that ropivacaine metabolism is less inhibited by propofol than is lidocaine metabolism, nor could we definitively conclude that all of the ropivacaine metabolism was inhibited in vitro. From another point of view, the findings that propofol inhibited both CYP1A2 and CYP3A4 in previous studies and the finding that the ropivacaine concentration after metabolism was increased as the dose of propofol increased in this study indicate that CYP1A2 at least did not induce or compensate for CYP3A4 activity by propofol.

Propofol concentration was not measured in this study. In a previous report, propofol metabolism was found not to be inhibited by some drugs used during general anesthesia [20]. In addition, whereas propofol inhibits lidocaine metabolism, propofol metabolism is not inhibited by lidocaine [9]. Considering these results, the affinity of propofol for CYP isoforms may be high compared with that of these drugs. In this study the dose-response curve in Fig. 1 is not seen as sigmoid. This reason may be that we did not assess it at a very low concentration of propofol.

Based on the Lineweaver–Burk plot, the inhibition pattern of PPX formation from ropivacaine by propofol occurred in a competitive manner. The Km of PPX formation from ropivacaine is smaller than that previously reported [16]. A small Km value indicates the presence of high affinity of the substrate for the enzyme. One of the probable factors that made the Km different is that ropivacaine concentrations were different for the studies. The ropivacaine concentration was as high as 1 mmol·l^{-1} in a previous study, whereas it was only as

high as 20 μmol·l^{-1} in the present study. During epidural anesthesia the ropivacaine concentration has been estimated to be about 3.0–12.5 μmol·l^{-1} [21]; therefore, we investigated ropivacane metabolism in the near-clinical range.

This experiment was conducted in vitro, and it is worth noting that in vivo factors such as protein binding and metabolism in organs other than the liver have a clinical influence on ropivacaine metabolism. Furthermore, it is not known whether this inhibitory effect is seen clinically. We chose ropivacaine and propofol concentrations for the reaction based on clinical blood concentrations, but it is not known if the blood concentration represents the tissue concentration. It is important to evaluate in vivo whether this result has importance in a clinical setting. Furthermore, reports on ropivacaine interactions have been based on studies in healthy volunteers. Recently, ropivacaine blood concentration and the area under the curve (AUC) were found to be increased in patients with renal failure [22]. It is thus important to examine the risk of ropivacaine interaction in critically ill patients undergoing general anesthesia.

In summary, propofol inhibited ropivacaine metabolism to PPX competitively in human liver microsomes within the range of clinical concentrations of both drugs. Our results suggest that propofol inhibits CYP3A4 activity. Further study is needed to evaluate this finding clinically.

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