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Subcellular expression of UGT1A6 and CYP1A1 responsible for propofol metabolism in human brain

ZHANG Shi-Hai[1], LI Qing, YAO Shang-Long, ZENG Bang-Xiong (*Department of Anesthesiology, Union Hospital, Huazhong University of Science & Technology, Wuhan 430022, China*)

KEY WORDS propofol; polymerase chain reaction; brain; microsomes; mitochondria; glucuronosyltransferase; cytochrome P-450

ABSTRACT

AIM: To observe the subcellular expression of UDP-glucuronosyltransferase 1A6 (UGT1A6) and cytochrome P-450 1A1 (CYP1A1) mRNA in the human brain and investigate the effect of brain on extrahepatic metabolism of propofol. **METHODS**: Fifteen patients scheduled for craniotomy under propofol anesthesia were selected. Brain tissue was taken and blood samples from the radial artery and the internal jugular vein were drawn simultaneously. PCR technique was used to detect UGT1A6 and CYP1A1 mRNA in the microsomes and mitochondria. Enzymatic activities were assayed. Blood propofol and propofol-glucuronide concentrations were measured with HPLC and GC, respectively. **RESULTS**: UGT1A6 mRNA was located mainly in the microsomes, and CYP1A1 mRNA was mainly in the mitochondria. The higher blood concentration of propofol and lower blood concentration of propofol-glucuronide were observed from the radial artery than from the internal jugular vein, respectively. The K_m of UGT1A6 and CYP1A1 was (0.74 ± 0.21) mmol and (548 ± 50) mmol, respectively. The V_{max} was (536 ± 98) $nmol \cdot h^{-1} \cdot mg^{-1}$ and (37 ± 5) $nmol \cdot h^{-1} \cdot mg^{-1}$, respectively. **CONCLUSION**: The human brain is an important organ for extrahepatic metabolism of propofol. The metabolism occurs within microsomes and mitochondria in brain.

INTRODUCTION

Propofol, a rapid acting intravenous anesthetic, is widely used for anesthetic induction and maintenance. The main propofol metabolism site is liver, but extrahepatic clearance of propofol has been suggested because systemic propofol clearance exceeds hepatic blood[1,2]. Furthermore, extrahepatic metabolism of propofol has been confirmed in patients during the anhepatic phases of liver transplantation[3]. The extrahepatic propofol metabolism sites remain unclear. Kidney, gut, and lung may be the possible sites[4,5]. However, brain as the anesthesia acting target organ of propofol, its metabolizing effect on propofol has never been studied. Because UDP-glucuronosyltransferase 1A6 (UGT1A6) and cytochrome P-4501A1 (CYP1A1) are two known enzymes responsible for propofol metabolism in the liver. Therefore, in the present study, we intended to identify and locate UGT1A6 and CYP1A1 mRNA subcellularly in the human brain and measure their activities in metabolism of propofol.

MATERIALS AND METHODS

Patients and anesthesia Fifteen adult patients with neuroglioma scheduled for elective craniotomy were selected. No premedication was given to them (10 men and 5 women; age, 48 a ± 21 a; weight, 56 kg ± 19 kg). Before induction of anesthesia, the patients received a bolus dose of fentanyl 0.1 mg and were preoxygenated with 100 % oxygen for 5 min. Propofol was first injected as a bolus dose (2 $mg \cdot kg^{-1}$) and then continuously infused. The following infusion rates were used: 12 $mg \cdot kg^{-1} \cdot h^{-1}$ for the first 15 min, 9 $mg \cdot kg^{-1} \cdot h^{-1}$ for the next 25 min, and 6 $mg \cdot kg^{-1} \cdot h^{-1}$ thereafter. Endotracheal intubation was facilitated by vencuronium 0.05 $mg \cdot kg^{-1}$. After tracheal intubation, the lungs were ventilated to normocapnia with 100 % oxygen.

Before induction of anesthesia, a 18-gauge cannula was inserted in the large vein of forearm and was used solely for the infusion of propofol. After induction of anesthesia, two additional cannulas (20-gauge) were

[1]Correspondence to ZHANG Shi-Hai, MD.
Phn 86-27-8572-6389. Fax 86-27-8572-6406.
E-mail zhangshv@public.wh.hb.cn
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placed: one in the radial artery in the contralateral forearm (for blood pressure monitoring and blood sampling) and the other in the right internal jugular vein (for venous blood sampling).

Human brain tissue was taken at biopsy[6], and arterial and internal jugular venous blood samples were drawn simultaneously.

Isolation of microsomal and mitochondrial proteins from brain tissue To subcellularly localize UGT1A6 and CYP1A1 that responsible for propofol metabolism in the human brain, microsomes and mitochondria from brain tissue were prepared. Microsomes and mitochondria were prepared with the method described by Ghersi-Egea *et al*[7]. Microsomal UGT1A6 protein was isolated as below: brain tissue samples were pulverized under liquid nitrogen, resuspended in 1 mL of buffer (Tris-HCl 50 mmol, pH 7.4, $MgCl_2$ 10 mmol) and homogenized with a Potter-Elvehjem tissue grinder. The tissue homogenate was centrifuged at 10 000 × *g* for 5 min at 4 ℃, and the supernatant was collected. The pellet was resuspended in 0.5 mL of buffer and centrifuged, and the supernatant was collected. The combined supernatants were centrifuged at 150 000 × *g* for 60 min at 4 ℃, and the pellet was resuspended in 0.2 mL of buffer. Microsomal protein was stored at −70 ℃. Mitochondrial CYP1A1 protein was purified with the method described by Raza *et al*[8].

PCR procedures Microsomal and mitochondrial mRNA were extracted with the method described by Strassburg *et al*[9]. RNA concentrations were determined by spectrophotometry at 260 nm and 280 nm. Samples were stored in water at −70 ℃ until further analysis.

The forward primer for UGT1A6 was 5′-CTCTGAAAGGATGGCTTGCC-3′, and the reverse primer was 5′-TGGCCGAGTCCTTCAGGAGG-3′. The forward primer for CYP1A1 was 5′-TGGATGAGAACGC-CAATGTC-3′, and the reverse primer was 5′-GGATGA-AGGCCTCCATATAG-3′. Primers for human β-actin were 5′-GGCGGCACCACCATGTACCCT-3′ and 5′-AG-GGGCCGGACTCTCATACT-3′. RNA extracted from microsomes and mitochondria were used to perform RT-PCR. PCR was performed on a thermal cycler PTC-150 (USA) with the following thermocycle parameters: a 5-min initial denaturation at 98 ℃ followed by 30 cycles of 45-s denaturation at 94 ℃, 45-s annealing at 60 ℃, 90-s extension at 72 ℃, and finally a 10-min extension at 72 ℃.

Quantification of PCR products Ten microliters of each PCR were resolved by electrophoresis in a 2 % agarose gel containing 1 mg · L^{-1} ethidium bromide. Gels were photographed using Polaroid positive/negative film (Polaroid, Cambridge, MA). Negatives were used for laser quantification in an LKB 2222-020 UltroScan XL densitometer (Pharmacia LKB, Bromma, Sweden). The area beneath the graph of each peak was determined, and means were calculated from samples. Peaks from human β-actin were used as internal standards for each RNA sample and used to determine the relative UGT1A6 and CYP1A1 bands intensity.

Enzyme kinetics In experiments performed to determine the apparent K_m and V_{max} values characterizing propofol UGT1A6 activity, the [^{14}C] uridine diphosphoglucuronic acid (UDPGA) (10.36 TBq/mol, Dupont, USA) concentration was kept constant (3 mmol/L). Enzymatic activity was measured as the transfer of [^{14}C] UDPGA to the aglycone to produce labeled glucuronide[10]. Microsomal protein 3 g · L^{-1} was prepared. Enzymatic activity was measured for six propofol concentrations in 0.2 to 5 mmol/L range. The incubation was for 15 min at 37 ℃. Mitochondrial CYP1A1 activity was assayed with the method described as the above with modification.

Propofol and propofol-glucuronide measurement Blood concentration of propofol from the radial artery and from the internal jugular vein was measured with HPLC (Hewlett-Packard 1090, USA) with a fluorescence detector (Model RF-535, Shimazu) and a reverse phase column (Lichrosphere RP-18100, 4 mm × 125 mm, 5-μm particle size, Merck, Darmstadt, Germany)[11]. Blood glucuroconjugated propofol metabolites were hydrolyzed to the free form by incubation with a specific β-glucuronidase and detected with GC (Model GC-14A, Shimazu)[12].

Statistics Data were expressed as $x \pm s$ and assessed by paired *t*-test.

RESULTS

Subcellular locations of UGT1A6 and CYP1A1 High level of UGT1A6 mRNA was detected in the microsomes of human brain. UGT1A6 mRNA level was low or undetectable in the mitochondria (64 ± 18 *vs* 6.0 ± 2.4, $t = 12.15$, $P < 0.01$, Fig 1 and 2). Higher level of CYP1A1 mRNA was found in mitochondria than in microsomes (67 ± 17 *vs* 17 ± 4, $t = 12.11$, $P < 0.01$, Fig 1 and 2).

UGT1A6 and CYP1A1 enzyme kinetics The

K_m of the microsomal UGT1A6 and mitochondrial CYP1A1 was (0.74±0.21) mmol and (548±50) mmol, respectively. The V_{max} was (536±98) nmol·h^{-1}·mg^{-1} and (37±5) nmol·h^{-1}·mg^{-1}, respectively (Tab 1).

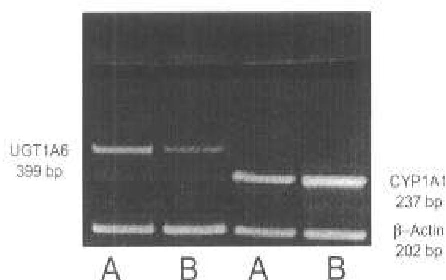

Fig 1. UGT1A6 and CYP1A1 bands in human brain subcellular fractions. A and B represent microsomes and mitochondria, respectively.

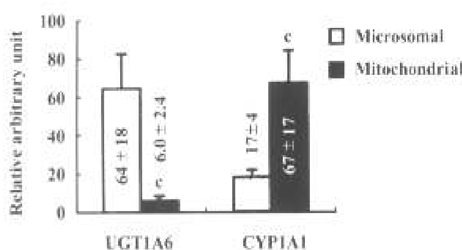

Fig 2. Relative arbitrary units of microsomal and mitochondrial UGT1A6 and CYP1A1 bands intensity. $n=15$. $\bar{x}\pm s$. $^cP<0.01$ vs microsomal.

Tab 1. Enzyme kinetics of UGT1A6 and CYP1A1. $n=15$. $\bar{x}\pm s$.

Enzymes	K_m/mmol	V_{max}/nmol·h^{-1}·mg^{-1}
Microsomal UGT1A6	0.74±0.21	536±98
Mitochondrial CYP1A1	548±50	37±5

Blood concentrations of propofol and propofol-glucuronide Drug concentration in blood from the radial artery was assumed as the same as from the internal jugular artery. Drug concentration in blood from the radial artery can represent that from the internal jugular artery in the course of calculation of brain drug metabolism. The higher propofol concentration in blood from the radial artery than from the jugular vein was observed ($t=17.92$, $P<0.01$, Tab 2). The higher propofol-glucuronide concentration in blood from the jugular vein than from the radial artery was measured ($t=4.76$, $P<0.01$, Tab 2). The C_a-C_{ijv} differences of propofol and propofol-glucuronide concentrations represent human brain metabolism rate of propofol. The C_a-C_{ijv} difference of propofol concentration (1.9 mg·L^{-1} ±0.4 mg·L^{-1}) was higher than C_{ijv}-C_a difference of propofol-glucuronide concentration (0.32 mg·L^{-1} ± 0.20 mg·L^{-1}, Tab 2).

Tab 2. Propofol and propofol-glucuronide concentrations in blood. $n=15$. $\bar{x}\pm s$. $^cP<0.01$ vs C_a.

Parameters	Propofol	Propofol-glucuronide
C_a(mg/L)	3.4±0.3	1.03±0.11
C_{ijv}(mg/L)	1.5±0.3^c	1.4±0.3^c
$\|C_a$-$C_{ijv}\|$(mg/L)	1.9±0.4	0.32±0.20

C_a, C_{ijv}, and $|C_a$-$C_{ijv}|$ represent arterial concentration, internal jugular venous concentration, and arterial – internal jugular venous concentration difference, respectively.

DISCUSSION

Marked interspecies differences in propofol clearance and metabolite profiles have been observed[13], so the present study was undergone in humans. The decrease of propofol concentrations and the increase of propofol-glucuronide in blood after its passage through the brain were observed. The presented results showed that not only the liver but also the brain is responsible for propofol metabolism in human organism.

As far as the liver is concerned, the clearance of propofol exceeds its capacity in respect of its total blood flow[1]. Moreover, no significant changes of propofol pharmacokinetics were observed elsewhere in patients with the liver function abnormalities or alterations in the liver blood flow, which suggested the presence of extrahepatic metabolism[3]. Propofol is a highly lipophilic intravenous anesthetic. It can pass easily through blood-brain barrier and perform general anesthesia action in the brain. The extrahepatic expression of UDP-glucuronosyltransferases and cytochromes are important in the detoxification of a number of endogenous and exogenous compounds. We hypothesize that UDP-glucuronosyltransferases and cytochromes P-450 exist in the human brain and be involved in extrahepatic metabolism of propofol. The hypothesis is confirmed by RT-PCR technique in this study. With specific designed primers for UGT1A6 and CYP1A1, UGT1A6 and CYP1A1 mRNA are detected in the human brain.

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Propofol interacts with the cytochrome P-450 enzymes of the liver to undergo oxidative metabolism producing 2, 6-diisopropyl-1, 4-quinol as its major metabolite[2,13]. The active sites of cytochrome P-450 protein molecule are lipophilic in nature; therefore, propofol has the potential for being a cytochrome P-450 substrate because it is both highly lipophilic and readily binds to protein. Propofol is rapidly metabolized mainly by glucuronidation[2] and producing 2, 6-diisopropyl-1, 4-quinol as its major metabolite, too. Glucuronidation is a major drug metabolic pathway, catalyzed by a group of closely related isoforms of uridine diphosphate glucuronosyltransferases (UGT). As the same metabolism procedure in the liver, uridine diphosphate glucuronosyltransferases and cytochrome P-450 are responsible for propofol metabolism in the human brain because the higher propofol-glucuronide concentration in blood from the internal jugular vein than from the radial artery was observed in the present study.

Unlike in the liver that uridine diphosphate glucuronosyltransferases and cytochrome enzymes are mainly in the microsomes[4], subcellular expression of UGT1A6 and CYP1A1 mRNA revealed that CYP1A1 was mainly in the mitochondria and UGT1A6 in the microsomes in human brain. This kind of enzyme distribution demonstrated that in the brain propofol could be metabolized not only in the microsomes as in the liver, but also in the mitochondria. In the present study, the K_m and V_{max} of microsomal UGT1A6 are similar to the propofol glucuronidation activities observed by Le Guellec *et al*[4] in the liver. The similarity shows that brain UGT1A6 maybe belongs to the uridine diphosphate glucuronosyltransferase superfamily responsible for many drug metabolism including propofol in the liver. The K_m and V_{max} of mitochondrial CYP1A1 do not agree with the findings observed by Guitton *et al*[14] in the hepatic microsomes. The reason of the disagreement is unclear. One explanation may be subcellular fractionation difference.

In the present study, propofol concentration difference (C_a-C_{ijv}) is much larger than propofol-glucuronide concentration difference (C_{ijv}-C_a). It demonstrated that only a small part of propofol was biotransformed in the human brain, a large part of propofol was just distributed in human brain tissue. The similar phenomenon of biotransformation and distribution of propofol was observed in human lungs[5].

In summary, high mRNA levels of enzymes responsible for propofol metabolism were detected within microsomes and mitochondria in the human brain and showed enzymatic activities to propofol. The results demonstrate that human brain is an important organ for extrahepatic metabolism of propofol.

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UGT1A6及CYP1A1在人脑亚细胞结构中的表达及对异丙酚的代谢作用

张诗海[1], 李　晴, 姚尚龙, 曾邦雄 (华中科技大学附属协和医院麻醉科, 武汉 430022, 中国)

关键词　二异丙酚; 聚合酶链反应; 脑; 微粒体; 线粒体; 葡糖醛酸基转移酶; 细胞色素 P-450

目的: 观察 UDP-葡萄糖醛酸基转移酶 1A6(UGT1A6)及细胞色素 P-4501A1(CYP1A1) mRNA 在人脑组织亚细胞结构中的表达并研究脑组织在异丙酚肝外代谢中的作用. **方法**: 选择在异丙酚麻醉下行开颅术的患者 15 例, 在抽取脑组织样本的同时抽取动脉和颈内静脉血样. 用聚合酶链反应(PCR)法测定微粒体和线粒体中 UGT1A6 及 CYP1A1 mRNA 的表达水平, 并测定二酶的活性. 用高效液相色谱和气相色谱法测定动脉血及颈内静脉血中异丙酚及异丙酚-葡萄糖醛酸结合物的含量. **结果**: UGT1A6 mRNA 主要表达于微粒体, CYP1A1 mRNA 主要表达于线粒体. 颈内静脉血异丙酚浓度显著低于动脉血, 异丙酚-葡萄糖醛酸结合物浓度显著高于动脉血. 二酶的 K_m 分别为 (0.74±0.21) mmol 和 (548±50) mmol, V_{max} 分别为 (536±98) nmol·h^{-1}·mg^{-1}及(37±5) nmol·h^{-1}·mg^{-1}. **结论**: 脑组织是参与异丙酚肝外代谢的重要器官, 代谢位于脑内微粒体和线粒体中.

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