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Research Article

UGT1A9 Single Nucleotide Polymorphisms do not Account for the Variability of Response to Propofol: A One-way Design with Multiple Levels Study of the Propofol Pharmacodynamics

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Abstract

There is remarkable individual variability of response to propofol during its clinical use, especially in its sedation effect. The UGT1A9 is a primary enzyme metabolizing propofol, whose expression level and activity can be affected by its Single Nucleotide Polymorphisms (SNPs). This article was to explore whether UGT1A9 SNPs contribute to the individual differences of propofol pharmacodynamics during general anesthesia. In the study, propofolin Target Controlled Infusion (TCI) was adopted for the anesthesia induction and maintenance for 150 female patients undergoing benign breast mass resection surgery. Patients were divided into 3 groups according to each SNP genetopes (Wild homozygotes group, heterozygous group and mutant homozygote group). Propofol was induced and maintained using a TCI system with a predicted plasma concentration (Cp) of 3.0 μg mL^{-1}. Bispectral index, time and effect site concentration were recorded when the Observer Assessment of Sedation (OAA/S) was up to 4. Time and effect site concentration were also recorded when BIS was up to 80. The UGT1A9 I399 genotype frequencies were TT 21%, TC 63% and CC 16%; -1818 genotype frequencies were TT 33%, TC 52%, and CC 15%; -1887 genotype frequencies were TT 81%, TG 19%. There were no significant associations between UGT1A9 SNPs and these pharmacodynamics parameters. It was concluded although great individual differences exist in the propofol pharmacodynamics. The UGT1A9 SNPs and its common haplotypes do not appear to account for these variations in propofol pharmacodynamics.

Key words: UGT1A9, SNP, propofol, TCI, pharmacodynamics

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Propofol (2,6-diisopropylphenol) because of its rapid onset of action, short awakening time, no accumulative effect upon repeated use or continuous infusion, is widely used in clinical anesthesia and ICU sedation. However, the pharmacokinetics and pharmacodynamics of propofol vary obviously among individuals. Clinical application of propofol indicates there are remarkable individual differences in its sedation effect. Progressive myocardial failure and dose-dependent hypotension also have been reported (Hansen, 2005; Iohom *et al*., 2007; Phillips *et al*., 2015). The UDP-glucuronosyltransferase 1A9 (UGT1A9) is the representative phase II enzyme, which plays an important role in the metabolism of propofol (Bray, 1998; Liang *et al*., 2011; Mukai *et al*., 2014). The expression levels of UGT 1A9 are associated with its genetic polymorphisms in human liver, contributing to different substrate metabolic activit (Girard *et al*., 2004; Kirby *et al*., 2009). Whether the genetic polymorphisms of UGT1A9 contribute to individual differences in the pharmacodynamics of propofol are not clear. This study was aimed to describe the genetic polymorphism of UGT1A9 in patients undergoing benign breast lumpectomy under total intravenous anesthesia (TIVA); determine whether there would be correlation between the studied pharmacodynamics parameters of propofol, UGT1A9 SNPs and its common SNPs interactions. To our knowledge, it is the first time to investigate the effect of UGT1A9 polymorphisms on the propofol sedation effect during the anesthesia recovery period.

MATERIALS AND METHODS

Subjects: A total of 150 American Society of Anesthesiologists (ASA) physical status-female patients (aged 20-50 years) with normal Body Mass Index (BMI) scheduling for elective benign breast lumps under general anesthesia were enrolled in this study. Ethical approval for this study was provided by the ethical committee of the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China. Informed consent was obtained from all patients enrolled in this study. Exclusive criteria included severe neurological, respiratory or cardiovasular diseases, a history of smoking or alcoholism, hyperthyroidism, hepatic and renal dysfunction, pregnancy and lactation. Patients meeting the inclusive criteria received a same standardized anesthesia during the surgery.

Anesthetic procedure: No premedication was administered. On arrival to the room, two 18-G intravenous cannulas were inserted into the fore veins (one for blood sampling, the other for administration of medicine and infusion). Bispectral index (software version 3.31; Aspect Medical Systems Inc., Natick, MA, USA) electrocardiogram, non-invasive blood pressure, pulse oximetry (SpO_2) and end-tidal carbon dioxide measurement ($EtCO_2$) were monitored for all the patients. All patients received standardized anaesthetic techniques consisting of propofol TCI.

Propofol was administered by target controlled infussion, induction of anaesthesia: plasma concentration of propofol TCI was 3 μg mL^{-1} (Graseby 3500 infusion pump; Sims Graseby Ltd., Watford, UK) intravenous remifentanil 1.5 μg kg^{-1} (60s within a constant speed of infusion) and cis-atracurium 0.15 mg kg^{-1} after the patient lost consciousness. Laryngeal mask was placed by a skilled anesthetist and mechanical ventilation was adjusted according to the patient's weight and condition. Anesthesia was maintained using propofol TCI with a Cp of 3 μg mL^{-1} and continuous infusion of remifentanil 0.2-0.3 μg kg^{-1} min^{-1}. The dose of remifentanil was adjusted according to the depth of anesthesia to ensure the maintaining of more stable vital signs. Remifentanil and cis-atracurium infusion was stopped about 10 and 30 min before the end of surgery, respectively. Propofol TCI was abruptly stopped after surgery. A normal tone voice was used repeatedly to wake the patients until the eyes of the patients were opened and the satisfactory OAA/S was up to 4. The awakening time (T_1, OAA/S to 4) BIS value and the effect site concentration at OAA/S4 (C_1) were recorded. The time of BIS value rising to 80 (T_2) and the effect site concentration at BIS 80 (C_2) were also recorded.

Blood sampling and genetyping: A venous blood sample (1 mL) was withdrawn into an EDTA (ethylenediamine tetraacetic acid) anticoagulant tube before the surgery, DNA was extracted from this human whole blood using DNA extraction kit (Takara Bio Co., Ltd.) for genotyping. The polymorphic sites of the UGT1A9 I399 C>T, -1818T>C and -1887T>G alleles were analyzed by Polymerase Chain Reaction (PCR) and gene sequencing technology. Hardy–Weinberg equilibriumtesting was analyzed using appropriate X^2 testing by the software SHEsis (Shi and Lin, 2005). The UGT1A9 promoter region from -2224 to +2 and the intron region were amplified using PCR. The total volume for each reaction was 25 mL, including template 5 ng, Taq enzyme 0.125 U, $MgCl_2$

1.5 mL (25 mM) dNTP 2 mL (25 mM) 10×buffer 2.5 mL and primers 0.5 mL of each (20 mM) (TaKaRa Biotechnology Co., Ltd., Dalian, China).

Mutations of the promoter region were -1887T/G and -1818T/C. Forward primers: TTTTCAATTGTTCATTGCTA and Reverse primers: CTACTC AATGGAGGACAATC and both amplification products were of 664 bp. The PCR conditions: 98°C 2 min, 96°C 30 sec for 11 cycles, 60°C 1 min (decrease 1°C for each cycle) 72°C 45 sec, then for another 35 cycles: 96°C 30 sec, 55°C 40 sec, 72°C 45 sec. The reaction was completed by 7 min extension at 72°C.

Mutations of the intron region was I399, Forward primers: TATATGCCCGCCCCAGAG and Reverse primers: TATGTCCAGCCCAATACTAGATTTT and the amplification products were of 145 bp. The PCR conditions: 95°C 3 min, then for 36 cycles: 94°C 30 sec, 52°C 30 sec and 70°C 30 sec, then 70°C 2 min to extend.

The PCR products were sequenced with primers using an ABI PRISM 3700 DNA Analyser (Applied Biosystem, Foster City,CA, USA). Ambiguous sequencing chromatograms and samples with SNPs were systematically reamplified and resequenced.

Statistical analysis: Statistical analysis was performed with the SPSS 17.0 (Spss. Inc., Chicago, Ill., USA) for windows and a two-tailed p-value of <0.05 was considered to be statistically significant. Data were expressed as Means±SD. Data were compiled according to genotypes and allele frequencies were estimated from the observed numbers of each specific SNP. The Chi-square tests were used to vefify the Hardy-Weinberg equilibrium. The effects of genotypes on propofol pharmacodynamics and pharmacokinetics were compared by one-way analysis of variance with post-hoc Bonferroni correction for multiple comparisons before and after adjusting for the age, weight, height, BMI and duration of the surgery. A likelihood analysis determining the association of major haplotypic groups with the outcomes (time to OAA/S 4 and BIS 80) using the haplotype score software (www.mayo.edu/hsr/people/schaid.html) (Schaid *et al*., 2002).

RESULTS

There was no significant difference among the every three groups with regard to age, weight, BMI and operation time (p>0.05). There was no further adjustment of propofol infusion rate because of hypotension or hypertension and no adverse effect was recorded.

Table 1: Genotype and gene frequency of UGT1A9

Site	SNP	n/N	Genotype frequency (%)	Gene	Gene frequency (%)
I399	C/C	24/150	16.00	T	52.33
	C/T	95/150	63.33	C	47.67
	T/T	31/150	20.67		
-1818	T/T	50/150	33.33	T	59.00
	T/C	77/150	51.33	C	41.00
	C/C	23/150	15.33		
-1887	T/T	122/150	81.33	T	90.67
	T/G	28/150	18.67	G	9.33

($\bar{x} \pm s$, N = 150)

Table 2: Individual differences in the propofol sedation effect during the recovery period

Parameters	Item	Range	Mean value	Fold
OAA/S to 4	T_1	1~18 min	5[#] 3, 7 min	18
	BIS value	51~80	69±7	1.9
	C_1	1.2~2.9 μg mL^{-1}	2.2±0.4 μg mL^{-1}	2.5
BIS back to 80	T_2	2~29 min	8[#] 6, 11 min	15
	C_2	0.9~2.8 μg mL^{-1}	1.9±0.4 μg mL^{-1}	3.3

[#]Median, T_1: The awakening time (OAA/S to 4), C_1: Effect site concentration at OAA/S 4, T_2: The time of BIS value rising to 80 and C_2: Effect site concentration at BIS 80

The frequency of UGT1A9 I399 C>T, -1818 T>C, -1887 T>G allele in Han Chinese patients with breast surgery was 52.3, 41 and 9.3%, respectively (Table 1). The allele frequencies were in Hardy-Weinberg equilibrium (p>0.05) which indicated the patient pool in our study was likely representative of the population being studied.

The results showed the pharmacodynamics parameters varied obviously during the recovery time of the anesthesia. The T_1 varied from 0-18 min, showing an 18 fold inter-patient variability. The C_1 fluctuated from 1.2-3.0 μg mL^{-1}, showing a 2.5 fold inter-patient variability. The BIS value at OAA/S 4 varied from 43-80, showing a 1.9 fold inter-patient variability. The T_2 varied from 0-29 min, showing a 29 fold inter-patient variability. The C_2 varied from 0.9-3.0 μg mL^{-1}, showing a 3.3-fold inter-patient variability (Table 2).

Patients were divided into three genotyping groups of homozygous wild group, heterozygous and mutant homozygous group according to the genotypes of UGT1A9 I399C>T, -1818T>C and -1887T>G. No statistical differences in propofol sedative effects were detected after the stopping of propofol TCI to the patients OAA/S 4 among the every three genotyping groups (p>0.05). After analyzed of age, weight, height and duration of anesthesia using covariate analysis of covariance, we found no significant difference in propofol sedative effects, either (p>0.05) (Table 3). After compared the interaction effect of UGT1A9 I399C>T and -1818T>C. There was no interaction effect of the UGT1A9 I399C>T and

Table 3: Propofol sedation effects among UGT1A9 genotype groups

	OAA/S to 4					BIS back to 80	
	Genotype	n	T_1	BIS value	C_1	T_2	C_2
I399	C/C	24	4.67±3.62	69±7	2.39±0.41	7.75±3.52	1.98±0.38
	C/T	95	5.58±3.27	69±7	2.26±0.41	9.20±4.56	1.85±0.43
	T/T	31	5.10±2.52	68±7	2.34±0.33	9.55±6.69	1.96±0.49
	p-value		0.266	0.608	0.212	0.138	0.159
-1818	T/T	50	5.22±3.03	68±7	2.29±0.36	9.18±4.70	1.89±0.39
	T/C	77	5.56±3.49	70±7	2.28±0.43	9.17±5.19	1.87±0.48
	C/C	23	4.83±2.37	68±7	2.37±0.34	8.30±4.67	1.98±0.39
	p-value		0.652	0.487	0.805	0.775	0.602
-1887	T/T	122	5.29±3.19	69±7	2.31±0.39	9.10±5.09	1.89±0.39
	T/G	28	8.79±4.28	70±7	2.24±0.40	8.79±4.28	1.87±0.39
	p-value		0.835	0.851	0.520	0.851	0.711

T_1: Awakening time (OAA/S to 4), C_1: Effect site concentration at OAA/S 4, T_2: The time of BIS value rising to 80 and C_2: Effect site concentration at BIS 80 ($\bar{X}\pm s$, N = 150)

Table 4: Interaction effect of UGT1A9 I399 C>T and -1818 T>C on propofol pduring the recovery period

Group			OAA/S to 4			BIS back to 80	
I399	-1818	n	T_1 (min)	BIS	C_1 (μg mL^{-1})	T_2 (min)	C_2 (μg mL^{-1})
T/T	C/T	11	4.82±2.32	68.64±6.93	2.38±0.33	9.82±6.77	1.93±0.53
C/T	C/C	8	4.38±2.50	70.00±5.73	2.43±0.40	7.63±2.50	1.96±0.35
C/T	C/T	56	6.09±3.70	69.91±7.06	2.21±0.45	9.57±5.08	1.82±0.47
C/T	T/T	31	4.97±2.36	68.10±6.96	2.31±0.33	8.94±3.91	1.89±0.39
C/C	T/T	14	5.57±4.13	67.50±7.84	2.26±0.41	8.86±3.61	1.88±0.33
T/T	C/C	15	5.07±2.34	66.80±8.21	2.33±0.31	8.67±5.54	1.99±0.44
C/C	C/T	10	3.40±2.41	71.30±5.48	2.57±0.33	6.20±2.86	2.12±0.41
T/T	T/T	5	5.80±3.77	69.60±3.21	2.26±0.42	11.6±10.29	1.90±0.63

T_1: Awakening time (OAA/S to 4), C_1: Effect site concentration at OAA/S 4, T_2: The time of BIS value rising to 80 and C_2: Effect site concentration at BIS 80 ($\bar{X}\pm s$, N = 150

-1818T>C alleles on the propofol pharmacodynamics during the recovery period, either (Table 4).

DISCUSSION

Glucuronidation is a primary metabolic pathway of propofol (Restrepo *et al*., 2009). The UGT1A9 is a class of UGT enzyme with high expression in the liver and other tissues (Gong *et al*., 2001) plays very important role in the glucuroniding of propofol. As a representative phase II enzyme (Allegaert *et al*., 2008). Propofol is mainly metabolized by UGT1A9 in the liver and excreted with inactive product (Al-Jahdari *et al*., 2006; Allegaert *et al*., 2008). A lot of evidences suggest that there are obvious individual differences in the expression of UGT1A9 and its glucuronidation of regulation (Gagne *et al*., 2002). It was assumed that UGT1A9 genepolymorphisms might be reasons for the variability in propofol sedeative effect. So, in this study, investigation on the UGT1A9 genepolymorphisms and inter-patient variability in the propofol TCI pharmacodynamics was done.

It is reported that propofol is the most suitable induction agent to reduce the hemodynamic responses to intubation (Kovac, 1996). When intermittent boluses of propofol are given during the induction and maintenance of anesthesia, inconsistencies in dosage and hemodynamic instability can be seen often, but this can be avoided when a target-controlled infusion of propofol is used. The use of propofol for induction and maintenance of anesthesia has gained great popularity among anesthetists. The TCI incorporates the pharmacokinetic variables of an intravenous drug to facilitate safe and reliable administration. If the anesthesiologist changes the target concentration of propofol, the TCI system continuously adjusts the required infusion rate. The total dose of propofol administered tends to be less when a TCI is used, allowing a more rapid recovery. Propofol administration becomes as simple as changing the inspired concentration of an inhaled anesthetic.

The BIS monitoring can provide an objective measurement of the level of consciousness in sedated patients, which has a good relation with the propofol sedation level (Kurita *et al*., 2001). It is widely used as an indicator of the level of depth of anesthesia, measuring the degree of depression in the central nervous system (Milne *et al*., 2003). The target BIS of 40-50 is an appropriate anaesthesia depth for surgical (Johansen and Sebel, 2000). Previous studies have evaluated the relationship of the predicted Ce of propofol with the depth of anesthesia (Irwin *et al*., 2002; Milne *et al*., 2003). The predicted plasma concentration and Ce of propofol exists

a considerable discrepancy, which suggests that the predicted Ce of propofol may be a more useful clinical correlate than the predicted plasma concentration during induction and recovery (Wakeling *et al*., 1999). So, we adopted a strict anesthesia using propofol TCI under the BIS monitoring.

Remifentanil is an opioid agent with unique structure, which contains an ester bond rendering it to rapid hydrolysis by nonspeci c esterases from blood and tissue. It has a very short half-life. Because of its special metabolism ways, remifentanil does not affect the metabolism of propofol. Therefore, remifentanil was used as an anesthetic adjuvant in this study.

The OAA/S scores have been shown to have a good correlation with the levels of sedation (Glass *et al*., 1997). So, we adopted the OAA/S score, Ce of propofol and BIS as clinical parameters to predict the sedation and hypnosis induced by propofol.

Inter-patient variances in responding to propofol sedative effect of 18-fold, 2.5-fold, 1.9-fold, 29-fold were observed in T_1, C_1, T_2, C_2, respectively. Our results showed there really were obvious individual differences in propofol sedative effect.

Variability in drug responses could be result from both genetic and environmental factors. To insure our results would not affected by the confounding factors. Patients with cardiopulmonary disease, renal and hepatic dysfunction, or central nervous system disease were excluded from the study. Propofol clearance mainly depends on hepatic blood flow and metabolic enzyme activity. It is reported that within 20 min following the steady infusion of propofol, the plasma concentration approaches steady state (Morgan *et al*., 1990). There will be no significant difference among the groups with regard to the plasma concentrations of propofol at 20 min after the start of TCI 3 μg mL^{-1}. Propofol is a highly lipophylic compound and therefore exhibits rapid distribution from blood into subcutaneous fat and the central nervous system where it exerts its pharmacodynamic effects with subsequent redistribution. So, distribution of propofol may be the main reason for its individual differences in pharmacodynamic. Gender is one of the factors that affect the propofol pharmacodynamic (Loryan *et al*., 2012). So, all female patients selected with age of 20-50 yeasr in this study.

After unified all the confounding factors, patients were divided into homozygous wild group, heterozygous and mutant homozygous group according to the genotypes of each UGT1A9 allele. There was no difference among the every three groups with regard to age, weight, BMI and operation time. Then we analyzed three UGT1A9 SNP_S and the propofol sedative effect during the recovery period of anesthesia. The three SNP_S had the following distribution in the population studied: I399C>T: T/T 20.67%, T/C 63.33%, C/C 16%; -1818C>T: T/T 33.33%, T/C 51.33%, C/C 15.33%; -1887T>G: T/T 81.33%, T/G 18.67%. The UGT1A9 I399 C>T allele frequency in our study was 52.3%, similar to that reported in the study of the Chinese Han (55.0%) (Guo *et al*., 2013) but lower than the Japanese population (64.4%) (Inoue *et al*., 2007) and higher than the Caucasus people (38%) (Levesque *et al*., 2007).

No difference in pharmacodynamic parameters was found between the carriers of the mutunt T allele and the C/C genotype in relating to both SNP I399C>T and -1818C>T. As the allele frequency of -1887 T>G was low, we only analyzed the interaction effect of UGT1A9 I399C>T and -1818T>C on the propofol pharmacodynamics. There was no significant interaction relating to the propofol pharmacodynamic parameters, either. The results showed that enzyme UGT1A9 SNPs might not be account for these propofol sedative effect variations *in vivo*.

A variety of P450 isozymes (CYP2B6, CYP2C9, CYP2C8, CYP2C18, CYP2C19, CYP2A6) are involved in the propofol metabolism, however, all the metabolic rates are very low (Guitton *et al*., 1998; Oda *et al*., 2001). There was report that CYP2B6 polymorphism is the main determinant of individual differences about propofol hydroxylation *in vitro* liver microsomal study (Court *et al*., 2001) but the *in vivo* studies had showed it was not the reason for inter-individual differences in propofol metabolism.

The results showed that UGT1A9 polymorphism was not the genetic factors leading to individual differences in propofol pharmacodynamics. There was a limitation of present study the sample size were a little small. Furthermore, whether there are other factors can influence the metabolic enzyme such as, its epigenetic regulation on the post-transcription level are unknown. The SNP of one metabolic enzyme or in one organ site in DNA level may not be able to explain propofol pharmacodynamics of individual differences. So, large sample size research, different levels regulation of the propofol metabolism enzymes are needed to be carried in the future to illustrate the inter-individial difference in propofol sedative effect.

CONCLUSION

In conclusion, the results demonstrated that UGT1A9 SNPs in this study were not genetic factors for individual differences in propofol TCI pharmacodynamics.

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