Note

Association of the CYP2B6 c.516G>T Polymorphism with High Blood Propofol Concentrations in Women from Northern Greece

Orthodoxia MASTROGIANNI1, Emma G8ANDI2, Amvrosios ORPHANIDIS1, Nikolaos RAIKOS1, Evangelia GOUTZIOMITROU3, Efstratios M. KOLIBIANAKIS1, Basil C. TARLATZIS3 and Antonis GOULAS2,*

1Department of Forensic Medicine and Toxicology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece
2Department of Pharmacology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece
3Unit for Human Reproduction, 1st Department of Obstetrics and Gynecology, Papageorgiou General Hospital, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

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Summary: Cytochrome P450 2B6 (CYP2B6) is responsible for the initial biotransformation of propofol, an extensively metabolized intravenous anesthetic. In this study we examined the effect of the apparently functional CYP2B6 c.516G>T polymorphism on the distribution of propofol concentrations, quantified by GC/MS analysis following a single bolus dose, in the blood of 44 Greek women undergoing oocyte retrieval. Univariate analysis using age, height, weight and smoking status as covariates, as well as the Mann-Whitney non-parametric test, revealed a strong trend of association of the T allele with high propofol concentrations determined in whole blood, shortly after a single bolus dose. Propofol concentrations which were higher than one standard deviation of the mean were almost invariably associated with carriage of the T allele.

Keywords: blood propofol; GC/MS; CYP2B6; gene polymorphism

Introduction

Propofol is a fast-acting, intravenous anesthetic agent, commonly used for the induction and maintenance of anesthesia. Propofol is characterized by widely variable pharmacokinetics and pharmacodynamics among patients of the same ethnic origin, reflected in different dose requirements and recovery time. This variability has been attributed mainly to interindividual differences in biotransformation, arising from genetic (polymorphisms in genes coding for propofol biotransformation enzymes) and non-genetic factors (weight, height, lean body mass, sex) or interactions thereof. The extensive biotransformation of propofol (approximately 90% ends up in the form of conjugated metabolites in urine) is mostly catalyzed by various members of the UDP-glucuronosyltransferase (UGT) family (mainly UGT1A9) and the cytochrome P450 2B6 (CYP2B6) enzyme.

The human gene coding for CYP2B6 (CYP2B6) is highly polymorphic while the hepatic content and activity of the enzyme itself is subject to wide variation. Of the known functional single nucleotide polymorphisms, c.516G>T (g.15631, Q172H, rs 3745274) in exon 4, is the most common, and displays considerable inter-ethnic variability; Estimates of minor allele (T) frequency range from 14 to 21% in Asians, to as high as 49% in Africans. The polymorphism has been consistently associated with lower expression of functional mRNA and protein—an effect mainly attributed to erroneous splicing—but also to changes in catalytic activity, the latter being apparently substrate-specific. As CYP2B6 was shown to be responsible for interindividual variability of propofol hydroxylation, it was not unreasonable to expect that the CYP2B6 c.516G>T polymorphism would affect blood propofol levels and efficacy. Indeed, Kansaku et al. have found that the maximum concentrations of propofol, following infusion, are significantly correlated with awakening time, and proposed a propofol risk index score which includes carriage of the CYP2B6 c.516T allele as a risk factor, on the basis of their evidence showing some—albeit non-statistically significant—effect of the polymorphism on maximum propofol concentrations. Recent attempts to detect an association between CYP2B6 c.516G>T and
propofol pharmacokinetics, by other investigators, provided no such evidence however. In an attempt to obtain additional information as to the effect of this polymorphism on propofol pharmacokinetics, we used a highly sensitive GC/MS method developed in our laboratory (see Methods) to determine propofol concentrations in blood samples obtained shortly after (4 min) the administration of a single bolus dose of propofol, and subsequently examined their possible association with CYP2B6 c.516G>T, in a group of women residents of northern Greece.

Methods

Patients: Forty-four Greek women, undergoing oocyte retrieval for purposes of in vitro fertilization, in the 1st Department of Obstetrics and Gynecology, Papageorgiou General Hospital, Faculty of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece, participated in this study. The study was approved by the Ethics Committee of the Faculty of Medicine of the Aristotle University of Thessaloniki. All participants gave their informed consent. Demographics of the participants were as follows: age (±SD) = 37.32 ± 5.22 years, weight (±SD) = 66.91 ± 12.33 kg, height (±SD) = 1.65 ± 0.06 m. Twenty-six out of the forty-four (57.8%) were smokers. No history of alcohol abuse was reported. All women were administered an initial propofol bolus dose of 2.5 mg/kg. Peripheral blood was removed at 4 min following administration of propofol and stored at −70°C awaiting analysis of propofol and DNA isolation. Individually adapted additional doses of propofol were given at later times and did not affect the results of the study.

Method of analysis: Analysis of propofol was performed in whole blood (2 mL) following liquid-liquid extraction with butyl acetate. Following evaporation of the organic phase and resuspension of the dried residue in 100 µL of butyl acetate, one μL of the resulting solution was subjected to GC/MS analysis using electron impact ionization (Agilent Technologies, Santa Clara, CA, USA, 7890A with a MS 5975C inrXL, EI/CI MSD with a Triple-Axis detector). Propofol quantitation was accomplished using selected ion monitoring (target ion: 163, qualifying ions: 178, 117, 91). Lidocaine was used as the internal standard. The tested calibration range was from 2 to 200 ng/mL. Twelve calibration blood samples at six different levels and 2 blank samples with and without the internal standard were included in every validation sequence. The method showed satisfactory linearity with correlation coefficients greater than 0.99 in all validation runs. Accuracy and precision were determined analyzing a quality control (QC) level of spiked blood (100 ng/mL) and estimated by 6 replicate analyses of the QC samples. The precision was equal to 1.1% CV and the accuracy 100.6%. For real blood samples with propofol concentrations higher than 200 ng/mL, the final dry residue was reconstituted in the proper butyl acetate volume instead of that of 100 µL.

Genotyping: Genotyping for the CYP2B6 c.516G>T polymorphism was accomplished with a previously published PCR-RFLP method. In short, genomic DNA isolated from peripheral blood with a commercial kit (Ron’s Blood DNA minikit, Bioron GmbH, Ludwigshaften, Germany), was PCR-amplified using the following primers: forward, GGTCTGCCCATCTATAAAC; reverse, CTGATTCTTCACATGTCTGCG. The annealing temperature was 56°C. The amplified DNA was then digested with the RsaI restriction endonuclease (Thermo Fisher Scientific Inc., Waltham, MA). Presence of the CYP2B6 c.516G allele was inferred by the identification of 241 and 268 bp fragments, whereas that of the CYP2B6 c.516T allele by the presence of a 509 bp fragment, using agarose gel electrophoresis.

Statistical analysis: A univariate analysis with type III sum of square statistics was initially used to explore the association of CYP2B6 c.516G>T with blood propofol concentrations, testing age, smoking status, weight and height as covariates. Due to the non-normal distribution of the detected blood propofol values (see Results), the Mann-Whitney non-parametric test was also used to examine the effect of the polymorphism, as well as that of smoking status, on propofol distribution. The correlation of age, height, and weight with propofol levels was tested with Spearman’s test. p = 0.05 was used as the limit for statistical significance. The SPSS statistical package (version 20.0) was used for all calculations.

Results

Blood propofol concentrations varied widely and followed a skewed distribution (mean = 2,256.0 ng/mL, SD = 2,921.9; median = 1,512.5 ng/mL, 95% CI = 1,367.6–3,144.3; skewness = 2.5; Fig. 1A). The distribution of CYP2B6 c.516G>T genotypes and alleles was as follows: GG = 19 (43.2%), GT = 24 (54.5%), TT = 1 (2.3%); G = 70.5%, T = 29.5%. The genotype distribution did not deviate significantly from the one corresponding to the Hardy-Weinberg equilibrium (p = 0.121). Stratification according to carriage of the T allele showed that, on average, T carriers appeared to display higher propofol values (Fig. 1B). Univariate analysis, using carriage of the T allele as the fixed factor and propofol concentration as the dependent variable, produced a marginally significant term (p = 0.035). Inclusion of age, smoking status, weight and height reduced the significance of the association (p = 0.094), even though none of these covariates was independently associated with propofol levels. Comparison of propofol distributions with the Mann-Whitney non-parametric test produced a similar trend (p = 0.086). Again, stratification according to smoking status did not produce a statistically significant difference (p = 0.749), nor did the bivariate correlation of propofol with age (p = 0.667), weight (p = 0.934) or height (p = 0.507). Moreover, of the women displaying propofol levels higher than one standard deviation of the mean (2,921.9 ng/mL), nine out of ten were T carriers, as opposed to a much more balanced distribution of carriers and non-carriers at lower propofol values (p = 0.027, Table 1).

Discussion

In this study, we were able to detect a trend of association between the CYP2B6 c.516G>T polymorphism and blood propofol concentrations measured shortly after a single bolus administration of the drug, which reached statistical significance when limited to high propofol values (higher than one SD). Our results are in apparent contrast to two recent reports, from the same group, according to which no significant association was found between the same polymorphism and plasma levels of propofol or its metabolites. We attribute our finding to a number of reasons. First, we have limited our study to a time point (4 min post-administration) at which CYP2B6 exerts its maximum effect on propofol biotransformation, and its product, 4-OH-propofol, is by far the most abundant metabolite following a bolus administration of the drug. Second, it is under these conditions that blood
propofol levels display their widest variation compared to the rest of the AUC curve. Finally, our study sample was composed of individuals of the same sex (women) with little variation in terms of age, weight or height, which can act as confounding factors. A number of studies have shown that the CYP2B6 c.516T variant is associated with decreased protein levels in the liver,10–12. Thus, under the assumption that the CYP2B6 c.516G>T polymorphism does affect enzyme expression and activity, we have used conditions that would theoretically increase the chance of detecting an association between the CYP2B6 c.516G>T polymorphism and blood propofol levels. It should be pointed out that where no such association was detected—following a bolus administration of propofol—no effect of the polymorphism on CYP2B6 levels was detected either, even though an association between CYP2B6 and the rate of propofol 4-hydroxylation has clearly been established.3 On the other hand, in the study by Kansaku et al.,5 the highest maximum plasma concentrations of propofol—normalized for the duration of infusion—were largely displayed by carriers of the T allele, as was the case in our study.

A more direct demonstration of an effect of the CYP2B6 c.516G>T polymorphism on blood propofol levels would arguably demand a measurement of propofol’s hydroxylated metabolites, for which CYP2B6 activity is the rate-limiting step, in comparison to that of the non-hydroxylated propofol glucuronide which is formed by UGT1A9. Indeed, it has been proposed that differences in the ratio of hydroxylation to direct glucuronidation are, in effect, responsible for the observed interpatient variability of propofol levels.13 In addition, polymorphisms of the UGT1A9 gene have been shown to affect propofol glucuronidation in vitro,14,15 and could thus affect propofol levels in vivo as well. On the other hand, as 4-hydroxypropofol appears to be the most abundant and most rapidly formed metabolite following a bolus dose, it is highly unlikely that those polymorphisms would have a measurable effect under the conditions used in this study.

In conclusion, the CYP2B6 c.516G>T polymorphism may well affect maximum blood concentrations of propofol, even though its effect appears to be more modest than originally envisaged. Hopefully, future studies will advance our still limited understanding of the effects of CYP2B6 polymorphisms on propofol pharmacokinetics and the clinical significance thereof.

References
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