Propofol metabolites in man following propofol induction and maintenance

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Background. The pharmacokinetics of propofol in man is characterized by a rapid metabolic clearance linked to glucuronidation of the parent drug to form the propofol-glucuronide (PG) and sulfo- and glucuro-conjugation of hydroxylated metabolite via cytochrome P450 to produce three other conjugates. The purpose of this study was to assess the urine metabolite profile of propofol following i.v. propofol anaesthesia in a Caucasian population.

Methods. The extent of phase I and phase II metabolism of propofol was studied in 18 female and 17 male patients after an anaesthesia induced and maintained for at least 4 h with propofol. The infusion rates (mg kg⁻¹ h⁻¹) of propofol were (mean (SD)) 4.1 (1.0) and 4.5 (1.3) for males and females, respectively. Urine was collected from each patient for the periods 0–4, 4–8, 8–12, and 12–24 h after the start of propofol administration. In a preliminary study, the three main glucuro-conjugated metabolites were isolated from urine and characterized by magnetic resonance spectroscopy. The quantification of these metabolites for the different collection periods was then performed by a HPLC–UV assay.

Results. Total recovery of propofol in the metabolites studied amounts to 38%, of which 62% was via the PG metabolite and 38% via cytochrome P-450. This percentage is significantly higher than that previously reported from patients after a bolus dose of propofol. Extreme values for PG (0–24 h period) were included from 73 to 49%. There was no significant difference between female and male patients in the metabolite ratio.

Conclusions. We conclude that the extent of hydroxylation in propofol metabolism was higher than in previous findings after administration of anaesthetic doses of propofol. Moreover, the ratio between hydroxylation and glucuronidation of propofol is subject to an inter-patient variability but this does not correlate with the dose of propofol. However, the variation of the metabolite profile observed in the present report does not seem to indicate an extended role of metabolism in pharmacokinetic variability.

Br J Anaesth 2002; 88: 653–8

Keywords: anaesthetics i.v., propofol; pharmokinetics, propofol

Accepted for publication: December 3, 2001

The pharmacokinetics of propofol in humans has been widely studied after either continuous infusions or bolus doses.¹ The elimination kinetics are triphasic and characterized by a rapid metabolic clearance.² Propofol exhibits a rapid distribution from blood into tissues and slow return of the drug from deep peripheral compartments into the
blood. Thus, the clearance of propofol depends on both metabolism and distribution. Propofol is a highly lipophilic drug and is excreted mainly in the urine after glucuro-conjugation of the parent drug (to form the propofol-glucuronide (PG) and sulfo- and glucuro-conjugation of the hydroxylated metabolite to form 4-(2,6-diisopropyl-1,4-quinol)-sulphate (4-QS), 1-, or 4-(2,6-diisopropyl-1,4-quinol)-glucuronide (1-QG and 4-QG), respectively. Thus, the metabolism of propofol to 2,6-diisopropyl-1,4-quinol (1-4-quinol) by cytochrome P-450 (CYP450) is the rate-limiting step in the formation of 1-QG, 4-QG, and 4-QS. In humans, 4-QS, 1-QG, 4-QG, and PG were identified as the main metabolites, and at least four other minor conjugated metabolites were also described. To our knowledge, only two studies using a direct quantification of the main metabolites were carried out in patients with the purpose of assessing the metabolite profile of propofol. However, their respective patient inclusions were limited as one study was performed in six female Caucasians, and the other in only seven males and one female of Japanese origin. In both cases, propofol was administered in a bolus dose to induce anaesthesia which was then maintained with another anaesthetic such as enflurane. A third study was performed in male volunteers following administration of a subanaesthetic dose of propofol. From these studies, it was suggested that the proportion of phase I and phase II metabolism from parent drug was dependent on the administered dose of propofol and appears subject to inter-individual variation. It is important to investigate the extent of phase I metabolism in propofol as this was recently described as variable with a 19-fold variation of 1-4-quinol synthesis via CYP2B6.

The aim of this study was to assess, first, the metabolite profile of propofol after an i.v. propofol anaesthesia which was maintained for at least 4 h in a larger Caucasian population, and secondly to compare female and male patient profiles.

**Patients and methods**

**Patients, anaesthetic procedure, and urine specimens**

With ethics committee approval and patient informed consent, 35 Caucasian patients (18 females and 17 males) were included in the study. All patients were ASA physical status I or II and hospitalized to undergo inner ear surgery (for acoustic neurinoma). Patients with hepatic, respiratory, cardiovascular, or renal diseases were not included in the study. Four male patients were smokers. Propofol was infused using a Target Controlled Infusions system (Diprifusor-TCI, Zeneca). Infusion was set at a target plasma concentration of 6 μg ml⁻¹ for a few minutes and was then followed by an infusion at the target plasma concentration of 2 μg ml⁻¹. Pre-medication was given orally 2 h before induction of anaesthesia and consisted for all patients of hydroxyzine, alprazolam, and domperidone. Ringer’s solution was administered continuously at a rate of 5 ml kg⁻¹ h⁻¹. During the surgical procedure, prophylactic antimicrobial chemotherapy was given (ceftriaxone or cefazolin). Analgesia was provided by remifentanil 1 μg kg⁻¹ i.v. followed by a continuous infusion of 0.2 μg kg⁻¹ min⁻¹. Postoperatively, patients received acetaminophen (2 g 8 h⁻¹ i.v.) and ketoprofen (100 mg 8 h⁻¹ i.v.), for at least 24 h.

All urine was collected from each patient for the periods 0–4, 4–8, 8–12, and 12–24 h after the start of propofol administration. The volume of urine for each period was measured and after thorough mixing, a 40-ml aliquot was added to polypropylene haemolysis tubes and stored at −25°C before analysis.

**Drugs and chemicals**

Acetonitrile (HPLC grade) was purchased from Merck (Darmstadt, Germany). Ethyl acetate, 1-naphthyl-sulphate (1-NS) and acetic acid were obtained from Sigma (St Quentin Fallavier, France).

**Analytical procedure**

1-QG, 4-QG, and PG were extracted from human urine samples included in this study by liquid/liquid extraction using ethyl acetate. The mixture was mixed for 2 h, then the organic layer was removed and evaporated to dryness under nitrogen at 40°C. The residue was re-dissolved in water acidified with acetic acid and several fractions were injected onto the preparative chromatograph apparatus. Preparative chromatography was performed using a stainless steel column (250×20 mm, dp; 10–25 μm, 120 Å) packed with Shiseido Capcell Pak C18 from Interchim (Montluçon, France). The metabolites were separated with a linear gradient using water–acetic acid (pH 3.8) and acetonitrile as solvents. Aliquots corresponding to each metabolite were freeze-dried to obtain a lyophilized powder. Characterization of the glucuro-conjugate metabolite was performed using 1H-nuclear magnetic resonance spectroscopy (NMR) in a Spectrospin 500 MHz NMR spectrometer from Bruker (Wisssembourg, France).

Chromatographic conditions for the quantification of glucuro-conjugated metabolites were described previously. Urine samples were thawed, mixed, and centrifuged at 4000g (20 min, 4°C). The sample was then diluted (1:5, v/v) with phosphate buffer (25 mM, pH 3.8), and 20 μl of 1-NS, used as internal standard, was added to a final concentration of 40 μM. This mixture was then vortexed and 20 μl was injected onto the HPLC column. Stock solutions of 1-QG, 4-QG, and PG were prepared in phosphate buffer (25 mM, pH 3.8), then serially diluted to concentrations of 10, 25, 50, 100 and 250 μg ml⁻¹ in blank urine. These concentrations were used to assess the accuracy and linearity of the method on 3 different days for the three metabolites.
Table 1 Patient characteristics and anaesthetic procedure. Values are expressed as mean (SD). For age, weight, and total dose of propofol administered (induction+infusion) parameters’ range is indicated in square brackets. Data concerning the characteristics of patients were compared by a Mann–Whitney U-test. *The statistical differences in weight were considered as physiological

<table>
<thead>
<tr>
<th></th>
<th>Male (n = 17)</th>
<th>Female (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>56.2 (12.2)</td>
<td>52.2 (13.2)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81.9 (12.3)</td>
<td>62.3 (10.8)</td>
</tr>
<tr>
<td>Ureaemia (mM)</td>
<td>6.4 (2.1)</td>
<td>4.9 (2.3)</td>
</tr>
<tr>
<td>Creatininemia (µM)</td>
<td>94.6 (27.3)</td>
<td>74.9 (19.2)</td>
</tr>
<tr>
<td>Urine volume on 24 h (ml)</td>
<td>2922 (1053)</td>
<td>2797 (939)</td>
</tr>
<tr>
<td>Propofol dose at induction (mg)</td>
<td>227 (66)</td>
<td>197 (40)</td>
</tr>
<tr>
<td>Propofol infusion dose (mg kg⁻¹ h⁻¹)</td>
<td>4.1 (1.0)</td>
<td>4.5 (1.3)</td>
</tr>
<tr>
<td>Duration of propofol infusion (min)</td>
<td>328.4 (107.5)</td>
<td>322.0 (83.8)</td>
</tr>
<tr>
<td>Total dose of propofol (g)</td>
<td>2.08 (0.80)</td>
<td>1.72 (0.53)</td>
</tr>
</tbody>
</table>

Table 2 Proportion of the main metabolites of propofol in urine (0–24 h) of female and male patients in the present study. Comparison with results obtained in female Caucasian patients from a study by Sneyd and colleagues. Data are mean values (SEM) in both cases. *Differences (P<0.05) from respective metabolites, between females and males in the present study, were calculated using analysis variance, followed by the Scheffe post-hoc test. **Differences (P<0.05) from respective metabolites, between the present study (n=35) and Sneyd and colleagues study (n=6), were calculated using a Mann–Whitney U-test.

<table>
<thead>
<tr>
<th></th>
<th>4-QS</th>
<th>1-QG</th>
<th>4-QG</th>
<th>PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (n=18)</td>
<td>6.5 (0.7)</td>
<td>18.5 (1.0)</td>
<td>12.3 (0.6)</td>
<td>62.7 (1.5)</td>
</tr>
<tr>
<td>Male (n=17)</td>
<td>7.0 (0.6)</td>
<td>17.7 (0.9)</td>
<td>14.3 (0.6)</td>
<td>61.1 (1.6)</td>
</tr>
<tr>
<td>Whole population (n=35)</td>
<td>6.7 (0.5)</td>
<td>18.1 (0.7)</td>
<td>13.2 (0.5)</td>
<td>61.9 (1.1)</td>
</tr>
<tr>
<td>Sneyd and colleagues’ study</td>
<td>4.0 (1.2)*</td>
<td>11.8 (1.2)*</td>
<td>8.0 (1.8)*</td>
<td>76.0 (4.0)*</td>
</tr>
</tbody>
</table>

Statistical analysis

Patient characteristics were compared using a Mann–Whitney U-test. The differences in metabolite profile between males and females or between the four collection periods for the same metabolite, were evaluated using analysis variance followed by the Scheffe post-hoc test. Comparison of metabolite profiles between the study of Sneyd and colleagues and the present study were by Mann–Whitney U-test. For analytical validation, the significance of the slope and the validity of the linear calibration curves were confirmed using Fisher–Snedecor’s F-test. For each metabolite, the homoeocedasticity for the calibration curve was tested using Cochran’s test. In all cases P<0.05 was taken as the minimum level of statistical significance.

Results

Patients

Thirty-five Caucasian patients were studied and their characteristics are summarized in Table 1. There were no statistically significant differences for gender with age, uraemia, creatininemia, and in urine volume excreted during 24 h. Statistical differences in weight were considered physiological. There were no differences in propofol dose of induction, dose at infusion, and duration of infusion between female and male patients.

Analytical validation

The results obtained from NMR analysis have allowed clear identification unambiguously of the three metabolites 1-QG, 4-QG, and PG (data not shown). NMR spectra were identical to those described previously using the same analytical conditions. For 1-QG and 4-QG a weighted least-squares linear regression was applied (weight factor 1/ x²), whereas for PG an unweighted least-squares linear regression was used. For the three metabolites, the intercept was not statistically different from zero (Student’s t-test P<0.05). Determination coefficient (r²) of the linear regression was equal to 0.996 (0.006) (mean (SD)). Mean recovery from the five calibration points was 98.9 (5.1), 99.9 (6.6), and 100.6 (6.6)% for 1-QG, 4-QG, and PG, respectively. The limit of detection and the limit of quantification were equal to 0.2 and to 2.5 µg ml⁻¹, respectively. As 4-QS was not available as a pure compound from the isolation procedure, its quantification was performed arbitrarily using the 4-QG equation as the conjugation for both structures occurs at the same position and their UV spectra were very close.

Metabolite profile

The proportion of the main urinary conjugated metabolites for the 0–24 h period is shown in Table 2. There was no significant difference between female and male patients for 1-QG, 4-QS, and PG. For 4-QG a small but significant difference was observed (P=0.04). However, this small difference can be considered, from a metabolic and clinical viewpoint, as negligible. PG is the main metabolite and accounts for approximately 62% of the total metabolite profile for the 0–24 h period. Extreme values for PG (0–24 h period) of 70–73% for two females and one male and 49–52% in one male were recorded. The production of 1-QG was greater than 4-QG, and this difference was statistically significant.

The proportion of metabolites for the four collection periods and the significant difference between each period for each metabolite is shown in Table 3. The decrease in the proportion of PG over time was mirrored by an increase of the proportion of 1-QG. The proportion of 4-QG remained essentially unchanged. 4-QS increased during the last 12–24 h period in comparison with the 8–12 h period. These findings indicate that direct glucuronidation is involved particularly when the concentration of propofol was elevated illustrating that this pathway has a high capacity. During the first (bolus-infusion) and second collection period (duration of infusion was equal, in mean, to 320 min) extreme values for PG were included from 81 to 55% and from 75 to 50%, respectively, indicating inter-individual
Table 3 Relative percentage of propofol-conjugated metabolites in human urine (n=35) for each collection period (time in hours). Data are mean values (SEM). Significance of differences (P<0.05) was calculated using analysis of the variance, followed by the Scheffe post-hoc test. For 1-QG, P1=P2, P1=P3, P1=P4, P2=P3, P2=P4. For 4-QG, P1=P3, P1=P4. For 4-QS, P1=P3, P1=P4, P2=P4, P3=P4. For PG, P1=P3, P1=P4, P2=P4

<table>
<thead>
<tr>
<th>Period (h)</th>
<th>P1 (0–4)</th>
<th>P2 (4–8)</th>
<th>P3 (8–12)</th>
<th>P4 (12–24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-QG</td>
<td>13.4 (0.6)</td>
<td>18.5 (0.8)</td>
<td>22.7 (0.9)</td>
<td>22.9 (0.9)</td>
</tr>
<tr>
<td>4-QG</td>
<td>15.6 (0.8)</td>
<td>14.3 (0.6)</td>
<td>12.6 (0.6)</td>
<td>13.6 (0.6)</td>
</tr>
<tr>
<td>4-QS</td>
<td>4.7 (0.5)</td>
<td>4.3 (0.3)</td>
<td>5.7 (0.4)</td>
<td>8.6 (0.7)</td>
</tr>
<tr>
<td>PG</td>
<td>66.3 (1.6)</td>
<td>62.9 (1.3)</td>
<td>59.1 (1.3)</td>
<td>54.9 (1.5)</td>
</tr>
</tbody>
</table>

variation. The proportion of the three metabolites depending on the first step of the hydroxylation pathway varied from 33.7 to 45.1% as a function of the collection period.

Table 2 shows a comparison of our results with those of Sneyd and colleagues study performed in six female Caucasian patients.4 The proportion of PG excreted over 24 h in the present study was significantly lower (mean of 38 (2.0)% in all patients (mean (SEM)). Unquanti®ed amounts of unchanged propofol were detected in urine samples.

Discussion

This study indicates the importance of CYP450 in propofol metabolism in a clinical situation, a role which seems to have been underestimated in previous studies.4 8 Although PG is the main metabolite of propofol, followed by 1-QG, 4-QG, and 4-QS, the metabolite ratio (4-QS+1-QG+4-QG)/PG=0.61. The numerator corresponds to the sum of the metabolites for which CYP450 activity is the rate-limiting step in their further formation via the 1-4-quinol synthesis. No correlation was found between metabolite ratio and the administered dose of propofol (total dose, mean infusion rate, or induction dose). This finding was also observed for the proportion of PG in urine over the total period of 0–24 h and for each collection period, particularly the first two during which the blood concentration of propofol was more elevated. In the present study, we expected a greater proportion of PG with an increase in the administered dose of propofol as suggested previously by Sneyd and colleagues.4 In fact, our results indicate that the metabolite profile remains in favour of the glucuronidation pathway but to a lower extent even for high doses of propofol. Moreover, the level of glucuronidation vs the hydroxylation pathway from propofol is subject to inter-individual variation (49–73% for PG on 0–24 h). If it was established in humans that within the UDP-glucuronosyltransferase-1 gene family, propofol is a specific substrate for UGT1A8/9, it could be proposed that several CYP450 contribute to the para-hydroxylation of propofol with the predominant participation of CYP2B6 and CYP2C9.6 9 12 CYP2B6 was described recently as being responsible for inter-individual variability of propofol hydroxylation in vitro, and inter-individual variation in CYP2C9 expression has also been reported.9 12

Our anaesthetic procedure included the administration of remifentanil. This new opioid is metabolized by several non-specific esterases in blood and in tissues, conferring a metabolism independent of liver function.14 In the present study, no correlation was found between the proportion of any metabolite and the total dose of remifentanil. In addition, acetaminophen and ketoprofen, two drugs administered in the postoperative period, undergo glucuronidation as their main metabolic pathways. UGT1A6 and UGT2B7 are the most effective metabolizers of acetaminophen and ketoprofen via glucuronidation, respectively.15 Although one cannot rule out, definitively, on this basis a metabolic interaction between propofol and these drugs, a modification of the metabolite ratio of propofol was unlikely under these conditions. From a methodological point of view, the quanti®cation of 4-QS via the 4-QG equation has introduced an error in the amount of 4-QS. However, the UV spectra of both metabolites were very close, particularly in the region of 220 nm.10 Moreover, the recovery of 4-QS in urine was found to be relatively low, so a small deviation from the actual value would not modify markedly the relative proportions of the metabolites.

Differences between the present data, obtained from a larger population (n=35), and that obtained from Sneyd’s study in female patients could be explained by the limited size of their population (n=6), which may not have been representative, rather than by the different quantification methods that were used. Furthermore, the pharmacokinetics of propofol are well ®tted by a triexponential model, with a short ®rst elimination half-life assessed to 30–40 min after an infusion from 3 to 9 mg kg⁻¹ h⁻¹.2 16 Thus, our anaesthetic procedure favours metabolic pathways present during steady-state conditions (mean 320 min for the duration of propofol infusion) and they do not correspond to the single dose data. Total recovery of the administered dose, during the 0–24 h period, for all patients was lower (38.0 (2.0)%) than that observed in female Caucasian patients (50.9 (4.0)%). Several recent studies reported that remifentanil could decrease the clearance of propofol as a
result of decreased hepatic perfusion. This point is consistent with the fact that propofol has a high hepatic extraction (over 0.7) and thus, hepatic blood flow is a major determinant of its hepatic elimination. It has been established that modifications of drug disposition occur during surgery and anaesthesia, and the period of anaesthesia was markedly longer in our study (165±540 min) than in Sneyd and colleagues study (50–150 min). Consequently, this consideration explains the lower percentage of recovery of the propofol dose in the present study.

In the present study, the percentage of recovered conjugate was comparable with female and male patients in agreement with a previous study concluding that there were no gender differences in the pharmacokinetics of propofol in humans. None of the patients who smoked exhibited a different metabolite profile although an influence on acetaminophen glucuronidation was reported in male heavy smokers. However, the number of smoker patients in our study (n=4) was too small to draw any conclusions. Similarly, six elderly patients (over 70 yr old) were included. Their metabolite profile was in the same range as younger patients although a lower total body clearance has been described in the elderly.

In summary, the present study has documented that the extent of hydroxylation in propofol metabolism was higher than reported previously after administration of an anaesthetic dose of propofol. For the whole study group, the ratio of hydroxylation to glucuronidation was subject to inter-patient variability. The relative abundance of CYP2B6, CYP2C9, and UGT1A8/9 may perhaps explain this inter-individual variation. However, first, the metabolite profile does not correlate with propofol dose and secondly, no unusual pattern was observed in a particular patient. However, inter-patient variability was not great compared with other anaesthetics such as alfentanil which exhibits an important inter-individual variability in hepatic metabolism with clinical consequences.

Acknowledgements

We thank the recovery staff of the intensive care unit and ENT surgery. We also gratefully acknowledge the assistance of Dr L. Ettouati for preparative HPLC and provision of a freeze-dry device, Dr B. Fenet for NMR analysis, G. Charvet, and R. Gonin for use of HPLC apparatus.

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