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Propofol Reduces the Distribution and Clearance of Midazolam

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INTRODUCTION

Previously, we studied the influence of midazolam on the pharmacokinetics of propofol (1). The most important finding of that study was that midazolam increased blood propofol concentrations by 25% through a reduction in the metabolic, rapid, and slow distribution clearances of propofol. In addition, a reduction in mean arterial blood pressure was associated with propofol pharmacokinetic alterations that increased the blood propofol concentrations even further. In that study, the plasma midazolam concentration, as controlled by target-controlled infusion (TCI), was increased when administered in the presence of propofol, indicative of a possible influence of propofol on the pharmacokinetics of midazolam.

In clinical practice, midazolam is used for preoperative anxiolysis, to assure sedation during regional anesthesia and during ventilation in the intensive care unit and during prolonged procedures to induce and maintain surgical hypnosis perioperatively. In these settings, midazolam is occasionally combined with other sedatives and/or opioids to obtain the desired effect (hypnosis) and limit the side effects (hemodynamic or respiratory depression) (2-5). Various combinations of hypnotic drugs and/or opioids have been shown to exhibit both pharmacokinetic and pharmacodynamic interactions (6), often increasing the effect of the combination. (7-9)

Researchers studying the effect the propofol-midazolam interaction predominantly evaluated the pharmacodynamic interaction. (3,4,10,11) Only 1 study described the pharmacokinetic interaction between propofol and midazolam and reported that propofol affected the clearance of midazolam through a possible competitive inhibition of hepatic CYP 3A4.(8) However in that study, clearance was determined on the basis of the influence of just a 1-hour infusion of propofol on the pharmacokinetics of midazolam that was given as just a single bolus dose. Because propofol and midazolam are at times combined for prolonged periods of time, e.g., for intensive care unit sedation (10), we evaluated this interaction during prolonged infusion.

We hypothesized that prolonged infusion of propofol would affect the pharmacokinetics of midazolam and that hemodynamic factors might play a role in this pharmacokinetic interaction. Therefore, we studied the influence of a 7-hour infusion of propofol on the pharmacokinetics of midazolam and evaluated the influence of various hemodynamic variables.
**MATERIALS AND METHODS**

*Volunteers and Study Protocol*

After obtaining approval of the Medical Ethics Committee of the Leiden University Medical Centre and written informed consent, 8 healthy male volunteers, were studied. All volunteers were within 30% of ideal body weight, had no history of renal or hepatic disease standards and were not taking medication within 6 months before or during the investigation. All volunteers denied smoking or consumption of more than 20 g of alcohol per day. Before the investigation, a blood sample was taken for screening of renal or hepatic disease in accordance with Leiden University Medical Centre standards.

Volunteers were studied in a randomized cross-over manner during two sessions. During the first session volunteers received a midazolam bolus dose of 0.035 to 0.05 mg kg\(^{-1}\) in 1 min followed by an infusion of 0.035 to 0.05 mg kg\(^{-1}\) h\(^{-1}\) for 59 min (session A, control). During the second study session (session B) the volunteers received the same midazolam infusion scheme as during session A, but now in the presence of a TCI of propofol for 7 hours at a constant propofol target concentration (C\(_T\)) of 0.6 or 1.0 µg mL\(^{-1}\) using the Diprifusor\(^{®}\). The target controlled infusion of propofol was started 15 min before to the start of the midazolam administration to ensure a semi steady state concentration of propofol at the beginning of the midazolam infusion.

The 2 sessions were separated by a period of at least 2 weeks. Both the C\(_T\) (0.6 or 1.0 µg mL\(^{-1}\)) and the order of the 2 sessions were randomized, such that in half of the volunteers the control sessions preceded the other session and half of the volunteers received a C\(_T\) of 0.6 µg mL\(^{-1}\) and the other half 1.0 µg mL\(^{-1}\). Volunteers fasted from midnight on the night before the study until the last blood sample had been collected. During the administration of propofol, they breathed 30% oxygen in air. When indicated, ventilation was assisted using a face mask to maintain the end-tidal CO\(_2\) partial pressure at <50 mm Hg. After termination of session A and B, the subjects were monitored for another 4 h during which they could recover from residual sedation and then received a light meal before they were escorted to their homes.
Materials
The studies were performed in an operating room. An IV cannula was inserted into a large forearm vein for the infusion of propofol and midazolam and an arterial cannula was inserted into a radial artery for collection of hemodynamic data and blood samples. The electrocardiogram, respiratory rate, peripheral oxygen saturation, the bispectral index and intra-arterial blood pressure were monitored continuously throughout the study. Furthermore, the cardiac output was determined using the pulsecontour methodology on the basis of the intra-arterial blood pressure curve with the LiDCOplus monitor (LiDCOgroup plc, London). The LiDCO monitor was calibrated before each experiment. For this purpose, a lithium sensor was connected to the arterial cannula. After 0.2mmol lithium was injected IV, the LiDCO monitor was calibrated on the basis of the non-invasive online-determined arterial lithium concentration-time curve and the cardiac output calculated. The LiDCO has been found reliable for cardiac output monitoring when compared with traditional thermodilution cardiac output monitoring for up to 8 hours after calibration (LiDCO versus thermodilution; r = 0.86).(12) Blood samples were drawn from the arterial cannula, after calibration of the LiDCO.
Heart rate; cardiac output; cardiac index; systemic vascular resistance, the systolic, mean and diastolic arterial blood pressure were all recorded online and saved for further analysis. All volunteers received an infusion of saline of 2 ml.kg\(^{-1}\).h\(^{-1}\) during each session.

Blood Samples and Assays
During session A, a blank blood sample (10 mL) was obtained. This sample was used for calibration purposes. Additional arterial blood samples (5 mL) for the determination of the plasma midazolam concentration, were taken 1, 3, 5, 10, 20, 30, 45 and 60 min after the start of the midazolam infusion, and 1, 2, 3, 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min after termination of the midazolam infusion. Blood samples were taken into heparinised syringes for determination of the plasma midazolam concentration. These samples were centrifuged to obtain plasma which was subsequently stored at -20 °C until analysis. The concentration of midazolam in plasma was determined by reversed-phase high-performance liquid chromatography-UV detection at 216 nm (HPLC).(13) The intra- and interassay coefficients of variation of this method were 2.2% and 2.0 % respectively, for midazolam in plasma in the concentration range of 9.7-1120 ng.mL\(^{-1}\). Midazolam assays were conducted within 12 weeks.
During session B, in addition to the sample scheme in session A, an additional arterial blood sample (3 ml) was taken every 60 minutes for determination of the whole blood propofol concentration. These blood samples were stored at 4 °C. Propofol assays were carried out within 12 weeks. Propofol concentrations in blood were measured by HPLC-fluorescence at
276 nm. (14) The intra- and interassay coefficients of variation of this method were 4.3% and 3.7% respectively, for propofol in blood in the concentration range of 0.06-6.8 µg.mL⁻¹. The assays of midazolam and propofol did not interfere because the fluorescence wavelengths of midazolam (217 nm) and propofol (276 nm) do not overlap. This allows a distinct and accurate estimation of the 2 drug concentrations. Measured and predicted propofol concentrations were compared using the Wilcoxon signed rank test.

**Statistical Analysis**

A first exploratory analysis of hemodynamic differences between sessions 1 and 2 was performed using the Wilcoxon signed rank test (SPSS version 12.5 for windows; SPSS, Chicago, IL). A probability level <0.05 was considered significant. The aim of this analysis was to explore the significance of the hemodynamic changes by propofol and limit the number of hemodynamic variables to be tested as covariate in the population pharmacokinetic analysis by NONMEM (version VI 1.2).

Population pharmacokinetic parameters were estimated using the first-order conditional estimation method with η-ε interaction for a 3-compartment model (ADVAN11). A proportional error model was used with variance σ² of the intraindividual variability terms (ε). The interindividual variability of each model parameter was specified using a log-normal variance model:

\[ \Phi_i(t) = \Phi_{TV}(t)e^{\eta_i} \]

with

\[ \Phi_{TV}(t) = \Phi_i^0e^{\sum{\alpha_j (MD_{cov,j} - MD_{cov,j})}} \]

Where \( \Phi_i \) is the population value and \( \Phi_{TV} \) is the typical value with fixed effects taken into account of the pharmacokinetic parameter in individual \( i \) at time \( t \). \( \eta_i \) is the Bayesian estimate of the normally distributed random variable η (with mean zero and variance \( \omega^2 \)) in the individual \( i \) (which is estimated by NONMEM), typical (population) \( m \) is the number of covariates considered, \( \alpha_j \) is the value of a coefficient parameter describing the dependence of the pharmacokinetic parameter on covariate \( j \), and \( MD_{cov,j} \) is the median of the covariate \( j \) in the population. \( MD_{cov,j} \) is the median of 16 observations (8 volunteers times 2 sessions), except for the propofol concentration (only session B).

Coefficients of variation (CV%) were calculated as 100 times the square root of the variance \( \omega^2 \) of η and, parameter distributions being asymmetric, are only approximately the coefficients of variation as usually defined.
**Pharmacokinetic Data Analysis and Inclusion Procedure for Covariates**

A pharmacokinetic parameter set was determined on the basis of the plasma midazolam concentration-time data alone (without covariates) of the 16 sessions. Three compartment models were fitted to the data (number of components based on literature and experiment design) with parameters $V_1$, $V_2$, $V_3$ (central volume of distribution), $Cl_1$, $Cl_2$, $Cl_3$ (shallow peripheral volume of distribution), $Cl_4$, $Cl_5$, and $Cl_6$ (deep peripheral volume of distribution), and $Cl_7$ (elimination clearance), $Cl_8$ (rapid distribution clearance), and $Cl_9$ (slow distribution clearance).

To determine the influence of propofol on the 6 midazolam pharmacokinetic parameters, all 64 ($64 = 2^6$, 2 referring to the presence or absence of the covariate, 6 referring to the 6 possible pharmacokinetic parameters) possible combinations for the covariate propofol were evaluated. Propofol was treated as a time-independent covariate. The model with the lowest Akaike’s Criterium (AIC) value was considered best.

The hemodynamic parameters that differed significantly between sessions A and B were evaluated as potential covariates to further improve the predictability of the midazolam pharmacokinetic parameter set. The arrhythmic mean of these hemodynamic parameters of the time periods before a blood sample was taken for plasma midazolam concentration analysis were calculated. These data then were treated as time-dependent variables in the analysis. For each hemodynamic parameter, another 64 analysis runs were performed on the basis of the pharmacokinetic parameter set of midazolam with propofol as covariate included. Again, the combination with the lowest value for AIC was considered best.(15)

To assess the accuracy of the model, we calculated the weighted residual (WR) and the absolute weighted residual (AWR) for each sample.

$$WR_i = \frac{C_{meas,ij} - C_{pred,ij}}{C_{pred,ij}}$$

$$AWR_i = \frac{|C_{meas,ij} - C_{pred,ij}|}{C_{pred,ij}}$$

In which $C_{meas,ij}$ is the $j$th measured concentration of the $i$th individual, and the $C_{pred,ij}$ denotes the corresponding predicted value. The median values of the weighted residuals (MDWR) and the absolute weighted residuals (MDAWR) were used as overall measures of goodness of fit.

The likelihood profile method was used to assess statistical significance of the covariate coefficients. In this method, each coefficient is fixed to a range of values at which the -2 log likelihood (-2LL) is determined (by optimizing the remaining parameters). The 2 values of each coefficient that yield an increase of 3.84 in -2LL constitute the 95% confidence intervals. Finally, internal model selection validation was performed using the bootstrap. In this approach, 1000 bootstrap data sets were subjected to analysis with a set of models and the number of time each model was
selected was counted to assess replication stability.\(^{(16)}\) The set of models consisted of those with 1 covariate model added at a time in order of importance according to the objective function values. The final model parameter estimates were also used to obtain 95% confidence intervals (using the percentiles method).

**Computer Simulations**

The clinical consequences of the influence of propofol on midazolam pharmacokinetics were explored by computer simulation using the final midazolam pharmacokinetic parameter with propofol and heart rate as covariates in a 85-kg male.

Three computer simulations were performed. (1) A computer simulation exploring the influence of the blood propofol concentration of 0 or 1.5 µg.mL\(^{-1}\) (1.5 µg.mL\(^{-1}\) was the maximal blood propofol measured in this study) on the midazolam concentration-time profile in the presence of a stable heart rate of 63 beats/min. (2) A computer simulation to evaluate the effect of heart rate on the midazolam concentration-time relationship. For this purpose we explored the influence of a heart rate of 40 and 90 bpm on the midazolam concentration-time profile in the absence of propofol. (3) A computer simulation evaluating the influence of propofol on 50% (the context sensitive half-time) and the 80% decrement time of midazolam. For this purpose we used the final midazolam pharmacokinetic parameters in the presence of a blood propofol concentration of 0 or 1.5 µg.mL\(^{-1}\) with a stable heart rate of 63 bpm.
RESULTS
All volunteers completed the study without adverse events. Volunteers that received propofol in addition to midazolam were sedated for a longer period of time after the ending of the study. All volunteers stayed in the hospital for 4 h after the end of the study and then were fit to leave the hospital. The mean (± SD) age, weight and height of the volunteers were 25.5 ± 5.8 yr, 85 ± 8.2 kg and 188 ± 5 cm.

Blood propofol concentration analyses and plasma midazolam concentration analyses were performed within 12 weeks after the end of the study. Blood propofol concentrations were stable in each participant (fig. 1) and were similar as predicted (+2%, \( P = 0.378 \)) in those who received a \( C_T \) of 0.6 \( \mu \text{g.mL}^{-1} \) and significantly higher than predicted (+23%, \( P < 0.001 \)) in those who received a \( C_T \) of 1.0 \( \mu \text{g.mL}^{-1} \). None of the volunteers experienced significant respiratory depression and the end-tidal partial \( \text{CO}_2 \)-pressure never exceeded 50 mm Hg.

Figure 1. Blood propofol concentration-time curves of the individual subjects during session B. The continuous lines indicate subjects who received a target-controlled infusion of propofol with a target concentration of 0.6 \( \mu \text{g/mL} \); the dashed lines indicate those who received a target of 1.0 \( \mu \text{g/mL} \).
During the 16 study sessions 470 blood samples were collected for both midazolam and propofol concentration determination. The analysis of the pharmacokinetics of midazolam in this study is based on 368 measured plasma midazolam concentrations. In the presence of propofol mean arterial pressure, cardiac output and stroke volume were significantly lower and heart rate higher than when midazolam was given as sole drug (table 1). Because of a power failure, hemodynamic data were lost in 1 session. Consequently the pharmacokinetic parameter sets of midazolam without covariates (the naively pooled) and with propofol as covariate are based on the concentration time data of 16 sessions, whereas those with an additional hemodynamic parameter as covariate are bases on hemodynamic data of 15 sessions.

Table 1: Median (range) Hemodynamic parameters obtained during the 420 min study in session A (without propofol) and session B (in the presence of propofol)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Session A median (range)</th>
<th>Session B median (range)</th>
<th>Difference (%)</th>
<th>Significance P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>63.6 (42.5-79.2)</td>
<td>64.4 (49.1-84.0)</td>
<td>+1.2</td>
<td>0.003</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>72.8 (60.6-91.4)</td>
<td>70.3 (55.7-93.0)</td>
<td>-3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SVR (dynes.s⁻¹.cm⁻⁶)</td>
<td>664.6 (463.7-1934.6)</td>
<td>759.1 (524.5-1552.0)</td>
<td>+14.2</td>
<td>0.039</td>
</tr>
<tr>
<td>SV (mL/beat)</td>
<td>128.7 (49.8-174.4)</td>
<td>98.8 (69.9-170.8)</td>
<td>-23.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>7.78 (3.1-11.6)</td>
<td>6.39 (4.1-10.2)</td>
<td>-17.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 2 shows the measured plasma midazolam concentrations in the presence and absence of propofol when targeted at a target propofol concentration (Cₜ) of 0.6 and 1 µg.mL⁻¹, respectively. In the presence of a Cₜ: 0.6 µg.mL⁻¹ (mean measured blood propofol concentration of 0.62 µg/mL⁻¹) and Cₜ 1.0 µg.mL⁻¹(mean measured blood propofol concentration of 1.2 µg.mL⁻¹) the plasma midazolam concentrations were 5.0 ± 14.7% and 26.9 ± 9.4% higher than when midazolam was given as sole drug (P = 0.115 and <0.001, respectively).

The addition of propofol as covariate significantly improved the pharmacokinetic model of midazolam according to the AIC (Table 2). The pharmacokinetic parameters of midazolam influenced that were influenced by propofol were V₁, Cl₁ and Cl₂. With a blood propofol concentration increasing from 0 to 1.2 µg.mL⁻¹, V₁ of midazolam decreased from 5.37 to 2.98 L, Cl₁ decreased from 0.39 to 0.31 L.min⁻¹ and Cl₂ from 2.77 to 2.11 L.min⁻¹ (fig. 3). Various hemodynamic parameters, when included in the midazolam pharmacokinetic model, reduced the AIC and residual error (σ²) significantly. Of these hemodynamic covariates, heart rate
contributed most according to the AIC. Midazolam pharmacokinetic parameters influenced by heart rate were $V_3$, $Cl_1$ and $Cl_2$ (table 2, figure 4). Figure 5 shows the results of the optimization process and displays the measured versus the predicted midazolam concentrations for the model without any covariates (Fig 5A) and the population predicted (Fig. 5B) and the individual predicted (Fig. 5C) midazolam concentrations for the model with propofol and heart rate as covariates. In figure 6, the log likelihood profiles are shown. The plots contain lines that denote a 3.84 increase in -2LL from which the 95% confidence intervals for the parameters can be read.

The bootstrap model selection validation resulted in 0%, 10.2% 25.6%, and 64.1% selection frequencies for propofol as covariate on no parameters, $V_1$, $V_1$ and $Cl_1$, $V_1$, $Cl_1$ and $Cl_2$, and 0%, 13.2%, 36.6% and 50.1% with, in addition, heart rate on no parameters, $Cl_2$, $Cl_2$ and $V_3$ and $Cl_2$ $V_3$ and $Cl_1$. The 95% confidence intervals obtained from the bootstrap and likelihood profiles were similar to those that would be obtained by the normal approximations using values and SEs from table 2.

**Figure 2.** The mean (SE) plasma midazolam concentration-time curves in the volunteers in the presence (continuous line) or absence (dashed line) of a target controlled infusion of propofol of 0.6 $\mu$g.mL$^{-1}$ (A) or 1 $\mu$g.mL$^{-1}$ (B). The plasma midazolam concentration-time curves have been normalized to the same midazolam dosing scheme of 0.05 mg.kg$^{-1}$ in 1 minute followed by an infusion of 0.05 mg.kg$^{-1}$.hr$^{-1}$ for 59 minutes.
Values in parenthesis are -2LL and AIC for 15 volunteers. Parameters $V_1$ are the parameters of an individual with median covariate values. The median covariate values are 461.697 ng.mL$^{-1}$ for propofol and 63.421 beats/min for heart rate. For example $Cl_1 = 0.36 \times e^{(-0.000188(C_{prop} - 461.697) + (0.00895 (HR-63.241))}$.

$V_1$ = central volume of distribution, $V_2$ = rapidly equilibrating peripheral volume of distribution, $V_3$ = slowly equilibrating peripheral volume of distribution, $Cl_1$ = elimination clearance, $Cl_2$ = rapid distribution clearance, $Cl_3$ = slow distribution clearance; CV = coefficient of variations; SE = standard error of estimate; $\alpha$ = measure of covariate importance (when omitted the covariate is not significant); Prop = concentration of propofol; -2LL = -2 X log likelihood; AIC = -2 LL + 2P, where P is the number of nonfixed parameters; AIC = Akaike's Information-theoretic Criterion; MDWR = Median Weighted Residual; MDWAWR = Median Absolute Weighted Residual; $\sigma^2$ = relative residual error

(a) Significant covariate (95% confidence interval obtained from the likelihood profile does not contain 0)

### Table 2. Population Pharmacokinetic Parameters of Midazolam in the absence of any Covariates (First 3 columns), in the presence of Propofol as covariate (second 3 columns) and with Propofol and Heart Rate as covariates (last 3 columns)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No covariates</th>
<th>Propofol as covariate</th>
<th>Propofol and Heart Rate as covariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value SE CV%</td>
<td>Value SE CV%</td>
<td>Value SE CV%</td>
</tr>
<tr>
<td>$V_1$</td>
<td>4.03 0.35 34.5</td>
<td>4.03 0.26 25.3</td>
<td>4.28 0.30 22.9</td>
</tr>
<tr>
<td>$V_2$</td>
<td>26.9 1.12 15.7</td>
<td>26.9 1.14 15.8</td>
<td>26.2 1.13 15.4</td>
</tr>
<tr>
<td>$V_3$</td>
<td>54.2 3.65 23.2</td>
<td>54.1 3.61 22.4</td>
<td>48.6 2.85 19.0</td>
</tr>
<tr>
<td>$Cl_1$</td>
<td>0.36 0.02 17.5</td>
<td>0.36 0.01 14.0</td>
<td>0.36 0.01 12.6</td>
</tr>
<tr>
<td>$Cl_2$</td>
<td>2.90 0.18 26.4</td>
<td>2.90 0.19 23.4</td>
<td>2.49 0.16 20.1</td>
</tr>
<tr>
<td>$Cl_3$</td>
<td>0.38 0.02 14.0</td>
<td>0.38 0.02 14.2</td>
<td>0.36 0.02 11.5</td>
</tr>
<tr>
<td>Covariates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha_{propofol, V_1}$</td>
<td>-4.53 X 10$^{-4}$</td>
<td>1.27 X 10$^{-4}$</td>
<td>-4.90 X 10$^{-4}$</td>
</tr>
<tr>
<td>$\alpha_{propofol, V_2}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha_{propofol, V_3}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha_{propofol, Cl_1}$</td>
<td>-2.05 X 10$^{-4}$</td>
<td>7.69 X 10$^{-5}$</td>
<td>-1.88 X 10$^{-4}$</td>
</tr>
<tr>
<td>$\alpha_{propofol, Cl_2}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha_{propofol, Cl_3}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha_{HR, V_1}$</td>
<td></td>
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<tr>
<td>$\alpha_{HR, V_2}$</td>
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<td></td>
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</tr>
<tr>
<td>$\alpha_{HR, V_3}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha_{HR, Cl_1}$</td>
<td>8.95 X 10$^{-3}$</td>
<td>6.08 X 10$^{-3}$</td>
<td>3.10 X 10$^{-3}$</td>
</tr>
<tr>
<td>$\alpha_{HR, Cl_2}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha_{HR, Cl_3}$</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
| Performance measures | -2LL | AIC | MDWR (%) | MDAWR (%) | $\sigma^2$
|                  | 1622.991 | 1648.991 | 0.62 | 10.82 | 0.00515 |
|                  | 1604.449 (1495.260) | 1636.449 (1527.260) | 1.55 | 10.46 | 0.00516 |
|                  | 1473.541 | 1511.541 | 0.98 | 9.87 | 0.0048 |

Values in parenthesis are -2LL and AIC for 15 volunteers. Parameters $V_1$-$Cl_3$ are the parameters of an individual with median covariate values. The median covariate values are 461.697 ng.mL$^{-1}$ for propofol and 63.421 beats/min for heart rate. For example $Cl_1 = 0.36 \times e^{(-0.000188(C_{prop} - 461.697) + (0.00895 (HR-63.241))}$.

HR = Heart Rate, $V_1$ = central volume of distribution, $V_2$ = rapidly equilibrating peripheral volume of distribution, $V_3$ = slowly equilibrating peripheral volume of distribution, $Cl_1$ = elimination clearance, $Cl_2$ = rapid distribution clearance, $Cl_3$ = slow distribution clearance; CV = coefficient of variations; SE = standard error of estimate; $\alpha$ = measure of covariate importance (when omitted the covariate is not significant); Prop = concentration of propofol; -2LL = -2 X log likelihood; AIC = -2 LL + 2P, where P is the number of nonfixed parameters; AIC = Akaike's Information-theoretic Criterion; MDWR = Median Weighted Residual; MDWAWR = Median Absolute Weighted Residual; $\sigma^2$ = relative residual error

(a) Significant covariate (95% confidence interval obtained from the likelihood profile does not contain 0)
Figure 3. Individual estimates of the initial volume of distribution ($V_1$), elimination clearance ($Cl_1$), and rapid distribution clearance ($Cl_2$) of midazolam obtained from the model without covariates as function of the blood propofol concentration. The regression line results from the NONMEM analysis.
Figure 4. Individual estimates of the slowly equilibrating volume of distribution ($V_3$), elimination clearance ($Cl_1$), and the rapid distribution clearance ($Cl_2$) of midazolam obtained from the model without covariates as function of the heart rate. The regression line results from the NONMEM analysis.
Figure 5. The measured versus the predicted midazolam concentrations for the pharmacokinetic model without covariates (A) and the population predicted (B) and the individual predicted (C) midazolam concentrations for the model with propofol and heart rate as covariates. The straight line indicates $x=y$. 
**Computer Simulations**

The 3 computer simulations using the final pharmacokinetic parameter set offer a clear view of the consequences of the propofol midazolam interaction on the midazolam dose-concentration relationship. (Table 3) In the presence of a blood propofol concentration of 1.5 µg.mL\(^{-1}\), midazolam concentrations are increased (Fig.7). The simulations revealed that in the presence of propofol the bolus dose of midazolam should be reduced by 25% for short term midazolam dosing schemes to obtain a similar midazolam plasma concentration-time profile as in the absence of propofol. When midazolam is given for an infusion of several hours the simulation suggest that an additional reduction of 15% in the midazolam infusion rate is required to obtain equal midazolam concentrations in the presence of and absence of propofol.

In figure 8 the influence of heart rate on midazolam pharmacokinetics is explored. The computer simulations show that varying the heart rate from 45 to 90 bpm the predicted midazolam concentration changes to a limited degree. Heart rate affects predominantly the initial distribution of midazolam. The influence of propofol on the pharmacokinetics of midazolam furthermore becomes evident in Figure 9. The concomitant administration of propofol at a blood concentration of 1.5 µg.mL\(^{-1}\) (the maximal measured blood propofol concentration in this study) leads to a slight increase in the CSHT and a significant lengthening of the 80% decrement time of midazolam.

<table>
<thead>
<tr>
<th>Table 3. Pharmacokinetic parameters of Midazolam (based on the final Pharmacokinetic Parameter Set with Propofol and Heart Rate as covariates) for various Propofol and Heart Rate Values as used in the Computer Simulations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Propofol (µg/mL)</strong></td>
</tr>
<tr>
<td><strong>Heart Rate (min(^{-1}))</strong></td>
</tr>
<tr>
<td>(V_1)</td>
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Figure 6. Likelihood profiles for the 6 covariate coefficients of the final model. The plots contain lines that denote a 3.84 increase in -2 log likelihood (-2LL) from which the 95% confidence intervals for the parameters can be read. The values for the x-axes have been multiplied with a value of 1000 for clarity.
Figure 7. The concentration-time profile of a simulated midazolam infusion scheme (0.05 mg.kg\(^{-1}\) in 1 minute followed by a continuous infusion of 0.05 mg.kg\(^{-1}\).hr\(^{-1}\) for 59 minutes) in the presence of a blood concentration of 0 and 1.5 µg.mL\(^{-1}\), with a steady heart rate of 63 bpm.

Figure 8. The concentration-time profile of a simulated midazolam scheme (0.05 mg.kg\(^{-1}\) in 1 minute followed by a continuous infusion of 0.05 mg.kg\(^{-1}\).hr\(^{-1}\) for 59 minutes) in the presence of a heart rate of 40 and 90 bpm, with a constant blood propofol concentration of 1.2 µg.mL\(^{-1}\). Simulation for the time span of 120 minutes (A) and for the initial 12-minute time period (B).
**DISCUSSION**

We studied the influence of propofol on the pharmacokinetics of midazolam. We hypothesized that propofol would alter the pharmacokinetics of midazolam. The results of this study confirmed our hypothesis. The most important finding of this study is that propofol ($C_{\text{blood}}: 1.2 \ \mu\text{g.mL}^{-1}$) increased midazolam concentrations by 26.9%. In the presence of propofol midazolam is administered in a smaller central compartment from which midazolam is cleared and distributed less rapidly to peripheral tissues.

Next to the primary findings of this study, we identified heart rate as the hemodynamic parameter that further improved the pharmacokinetic dataset of midazolam. Although heart rate improved the pharmacokinetic model of midazolam (as based on the AIC), computer simulations revealed this effect to be of limited clinical importance.

**Interaction Mechanisms**

The pharmacokinetic parameter set of midazolam without covariates described in this study corresponds well with midazolam pharmacokinetic parameter sets in the literature.(17-20) Our pharmacokinetic parameter set corresponds most closely with that by Buhrer et al.(18) probably because of similarities in the study design and the population studied, with comparable midazolam dose regimen.

Midazolam, with its metabolism mainly through cytochrome 3A3, 3A4 and 3A5, (21-23) is subject to numerous pharmacokinetic interactions on the basis of enzyme inhibition or induction in the liver and possibly the kidneys (24-26) The concentration shifts caused by these CYP 450 interactions that affect the clearance of midazolam are huge (up to 1000%), but in practice these interactions occur infrequently. (27) The interactions between anaesthetic drugs, however, occur more frequently, even daily, but induce concentration shifts that are less significant. (8,26)

In general, interactions between anaesthetic agents lead to an increase in the concentrations of the drugs combined. For example, alfentanil has been shown to increase blood propofol concentrations through a decrease in the elimination clearance and distribution clearance of propofol.(28) Propofol has been shown to increase alfentanil concentrations by decreasing the elimination clearance, rapid and slow distribution clearances of alfentanil.(29) Co-administration of propofol increased remifentanil concentrations through both a decrease in the central volume of distribution and distributional clearance of remifentanil by 41% and elimination clearance by 15%.(30)

The results of our study follow the above described patterns such that in the presence of propofol ($C_{\text{blood}}: 1.2 \ \mu\text{g.mL}^{-1}$), plasma midazolam concentrations were increased (26.9%). Both hemodynamic and enzymatic factors may be responsible for this interaction.

In contrast, to propofol that is known for its high hepatic extraction ratio (>0.9),(31) midazolam is a drug with an intermediate extraction ratio of 0.55(23). Therefore, the
clearance of midazolam may be affected by changes in hepatic blood flow, free fraction, and intrinsic hepatic enzyme activity. Propofol is generally known for its hemodynamic depressant effects and may reduce hepatic blood flow (32). In addition, in our study, the mean arterial pressure, stroke volume, and cardiac output were reduced in the presence of propofol (Table 1). This suggests that, at least to some extent, the reduction in clearance described in this study (Cl\textsubscript{1} from 0.40 to 0.31 L.min\textsuperscript{-1} -20%) may be caused by a propofol induced reduction in hepatic blood flow.

In addition, propofol is known as a CYP450 3A4 inhibitor (33). In contrast to enzyme induction that may take several weeks to develop, competitive inhibition of CYP activity may occur almost instantaneously because of the competition of two drugs (e.g. propofol and midazolam) for the enzyme’s active site. A short-term exposure to propofol at blood concentrations of 3 µg.mL\textsuperscript{-1} reduced the CYP 3A4 activity by approximately 37% (8). Therefore we conclude that the propofol induced reduction in the metabolic clearance of midazolam likely is the result of both the hemodynamic depressant and enzymatic inhibitory effects of propofol.

In addition to the propofol related reduction in midazolam clearance, hemodynamic alterations induced by propofol also influence the distribution pharmacokinetics of midazolam. Next to the influence of heart rate on the initial distribution, the hemodynamic-depressant effects of propofol are also responsible for the reduced transfer of midazolam to the peripheral tissues by reduction in Cl\textsubscript{2} by 44.5% from 2.77 L.min\textsuperscript{-1} to 2.11 L.min\textsuperscript{-1} in the controls. From table 1, the difference in heart rate between sessions A and B seems obscure and only significantly different between sessions because of the power of paired testing. Nevertheless, the addition of heart rate significantly reduced AIC (Δ-AIC, Table 2) the residual error (σ\textsuperscript{2}, Table 2) and the interindividual variability (CV%, Table 2).

Observation of the raw heart data and the residual errors in each individual finally taught us that this apparent discrepancy is explained by the fact that heart rate does not so much reduce the interindividual variability or the variability between sessions A and B but minimizes variability within each individual.

Finally model selection stability as assessed by the bootstrap showed that the replication stability was robust; in other words, the final models presented have a higher probability of being selected than simpler ones. The 95% confidence intervals as derived from the log likelihood profiles (Fig. 6) for the covariate effect on propofol on Cl\textsubscript{2} and heart rate on Cl\textsubscript{1}, included 0. Although these covariate effects did not attain statistical significance, inclusion of those effects may be still of importance for prediction because they were selected by AIC. This is in agreement with the arguments for predictor selection as described by Steyerberg (34).

In conclusion when midazolam and propofol are combined, (3-5,35) propofol increases the midazolam concentrations by a reduction in the central volume of distribution and the
metabolic and rapid distribution clearances of midazolam in a concentration dependent manner. Inclusion heart rate significantly improved the predictive performance of the midazolam pharmacokinetic model affecting the initial distribution of midazolam and reducing the intraindividual variability. The propofol-midazolam pharmacokinetic interaction allows for a 25% reduction of the midazolam bolus dose during short-term combined administration (2-3 hours). Although the influence of midazolam on propofol pharmacokinetics is predominantly by hemodynamic alterations (1), the results of this study suggest that propofol affects midazolam pharmacokinetics both through enzyme inhibition and hemodynamic alterations.

*Figure 9.* Context sensitive half-time (CSHT = 50 decrement time) and 80% decrement time of midazolam in the absence (continuous line) and in the presence of a blood propofol concentration of 1.5 µg.mL⁻¹ (dashed lines), using the final midazolam pharmacokinetic data set in the presence of a blood propofol concentration of 0 or 1.5 µg.mL⁻¹ with a stable heart rate of 63 bpm.
References


34. Steyerberg, E. Clinical Prediction Models. A practical approach to development, validation and updating. (Statistics for Biology and Health). Ref Type: Generic