Changes in the effect of propofol in response to altered plasma protein binding during normothermic cardiopulmonary bypass

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**Background.** During normothermic cardiopulmonary bypass (CPB), the effect on propofol pharmacokinetics of changes in its binding to plasma proteins is consistent with the predictions of the well-stirred model of hepatic elimination for nonrestrictively cleared drug. However, whether changes in binding lead to clinically significant changes in the drug effect remains unclear. The purpose of this study was to assess changes in the drug effect of propofol in response to altered plasma binding using quantitative EEG measurements.

**Methods.** Thirty patients undergoing cardiac surgery were assigned randomly to receive propofol infusions at 4 (Group P-4) or 6 (Group P-6) mg kg⁻¹ h⁻¹ during surgery. The concentration of propofol in blood samples, collected from the radial artery at predetermined intervals, was determined by HPLC. The unbound fraction of drug in plasma was estimated using equilibrium dialysis. Bispectral index (BIS) and burst suppression ratio (BSR) were measured at the time blood samples were collected.

**Results.** The total concentration of propofol in blood was unchanged during CPB relative to the pre-CPB value in both groups. However, the fraction of unbound propofol in blood increased by 2-fold during CPB. While BIS values were unchanged during CPB in Group P-4, there was a slight, but significant, decrease in Group P-6. In both groups, BSR significantly increased during CPB. BIS values showed a weak correlation with the concentration of unbound propofol ($r^2=0.19$, $P<0.001$). BSR showed a moderate correlation with the concentration of unbound propofol ($r^2=0.56$, $P<0.001$).

**Conclusions.** The anaesthetic effect of propofol significantly increased during CPB without any alteration in the total drug concentration. The enhanced efficacy may be caused by a reduction in plasma binding of the drug.

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**Keywords:** anaesthetics i.v., propofol, unbound fraction; monitoring, bispectral index; pharmacokinetics

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Changes in plasma protein binding frequently have little clinical relevance. However, as reported recently, it is likely to be clinically important for a limited number of highly cleared drugs that bind extensively to protein, and have a narrow therapeutically index.

Propofol is one such drug that is extensively bound to plasma proteins, with an unbound fraction of $<4\%$. Furthermore, propofol is highly extracted in the liver with a hepatic extraction ratio of $>0.8$ and a total body clearance of $\sim30$ ml kg⁻¹ min⁻¹. In addition, we have also demonstrated significant extrahepatic elimination of propofol by the kidney, with a renal extraction ratio of $\sim0.6$. Therefore, changes in plasma binding of propofol may lead to clinically significant changes in the drug effect. Intriguingly, in our previous study we observed a marked elevation in the unbound concentration of propofol in response to reduced plasma binding without any alteration in the total drug concentration during normothermic cardiopulmonary bypass (CPB) in patients receiving constant propofol infusion. This finding is consistent with predictions based on the well-stirred model of hepatic elimination for an i.v. infused high clearance drug.
of unbound propofol during CPB would be expected to increase the level of anaesthesia. However, whether this actually occurred remained unclear in our previous study. Thus, the present study was designed to investigate whether changes in plasma binding cause clinical effect for extensively bound and highly cleared drugs that are administered i.v.

The bispectral index (BIS) is a complex EEG parameter that integrates several disparate descriptors of the EEG into a single variable. The burst suppression ratio (BSR) is one of the subparameters incorporated in the BIS value, quantifying the degree of burst suppression. These values have been used clinically to quantify the pharmacodynamic action of anaesthetic drugs, including propofol.

However, there is a paucity of data concerning the pharmacodynamics of propofol during normothermic CPB. Recently, Yoshitani and colleagues reported an increase in BSR without a corresponding increase in the plasma concentration of propofol during normothermic CPB in patients receiving constant infusion of the drug. The enhanced effect of propofol might be caused by changes in plasma protein binding. By contrast, Hirschi and colleagues have reported that the BIS value remains unchanged with a continuous infusion of propofol during CPB compared with the pre-CPB level. These results do not appear to reflect the elevation in the concentration of unbound propofol during CPB.

In the present study, we have investigated propofol pharmacokinetics and dynamics during normothermic CPB to determine whether changes in plasma protein binding lead to clinically significant changes in drug effect as reflected in quantitative EEG measurements.

Methods

Patients, anaesthetic and CPB management

The study was approved by the Committee on Medical Ethics at the Saitama Cardio-Respiratory Centre. Thirty patients participated in this study after giving written informed consent. All subjects were selected according to the criteria of the New York Heart Association (NYHA) functional class I–II; each had a left ventricular ejection fraction (EF) of 40% or more and was scheduled for cardiac surgery with CPB (Table 1). Routine clinical laboratory tests indicated normal renal and hepatic function in all patients. Patients were randomly assigned to receive propofol at 4 mg kg\(^{-1}\) h\(^{-1}\) (Group P-4) or 6 mg kg\(^{-1}\) h\(^{-1}\) (Group P-6).

Anaesthesia was induced with propofol (0.5–1 mg kg\(^{-1}\)), fentanyl (4 μg kg\(^{-1}\)) and vecuronium (0.15 mg kg\(^{-1}\)) after the placement of peripheral i.v. and radial arterial cannulae. Anaesthesia was maintained with propofol (4 or 6 mg kg\(^{-1}\) h\(^{-1}\)), fentanyl (4 μg kg\(^{-1}\) h\(^{-1}\)) and vecuronium (0.1 mg kg\(^{-1}\) h\(^{-1}\)). Additionally, fentanyl (6 μg kg\(^{-1}\)) was given before skin incision. The infusion rate of each drug was kept constant until the end of the study. Anaesthesia was maintained by propofol and fentanyl as required clinically until the end of surgery. All patients were monitored with a pulmonary artery catheter (Vigilance, Swan-Ganz CCO Thermodilution Catheter, Baxter Co., USA).

After systemic heparinization (300 unit kg\(^{-1}\)), non-pulsatile normothermic CPB was started at a flow rate of 2.4 litre min\(^{-1}\) m\(^{-2}\). The priming volume of the system consisted of 1.6 litre of electrolyte solution, 50 ml of 7% sodium bicarbonate, with 20 g of mannitol. During CPB the following values were maintained: mean arterial pressure of 50–90 mm Hg, activated coagulation time >480 s and a haematocrit >20%. Nasopharyngeal temperature was maintained >35°C.

Blood sampling

Blood samples for the measurement of propofol concentration were collected into polyethylene tubes with ethylene-diaminetetraacetic acid from the radial artery at the times subsequently described. An aliquot of each sample was centrifuged for 10 min at 3000 g to separate plasma and then used immediately for measurement of protein binding. The remaining part of the sample was stored at 4°C until analysis. Haematocrit values and plasma albumin concentration were also determined for these samples. The time points (T1–T4) at which various procedures were performed during surgery were as follows: T1, just before skin incision, (range, 45–65 min after the infusion of propofol); T2, before administration of heparin; T3 and T4, corresponding to 30 and 60 min after the start of CPB.

Measurement of plasma unbound fraction by equilibrium dialysis

The extent of the unbound fraction of propofol to plasma protein was estimated immediately after collection using equilibrium dialysis as described previously. Briefly, samples (1 ml each) were dialysed against a buffer containing sodium phosphate 0.067 M and sodium chloride 0.05 M (pH 7.4, 1 ml) for 10 h at 37°C using a dialysis membrane with a molecular weight cut-off of 6000 Da (VB-8; Sanplatec, Osaka, Japan). Propofol concentrations were measured by high performance liquid chromatography (HPLC) in both the dialysate and the plasma after dialysis as described below. In the preliminary experiments, propofol recovery after dialysis (sum of concentration in dialysate

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Table 1: Characteristics of the patients. Data are mean (range) or mean (SD).

<table>
<thead>
<tr>
<th></th>
<th>Group P-4</th>
<th>Group P-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion rate (mg kg(^{-1}) h(^{-1}))</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>65 (43–77)</td>
<td>61 (48–76)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59 (7.2)</td>
<td>58 (6.9)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157 (8.6)</td>
<td>156 (9.1)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>11/4</td>
<td>10/5</td>
</tr>
<tr>
<td>NYHA (I/II)</td>
<td>4/11</td>
<td>3/12</td>
</tr>
</tbody>
</table>

There were no significant differences between Groups P-4 and P-6.
and plasma after dialysis) was >80%, and the reproducibility of measuring the plasma unbound fraction of propofol was confirmed with a coefficient of variation of <9.4%. The unbound fraction of propofol in plasma \( f_{U,P} \) was calculated according to the following equation:

\[
f_{U,P} = \frac{\text{concentration in dialysate}}{\text{concentration in plasma after dialysis}} \times 100. \tag{1}
\]

**Measurement of propofol by HPLC**

The propofol concentration was measured using HPLC for plasma (100 μl), lysed whole blood (500 μl), whole blood plus 400 μl distilled water, dialysate (500 μl) or plasma after dialysis (100 μl) as described previously. Each sample with internal standard (thymol in methanol) was buffered with 1 ml of 0.1 M phosphate buffer (pH 7.4) and extracted on a rotary mixer for 15 min with 5 ml of n-hexane. After centrifugation at 1600 g for 10 min, 30 μl of tetra-n-butyl-ammonium hydroxide solution was added to 4 ml of the organic phase and the solution was evaporated to dryness. The residue was resolved in methanol and an aliquot was injected into a HPLC (Waters 2690, Waters, USA). Propofol and thymol were detected with a fluorescence detector (Waters 474, Waters, USA, excitation: 276 nm, emission: 192 nm). The reproducibility of the measurement was confirmed with a coefficient of variation of <6% and the limit of quantification was 4 ng ml\(^{-1}\) using 500 μl dialysate.

**Calculation of the pharmacokinetic parameters of propofol**

Blood unbound fraction \( f_{U,B} \), blood unbound concentration \( C_U \), erythrocyte concentration \( C_{RBC} \) and apparent erythrocyte/blood partition coefficient \( K_{RBC/Blood} \) were calculated by the following equations:

\[
f_{U,B} = f_{U,P} \times \frac{C_P}{C_B}, \tag{2}
\]

\[
C_U = C_B \times f_{U,B}. \tag{3}
\]

\[
C_{RBC} = C_P + (C_B - C_P) \times \frac{100}{\text{Haematocrit}}, \tag{4}
\]

\[
K_{RBC/Blood} = \frac{C_{RBC}}{C_B}. \tag{5}
\]

where \( C_P \) and \( C_B \) represent the plasma and blood concentration, respectively. The tissue/blood partition coefficient determines the effective volume of the different tissues and the overall volume of distribution.

**Measurement of BIS and BSR**

Brain electrical activity was measured using a two channel bipolar frontal montage (A-2000 EEG monitor, Aspect Medical Systems, Newton, USA) which displayed BIS and BSR. The BIS value decreased continuously with decreasing level of consciousness (hypnosis). BSR quantifies the percentage of suppression during the burst suppression pattern. Burst suppression develops during deep anaesthesia. To calculate BSR, suppression is recognized as those periods >0.5 s during which EEG voltage amplitude is <5 mV. Time in suppressed state is measured and BSR is expressed as the function of the epoch where the EEG is suppressed. After alcohol cleaning, disposable sensor electrodes (BIS sensor plus, Aspect Medical Systems, Newton, USA) were applied on the forehead of patients according to the manufacturer’s recommendations. The raw EEG signals were band-pass filtered to 2–70 Hz and processed in real time using version 1.18 of the BIS algorithm. BSR was also calculated online. With the help of the serial port, the quantitative EEG variables were digitally recorded every 5 s for the duration of the study. Data were stored on a personal computer as text file and analysed off-line. BIS values showing sudden high values in electrocautery and electromyogram were identified as artifacts and eliminated in the off-line analysis. The values of EEG parameters at each time point were calculated by averaging the values during 240–300 s of artifact-free recording immediately before and after the selected time point.

**Statistical analysis**

The data are expressed as mean±SD. Statistical analysis was performed using a statistical software package (StatView-J 5.0 for Macintosh; SAS Institute, Cary, NC, USA). Analysis of variance for repeated measurements was used to detect significant changes. When significance was found, the Scheffe test was used as a post hoc comparison procedure to compare all data during CPB vs data collected before CPB. The differences in propofol concentration between groups were analysed by an unpaired t-test. Values of \( P<0.05 \) were considered to be significant. Linear regression analyses were used for correlation of variables (total and unbound propofol concentration vs the EEG variables).

**Results**

The total concentration of propofol in the blood was unchanged over time during the study period in both groups. The concentration in Group P-6 was ~1.5-fold higher than in Group P-4 in proportion to dosage \( (P<0.01) \). The unbound propofol concentration increased 2-fold during CPB in both groups (Fig. 1). Correspondingly, the unbound fraction of propofol in blood increased 2-fold during CPB in both groups. There were no significant differences in the unbound fractions between Groups P-6 and P-4 (Fig. 2). In both groups, the ratio of the propofol concentration in erythrocytes to that in blood \( (K_{RBC/Blood}) \) increased 1.6-fold during CPB (Fig. 3).

BIS values were similar at pre-CPB in both groups. In Group P-4, the values were unchanged during the study period. By contrast, in Group P-6 the values decreased slightly, but significantly, during CPB (Fig. 4). Burst suppression was not detected during the pre-CPB period in
either group. During CPB, however, the BSR increased significantly in Group P-6, whereas a gradual increase in BSR was found in Group P-4 (Fig. 4). BIS values showed a weak correlation with the concentration of total and unbound propofol, respectively ($r^2$: 0.15 vs 0.19, Fig. 5). BSR showed a stronger correlation with the concentration of unbound propofol than total drug ($r^2$: 0.56 vs 0.27, Fig. 6).

Plasma albumin concentration and hematocrit values decreased significantly during CPB. Cardiac index and nasopharyngeal temperature was constant throughout the study period. There were no significant differences in these physiological parameters between the two groups (Table 2).

Discussion

In the present study we observed a 2-fold increase in the concentration of unbound propofol, without any alteration in the total concentration of drug, during CPB in either group.
Effect of propofol to altered plasma protein binding

![Graph](image)

**Fig 5.** (A) The relationship between total propofol concentration in blood during constant-rate infusion of 4 (circles) and 6 (squares) mg kg\(^{-1}\) h\(^{-1}\) propofol and the BIS values with a regression slope \((r^2=0.15, P<0.001)\). (B) The relationship between unbound propofol concentration in blood and the BIS values with a regression slope \((r^2=0.19, P<0.001)\).

![Graph](image)

**Fig 6.** (A) The relationship between total propofol concentration in blood during constant-rate infusion of 4 (circles) and 6 (squares) mg kg\(^{-1}\) h\(^{-1}\) propofol and the BSR with a regression slope \((r^2=0.27, P<0.001)\). (B) The relationship between unbound propofol concentration in blood and the BSR with a regression slope \((r^2=0.56, P<0.001)\).

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**Table 2** Clinical data expressed as mean (sd). There were no significant differences between Groups P-4 and P-6. \(*P<0.01, \**P<0.001\), a significant difference compared with the value at T1.

<table>
<thead>
<tr>
<th>Group</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac index</td>
<td>P-4</td>
<td>2.3 (0.6)</td>
<td>2.5 (0.7)</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>P-6</td>
<td>2.5 (0.5)</td>
<td>2.6 (0.4)</td>
<td>2.4</td>
</tr>
<tr>
<td>Plasma albumin</td>
<td>P-4</td>
<td>3.5 (0.3)</td>
<td>3.4 (0.2)</td>
<td>2.4 (0.5)**</td>
</tr>
<tr>
<td></td>
<td>P-6</td>
<td>3.6 (0.2)</td>
<td>3.5 (0.3)</td>
<td>2.5 (0.5)**</td>
</tr>
<tr>
<td>Haemtocrit</td>
<td>P-4</td>
<td>34 (4)</td>
<td>33 (4)</td>
<td>25 (4)**</td>
</tr>
<tr>
<td></td>
<td>P-6</td>
<td>32 (6)</td>
<td>32 (5)</td>
<td>24 (4)**</td>
</tr>
<tr>
<td>Nasopharyngeal temperature (°C)</td>
<td>P-4</td>
<td>36.3 (0.6)</td>
<td>35.5 (0.8)</td>
<td>35.6 (1.0)</td>
</tr>
<tr>
<td></td>
<td>P-6</td>
<td>36.1 (0.7)</td>
<td>35.4 (0.6)</td>
<td>35.6 (0.8)</td>
</tr>
</tbody>
</table>

(Figs 1 and 2). According to the well-stirred model of hepatic elimination,\(^{17}\) the blood clearance and steady-state concentration of total drug is independent of plasma binding when it is administered i.v. and has a high extraction ratio. However, an increase in the level of unbound fraction results in an increase in the concentration of unbound drug in the blood. Because propofol is highly bound to plasma protein and efficiently extracted in the liver and kidneys,\(^{5,6,9}\) the observed changes in both total and unbound drug concentrations are consistent with these predictions.

An initial reduction in total drug concentration as a result of haemodilution and an increase in the volume of distribution would have occurred at the beginning of the CPB procedure as shown in our previous study.\(^5\) However, this effect is only transient and it returned to the pre-CPB level within 30 min because of the rapid distribution half-life and the large distribution clearance, and then remained constant throughout the rest of the study period. A possible confounding factor, namely, changes in hepatic blood flow is considered to be unimportant because cardiac output and nasopharyngeal temperature was kept constant by the CPB procedure. Also, the hepatic blood flow, as assessed by indocyanine green method, did not change during cardiac surgery when performed under the same anaesthetic condition and normothermic CPB management as used in this study.\(^{18}\) Propofol binds mostly to albumin and to erythrocyte membrane.\(^7\) The increase in the unbound fraction was probably caused by a lower concentration of albumin and haematocrit as a result of the sudden haemodilution (Table 2).

Generally, a drug’s pharmacological effects are a reflection of its unbound concentration in the circulation, as only drug which is not bound to plasma protein is able to pass through various membranes and reach target sites within tissues. Engdahl and colleagues\(^{19}\) have shown that equilibration of propofol across the blood–brain barrier is likely limited by plasma protein binding. The increased concentration of unbound propofol in the blood would be expected to lead to a similar increase in the concentration at its target site in the brain, thereby enhancing the level of anaesthesia. Indeed, the increased distribution of propofol into erythrocytes was matched by an increase in the amount of unbound fraction (Fig. 3). In fact, a significant increase in BSR was observed during CPB for both groups (Fig. 4). These
observations are similar to those reported by Yoshitani and colleagues.\textsuperscript{15} The enhanced efficacy of propofol is presumably caused by an increase in the concentration of unbound drug. BSR showed a moderate correlation with the concentration of unbound propofol ($r^2=0.56$, Fig. 6). Propofol induced a biphasic response on EEG.\textsuperscript{11} At low concentrations of propofol, both frequency and amplitude increased, whereas at higher concentrations, the EEG slowed and the amplitude decreased. A high concentration produced burst suppression. Doyle and colleagues\textsuperscript{20} reported that a mean infusion rate of 13.6 mg kg$^{-1}$ h$^{-1}$ (range 8.5–28.6 mg kg$^{-1}$ h$^{-1}$) of propofol was needed to suppress the EEG. VanHemelrijk and colleagues\textsuperscript{21} found that blood propofol concentrations of 6.3±1.4 μg ml$^{-1}$ were needed to cause burst suppression. In the present study, the infusion rate and observed total drug concentration in the blood required to produce burst suppression was about half that reported in these studies. However, the concentration of propofol at its target site in the brain would be similar because of an increase in the unbound fraction. Furthermore, because propofol has a very short pharmacokinetic–pharmacodynamic equilibration half-time (i.e. drug effects appear to be directly related to unbound drug concentration),\textsuperscript{11,22} an enhanced pharmacodynamic response could have occurred during the brief time in which the unbound blood concentration was elevated.

Similarly, the BIS values also decreased slightly but significantly during CPB in Group P-6. In contrast, the values remained unchanged during CPB in Group P-4 (Fig. 4). The observed changes in the BIS values in Group P-4 are similar to those reported by Hirschi and colleagues.\textsuperscript{16} A BIS value of between 40 and 50, observed for Group P-4, is generally acknowledged as being clinically acceptable during surgery. However, an increasing unbound propofol concentration in Group P-4 was not adequately reflected by the BIS value. In this range, the BIS algorithm results in a broad plateau underestimating any changes in plasma or effect site concentration of an anaesthetic.\textsuperscript{23–25} Also, Bruhn and colleagues\textsuperscript{26} reported that BIS values do not always correlate with the depth of anaesthesia from the beginning of burst suppression of the EEG up to 40% suppression ratio. Thus, BIS values would be expected to show a weaker correlation with the unbound propofol concentration than BSR (BIS; $r^2=0.19$ vs BSR; $r^2=0.56$). BIS would be suited to measurement in the range of sedation to hypnosis, not the deeper levels of anaesthesia.

The increased BSR at BIS values of ~40 would be too deep a state of anaesthesia. In principle, this should be compensated by a reduction in drug dosage. Schmidlin and colleagues\textsuperscript{27} found that the median BIS value was 49 with a mean propofol infusion rate of 1.6 mg kg$^{-1}$ h$^{-1}$ during normothermic CPB, suggesting that this dose was sufficient.

There are several limitations to this study. One is that the effect of CPB alone on the EEG cannot be excluded. It is not clear how CPB would have influenced the result of the current study. De Paepe and colleagues\textsuperscript{28} have shown that physiological stress alone, such as hypovolaemia, can increase the sensitivity of rats to the EEG effects of propofol. CPB has been recognized as a cause of complex systemic inflammatory and stress hormone responses, which may increase end organ sensitivity of the drug. Increased BSR and decreased BIS values during CPB may be partly attributed to physiological stress induced by CPB. Further study is needed concerning the effect of CPB on the EEG. Another limitation is that analysis of propofol pharmacokinetics and dynamics has been performed in the presence of an opioid, fentanyl, that induces progressive slowing in the EEG frequency with increasing serum concentrations and decreases BIS values.\textsuperscript{11,29,30} We chose the current fentanyl dosage to minimize the impact on the pharmacodynamic characterization of propofol and the haemodynamic changes induced by surgical procedures. However, because plasma fentanyl concentrations were not always constant during the study period, they still might have influenced BIS values.

In conclusion, we observed a marked elevation in BSR caused by an increase in the concentration of unbound propofol, without any alteration in the total drug concentration during CPB. Our findings document that changes in plasma binding of propofol can lead to clinically significant changes in the anaesthetic effect of the drug.

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