Propofol concentration in exhaled air and arterial plasma in mechanically ventilated patients undergoing cardiac surgery

M. Grossherr1*, A. Hengstenberg4, T. Meier1, L. Dibbelt2, B. W. Igl3, A. Ziegler3, P. Schmucker1 and H. Gehring1

1Department of Anaesthesiology, 2Institute of Clinical Chemistry and 3Institute of Medical Biometry and Statistics, University of Luebeck, Ratzeburger Allee 160, D-23538 Luebeck, Germany. 4Research Unit, Draegerwerk AG & Co. KGaA, Luebeck, Germany

*Corresponding author. E-mail: martin_grossherr@hotmail.com

Background.

Measuring propofol concentration in plasma (cPPL) and in exhaled alveolar gas (cPG) during constant infusion provides information about their respective time courses. In the present study, we compared these time courses in patients undergoing cardiac surgery from the beginning of propofol anaesthesia until eye opening upon awakening.

Methods.

The cPG was measured before, during, and after continuous infusion of propofol for general anaesthesia in 12 patients at two randomly allocated doses (3 or 6 mg kg\(^{-1}\) h\(^{-1}\)). Gas samples were collected on Tenax tubes. After thermodesorption, cPG was measured by gas chromatography mass spectrometry. Simultaneously with exhaled gas, arterial blood was sampled for measuring cPPL by reversed-phase high-performance liquid chromatography with fluorescence detection. In order to compare the time courses of cPPL and cPG as dimensionless values directly, each gas and plasma value was normalized by relating it to the corresponding value at the end of the initial infusion after 40 min.

Results.

The cPG ranged between 2.8 and 22.5 ppb, whereas the corresponding cPPL varied between 0.3 and 3.3 μg ml\(^{-1}\). Normalized concentration values showed a delayed increase in cPG compared with cPPL under constant propofol infusion before the onset of cardiopulmonary bypass, and a delayed decrease after stopping the propofol at the end of anaesthesia.

Conclusions.

Propofol can be measured in exhaled gas from the beginning until the end of propofol anaesthesia. The different time courses of cPPL and cPG have to be considered when interpreting cPG.


Keywords: anaesthetics i.v., propofol; measurement techniques, chromatography, mass spectrometry, drug concentration; monitoring

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The determination of propofol concentration in breath gas (cPG) opens new avenues for non-invasively estimating its plasma concentration (cPPL).\(^1\)-\(^3\) Moreover, online procedures using mass spectrometry and electrochemical sensors offer direct real-time measurement of breath gas concentrations.\(^4\)-\(^6\) Calibrated analytical procedures for measuring propofol concentration in breath gas have been described for ion reaction mass spectrometry in a clinical setting\(^5\) and for discontinuous gas chromatography-mass spectrometry (GC-MS) as a reference method in a clinical, headspace sampling setting\(^3\) and also in an experimental study involving TENAX sampling.\(^2\) However, no investigation has yet used the TENAX sampling procedure with the GC-MS method as a calibrated procedure to assess the propofol concentration in breath gas under clinical conditions.

Previous studies have compared propofol concentrations in plasma and breath gas after a steady state had been reached. It is not yet clear how the time courses of propofol concentration in plasma and breath gas are related to one another before this equilibrium is reached. It is also not clear whether the decrease in plasma propofol concentration that occurs during awakening until eye opening is reflected in the breath gas concentration measured by the calibrated GC-MS procedure.
By applying two distinct doses of propofol, our pilot investigation was designed to measure propofol concentration in breath gas, sampled on TENAX-tubes and analysed by calibrated GC-MS, and compared these values with plasma concentrations both at the beginning and at the end of propofol anaesthesia.

Methods

After approval had been obtained from the local ethics committee, 12 patients undergoing anaesthesia for cardiac surgery were randomly selected, and an informed written consent was obtained. On the day before the operation, the patients were randomly assigned to Group L (low dose) or Group H (high dose). For safety reasons, the sequence of doses was determined in advance. Exclusion criteria were cardiac or pulmonary insufficiency, pregnancy, and age <18 yr.

After the insertion of a peripheral venous and an arterial catheter under local anaesthesia, general anaesthesia was induced with etomidate 0.3 mg kg⁻¹ and sufentanil 0.5 μg kg⁻¹, and pancuronium 0.1 mg kg⁻¹ was used for muscle relaxation to facilitate tracheal intubation. For maintaining anaesthesia, propofol was infused according to the experimental protocol. Sufentanil 1 μg kg⁻¹ h⁻¹ was infused continuously until weaning from cardiopulmonary bypass (CPB). The lungs of the patients were ventilated using a breath rate of 10 min⁻¹, a tidal volume of 6–8 ml kg⁻¹, an inspiration–expiration ratio of 1:1, and a PEEP of 5 mbar (Primus, Draeger Medical, Luebeck, Germany; flow: 1 litre min⁻¹, fraction of inspired oxygen: 1.0, oxygen–air mixture), whereby the tidal volume was adjusted to maintain an arterial PCO₂ of between 5.0 and 5.6 kPa. The ventilation was continued in the intensive care unit (Evita XL, Draeger Medical; flow: 5 litre min⁻¹, fraction of inspired oxygen: between 0.3 and 0.6, oxygen–air mixture) to achieve an arterial oxygen saturation between 95% and 100% until extubation.

After systemic heparinization with 400 u kg⁻¹, a non-pulsatile mild hypothermic CPB was initiated at a flow rate of 2.5 litre min⁻¹ m⁻²; the circuit was primed with 1250 ml Ringer’s, 5000 u heparin, 250 ml mannitol 20%, and 20 ml sodium bicarbonate 8.4%. During CPB, mean arterial pressure was maintained at 60–70 mm Hg, activated clotting time >400 s, and haematocrit >20%.

In the propofol-free period after induction of anaesthesia, plasma and exhaled gas samples were collected for determining propofol blank values at time point (T) 0. Then, a continuous propofol infusion was started with 3 mg kg⁻¹ h⁻¹ in Group L and 6 mg kg⁻¹ h⁻¹ in Group H. At intervals of 10 min, further plasma and exhaled gas samples were obtained simultaneously at four time points (T₁–T₄). T₄ characterized the end of collecting samples before starting CPB. Because of our standard clinical practice, propofol 3 mg kg⁻¹ h⁻¹ was infused for all patients in both the groups until weaning from ventilation at the intensive care unit (T₅). Criteria for starting the weaning from ventilation included an exclusion of bleeding, a stable cardiopulmonary condition, and a core temperature higher than 36.0°C. Samples were obtained at T₅ and when the patient’s eyes opened (T₆).

Sedation with propofol was maintained at T₆ for one patient in each group as the gas exchange was observed to deteriorate in these individuals.

Over a period of 1 min, 100 ml of mixed inspiratory and exhaled air was led from the T-piece which was directly placed behind the tracheal tube through a TENAX tube.

The amount of air manually and continuously drawn through the TENAX tube was controlled with a 100 ml glass syringe. This procedure was immediately repeated once for each time point, so that duplicate samples were obtained. Both sampling time and the inspiration–expiration ratio were kept constant. This simple and easy-to-perform procedure can be carried out based on standard commercially available medical and analytical supplies.

Breath samples absorbed on TENAX tubes were thermally desorbed and transferred for GC-MS analysis using a cryotrap. Standardized conditions for chromatography were: carrier gas 1 ml min⁻¹ helium; column: optima 5 MS, 60 m×0.25 mm×0.25 mm (Macherey and Nagel, Dueren, Germany); and temperatures: injection/thermodesorption and detection 260°C, oven temperature 40–290°C. The retention time for propofol was 22.6 min. Further details have been described elsewhere.

Known quantities of 2,6-di-isopropylphenol, dissolved in cyclohexane (0.096–19.2 ng per tube in six steps), were applied to the TENAX tubes and examined using the above-described procedures. Repeated measurements (n=21) throughout the study period with the same amount of 2,6-diisopropylphenol (1.92 ng per tube) yielded an inter-assay coefficient of variation of 12.1%. The mean recovery of the propofol added to the TENAX polymer for these repeated measurements was 106% (range 84–136%).

Three arterial blood samples were drawn into lithium-heparinate-monovettes (Sarstedt, Nümbrecht, Germany) for determination of cPPL at each time point (T₀–T₆), simultaneously to the gas samples. Aliquots of plasma obtained by centrifugation were stored at −20°C until analysis. At the start (T₀) and end (T₆) of each measurement series, EDTA-treated blood and serum were additionally sampled for haematological (Advia 120 hematology analyzer, blood counts; Bayer, Fernwald, Germany) and clinical–chemical analyses (Aeriset clinical chemistry analyzer), determination of protein by a modified Biuret and of albumin by a dye-binding method (bromocresol purple) (Abbott, Wiesbaden, Germany).

After thawing, each plasma sample was mixed with two volumes of methanol containing the internal standard (2,4-di-i-butylphenol). After centrifugation, propofol was
determined in the supernatant by reverse-phase high-performance liquid chromatography using fluorimetric detection as described elsewhere. The measurable range of this method extended between 0.001 and 8.0 μg ml⁻¹. Pooled plasma samples repeatedly (n=20) tested for propofol yielded intra-assay coefficients of variation of 2.6%, 0.5%, and 0.7% at mean concentrations of 0.04, 0.49, and 2.85 μg ml⁻¹, respectively. Inter-assay coefficients of variation of 8.7% and 4.5% were obtained by analysing blank plasma spiked with propofol to concentrations of 0.05 and 1.00 μg ml⁻¹ (n=14); the mean recovery of added propofol was 103% and 100%, respectively (range 91–115% and 94–111%).

For each patient, we measured cPPL and cPG at the seven different time points T₀–T₆. In order to reduce variability, mean values of repeated concentration measurements were calculated per time point for each patient and used as our data set. In order to compare the observed cPPL and cPG, the ratio of each data point to the respective time course of cPPL was summarized for all the patients as a mean (SD). Wherever statistical hypotheses were being tested, the type 1 error rate was set to 5%. Owing to the explorative study design, P-values were not adjusted for multiple comparisons and were only used for descriptive purposes. Elsewhere in the manuscript, wherever a difference between the groups is stated, the corresponding test statistic produced a P-value of <5%. In doing this, we used non-parametric statistical test procedures to determine differences between samples. With two independent samples, we used the Mann–Whitney U-test; with two dependent samples, we used the Wilcoxon ranked-sum test; and with more than two dependent samples, we used the Friedman test. Data analysis was performed using the statistical software package SPSS 15.0 for Windows (Chicago, IL, USA).

Results

The patient characteristics are summarized in Table 1. Blood and serum concentrations of haemoglobin, protein, and albumin at the start (T₀) of measurement are given in Table 2. These data demonstrate the dilution of the concentrations of these parameters after CPB and operation (P=0.028), but reveal no difference between Group L and Group H.

<table>
<thead>
<tr>
<th>Gender (F/M)</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group H</td>
<td>1/5</td>
<td>58 (21–79)</td>
<td>176 (7.0)</td>
</tr>
<tr>
<td>Group L</td>
<td>2/4</td>
<td>69 (65–76)</td>
<td>168 (6.2)</td>
</tr>
</tbody>
</table>

Table 1 Patient characteristics. Mean (range) for age, mean (SD) for height and weight. Group L, low dose of propofol, 3 mg kg⁻¹ h⁻¹; Group H, high dose of propofol, 6 mg kg⁻¹ h⁻¹.

The time courses of cPG corresponded for the most part to the respective time course of cPPL, although the increase of cPG in the inflowing phase (T₀–T₃) was less prominent than the one for cPPL, as was the decrease in the purging phase (from T₃ to T₆).

Groups L and H differed with respect to cPPL at T₂ [1.6 (0.38) vs 2.6 (0.52) μg ml⁻¹, P=0.006], T₃ [1.3 (0.32) vs 2.5 (0.53) μg ml⁻¹, P=0.01], and T₄ [1.4 (0.41) vs 2.6 (0.27) μg ml⁻¹, P=0.004] (Fig. 1).

Further differences in the time course of cPPL and cPG between Group L and Group H are presented in Figure 1, and their values normalized with respect to T₄ as a ratio are illustrated in Figure 2. Differences between these ratios for cPPL and cPG were found between Group L and Group H (Fig. 2).

Discussion

In this pilot study, we determined cPG by using TENAX tubes for discontinuous gas sampling and GC-MS for the subsequent separation and quantification of propofol. We compared the time course of cPPL and cPG at the onset, before, and after CPB for cardiac surgery. Propofol was exhaled only in small amounts, since cPG ranged between 2.8 and 14.1 ppb, corresponding to cPPL between 0.53 and 2.6 μg ml⁻¹. The doubled dose generated a significant difference between the cPPL after 40 min propofol infusion compared with CPB onset, whereas with cPG only a tendency was revealed over this timescale (Fig. 1).

The slower increase in cPG compared with cPPL at the initial phase of propofol anaesthesia indicates that no equilibrium was reached between cPPL and cPG during the first 20 min of continuous propofol application (Fig. 2). During the ongoing application of propofol before CPB onset, cPG steadily increased although there was no change in cPPL after 10 min. cPG upon eye opening after cessation of propofol infusion and after 40 min at CPB onset did not differ whereas cPPL decreased. These facts suggest that upon awakening, no equilibrium existed between cPPL and cPG, although other explanations might also be entertained. First, the lung might be more permeable towards propofol after CPB-induced capillary
leakage. Secondly, the standardized but different ventilation scheme under anaesthesia after arrival in the intensive care unit after operation may have influenced the amount of exhaled propofol. Thirdly, the haemodilution by CPB reduces the binding of propofol by plasma albumin as suggested by Takizawa and colleagues. Our investigation, however, confirms that cPG after CPB can be measured using the described procedure.

Hornuss and colleagues measured cPG at the start and end of propofol infusion as was also done in our study. They determined relative signals as a surrogate for the cPG and also demonstrated an increase and decrease in cPG with a system using the ion-molecule reactions, coupled with quadrupole mass spectrometry, thus excluding a memory effect of the respiratory system for propofol. The combination with side-stream capnometry allowed the retrospective identification of the alveolar phase. They correlated these signals with blood concentrations, thus enabling an online monitoring of the cPPL without knowing the absolute cG values. Changes in cPPL exceeding 0.5 mg ml⁻¹ could be detected in real-time.

For the period after anaesthesia induction, Harrison and colleagues used continuous expiratory sampling by proton transfer reaction mass spectrometry in a feasibility study. cPG of 50 respectively 5 ppb was associated with cPPL of 8 respectively 4 μg ml⁻¹ as predicted values without

**Fig 1** Dosing and concentration profiles of propofol in patients with a low (3 mg kg⁻¹ h⁻¹, n=6) and a high (6 mg kg⁻¹ h⁻¹, n=6) dosage of propofol after induction of anaesthesia. The horizontal axis indicates the time points (T) studied: T₀ before propofol and T₁–T₄ under continuous infusion of propofol (sampling 10, 20, 30, and 40 min after initiation of the infusion). After CPB, on the intensive care ward, propofol 3 mg kg⁻¹ h⁻¹ was infused until T₅. Row 1: propofol concentration in expired gas (cPG); row 2: propofol concentration in plasma (cPPL); row 3: propofol dose. Differences in cPPL, between the groups, were measured at T₂ ($P=0.006$), T₃ ($P=0.01$), and T₄ ($P=0.004$). Differences between time points for Groups L and H are demonstrated for cPPL and cG ($P=0.031$).
defining their mode of calibration. For increasing doses and corresponding cPPL alone, Takita and colleagues measured the cPG by proton transfer reaction mass spectrometry and described the cPG as a function of the cPPL. They used the measured temperature to distinguish the alveolar fraction of the exhaled air. Understandably, cPG during inspiration decreased, but did not return to baseline.

During anaesthesia at steady state, Miekisch and colleagues discontinuously sampled breath gas by solid-phase microextraction of the headspace. They combined it with GC-MS to measure cPG and were able to calculate cPPL.

In general, there are various approaches for determining cPPL by measuring cPG. However, there is still a need to standardize the sampling of breath gas, and a need for a calibration procedure to measure cPG for comparing different methods and anaesthetic protocols.

In the present study, a continuous infusion was used before CPB and after induction of anaesthesia. An equilibrium between cPPL and cPG has to be striven for to describe the delayed time course of cPG. Our findings confirm those of Takita and colleagues who showed a delayed appearance of the cPG maximum after the bolus.

The small amount of exhaled propofol contradicts the idea that this pathway is responsible for the differences in cPG observed during lung passage, as was also described in other studies.

We found significant differences for the cPPL between doubled doses, whereas those for cPG were not found to be significant due to the higher variability and the small sample size.

Harrison and colleagues measured cPG in five patients and also suspected a variation between patients. Hornuss and colleagues confirmed the influence of inter-individual variability in cPG by demonstrating the highest correlations between direct in vitro measurement of cPPL in spiked blood and cPG in the above lying head space where the lung had no influence.

In our previous experimental study, cPPL and cPG were measured with the same analytical technique as in the present one, but there are five important differences between the two investigations. First, in the experimental study, the anaesthetic protocol was based on changing infusion rates yielding high cPPL just at the beginning in contrast to a continuous infusion. A correlation between cPPL and cPG was found. The present clinical investigation demonstrates the time course of cPPL and cPG at the beginning and at the end of propofol anaesthesia. Secondly, we applied different dosing protocols. In the experimental study, higher doses were infused especially at the beginning for the first 10 min with 16 and 32 mg kg⁻¹ h⁻¹ for goats and pigs, respectively. In the clinical investigation, propofol was constantly infused with 3 and 6 mg kg⁻¹ h⁻¹ at the beginning and 3 mg kg⁻¹ h⁻¹ during postoperative treatment at the intensive care unit. Similar cPPL for both animal species was determined which was two to three times higher than the ones measured in this clinical investigation. Thirdly, the breath sampling technique for determining cPG differed. In the experimental study, cPG was measured using end-tidal sampling whereas mixed inspiratory and expiratory breath gas was analysed in this clinical investigation. We address this point later in the discussion of the limitations of our study. Fourthly, the experimental study already indicated an increased propofol exhalation for pigs compared with goats. The results of this clinical investigation show that human lungs exhale relatively more propofol than those of pigs. Nearly identical concentrations of propofol were measured in patient’s breath gas whereas cPPL was only a half to a third of the values measured in pigs.

\[
\frac{C_{\text{PPL}}}{C_{\text{PG}}} = \frac{C_{\text{P}}}{C_{\text{G}}} = \frac{T_{\text{P}}}{T_{\text{G}}}
\]
These results point again to the species specific differences in propofol exhalation. Fifthly, in the experimental study, no indication was found that mixed venous $c_{PL}$ had a higher correlation to propofol concentration in alveolar gas than arterial $c_{PGL}$. To avoid further invasive interventions, we limited our clinical investigation to arterial $c_{PGL}$.

Currently, three continuous (proton transfer reaction, ion-molecule reaction–mass spectrometry, and electrochemical sensor)4–6 and two discontinuous methods (solid phase micro extraction and TENAX)2,3 exist for determining $c_{G}$. Continuous methods offer the opportunity to estimate changes in $c_{PL}$ in real time and have the potential to be used in monitoring devices. Discontinuous methods, such as the one applied in our study, are more suitable for comparing and validating different analytical procedures. Samples from a common sample site can be transferred to different laboratories to compare their analyses, whereas different methods used at different sites can be compared by transferring the samples to a central laboratory. For the calibration procedure of our study, known amounts of propofol were added to the TENAX tubes. The original $c_{G}$ may be slightly underestimated as the mean recovery rate was 106% in repeated tests.

The limitations of our study were related primarily to the gas sampling technique. Owing to the clinical handling with the patient, we introduced this simplified sampling technique in which a glass syringe was used without any differentiation of the alveolar gas. This procedure was, however, used for all sampling points. Our presented methods can be easily reproduced and performed by other investigators. Up to now, there exists no commercially available gas sampling device to obtain alveolar air. The impact upon measuring alveolar or mixed inspiratory and expiratory gas was described by Schubert and colleagues.14 For another CO$_2$-controlled sampling device, expiratory gas was described by Schubert and colleagues.14 For another CO$_2$-controlled sampling device, expiratory gas was described by Schubert and colleagues.14

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