Free and bound propofol concentrations in human cerebrospinal fluid

Andrzej L. Dawidowicz, Rafal Kalitynski & Anna Fijalkowska¹

Department of Chemical Physics and Physicochemical Separation Methods, Faculty of Chemistry, Maria Curie-Skłodowska University, PL 20-031 Lublin, pl. Marii Curie-Skłodowskiej 3, and ¹Department of Anaesthesiology and Intensive Therapy, University School of Medicine, PL 20-090 Lublin, ul. Jaczewskiego 8, Poland

Aims The aim of this study was to define the relationship between unbound propofol concentrations in plasma and total drug concentrations in human cerebrospinal fluid (CSF), and to determine whether propofol exists in the CSF in bound form.

Methods Forty-three patients (divided into three groups) scheduled for elective intracranial procedures and anaesthetized by propofol target control infusion (TCI) were studied. Blood and CSF samples (taken from the radial artery, and the intraventricular drainage, respectively) from group I (17 patients) were used to investigate the relationship between unbound propofol concentration in plasma and total concentration of the drug in CSF. CSF samples taken from group II (18 patients) were used to confirm the presence of the bound form of propofol in this fluid. The CSF and blood samples taken from group III (eight patients) were used to monitor the course of free and bound CSF propofol concentrations during anaesthesia.

Results For group I patients the mean (and 95% confidence interval) total plasma propofol concentration was 6113 (4971, 7255) ng mL⁻¹, the mean free propofol concentration in plasma was 63 (42, 84) ng mL⁻¹, and the mean total propofol concentration in CSF was 96 (76, 116) ng mL⁻¹ ($P < 0.05$ for the difference between the last two values). For group II patients the fraction of free propofol in CSF was 31 (26, 37)%. For group III patients the fraction of free propofol in CSF during TCI was almost constant (about 36%).

Conclusions The unbound propofol concentration in plasma was not equal to its total concentration in CSF and cannot be directly related to the drug concentration in the brain. Binding of propofol to components of the CSF may be an additional mechanism regulating the transport of the drug from blood into CSF.

Keywords: bound drug, free drug, human CSF, propofol, TCI

Introduction

Propofol is one of the most frequently used intravenous anaesthetic agents. Its properties and indications in anaesthesiology have been reviewed extensively [1–4]. The specific morphological properties of the choroidal epithelium and the existence of the cerebrospinal fluid (CSF) pathway for drug distribution to different targets in the central nervous system (CNS) suggest that the choroid plexus-CSF route is more significant than previously thought for brain drug delivery [5]. However,

information about the CSF pharmacokinetics of propofol, especially in humans, is still very limited [6–8].

Dutta *et al.* [9] have noted that the unexpectedly low effect-site equilibration half-life ($T_{1/2 k_{E0}}$) of propofol in rats can be explained by the provision that not only unbound drug, but also drug bound to plasma proteins and blood cells can participate in blood-brain propofol transport during capillary transit. Moreover, according to [9], (i) mechanisms other than free-water diffusion transport may be involved in the transition of propofol into the CSF, and (ii) the half-life of propofol complex dissociation may be several times shorter than the brain capillary transit time.

However, the hypotheses are based only on the transfer kinetics of propofol from blood into CSF. If these suggestions are true, CSF propofol concentration should be different from the unbound drug concentration in plasma under equilibrium conditions. For some other drugs,

Correspondence: Andrzej L. Dawidowicz, Department of Chemical Physics and Physicochemical Separation Methods, Faculty of Chemistry, Maria Curie-Skłodowska University, PL 20-031 Lublin, pl. Marii Curie-Skłodowskiej 3, Poland. Tel.: +48 81 537 5545; Fax: +48 81 533 3348; E-mail: dawid@hermes.umcs.lublin.pl

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measurements of their concentrations in different body fluids have led to the conclusion that their total concentration in saliva and CSF is comparable to the concentration of their free form in plasma [10]. This only applies to drug transport governed mainly by passive diffusion. Investigation of the relationship between the CSF and free plasma propofol concentration will help to elucidate if passive diffusion alone or other mechanisms are involved in the transport of the anaesthetic from blood to CSF.

The aims of the present study were to:

- characterize the relationship between unbound propofol concentration in plasma and the total drug concentration in human CSF;
- determine whether propofol binds to species within the CSF.

If the latter occurs, it may be responsible for the difference between the concentrations of propofol in the plasma and CNS.

Materials and methods

Study group and sampling

After obtaining approval from the University Ethics Committee and written consent from patients, the study was performed in patients who did not have elevated intracranial pressure and who were scheduled for the surgical removal of posterior fossa extra-axial tumours.

All patients were premedicated with 10 mg of diazepam administered orally 2 h before anaesthesia. Before induction, patients received a bolus of fentanyl 0.2 mg and were preoxygenated with 100% oxygen for 10 min.

Propofol was given using a Graseby 3500 pump as a target control infusion (TCI) to achieve blood induction concentrations between 4.5 and 5 $\mu\text{g ml}^{-1}$ and maintenance concentrations between 3.5 and 4 $\mu\text{g ml}^{-1}$ [11]. Tracheal intubation was facilitated using 0.15 mg kg^{-1} of *cis*-atracurium. After intubation the lungs were ventilated to normocapnia with an oxygen-air mixture (fraction of inspired oxygen = 0.33). In addition to the continuous infusion of propofol, anaesthesia was maintained with repeated doses of fentanyl and *cis*-atracurium. The infusion of propofol was stopped immediately after the end of surgery. When necessary, neuromuscular block was antag-

onized with 2.5 mg of neostigmine bromide preceded by 0.5 mg of atropine. Intraoperative fluid administration consisted of crystalloids initial infusion 7 ml kg^{-1} followed by 4 ml $\text{kg}^{-1} \text{ h}^{-1}$ of the same fluids [12].

Before the induction of anaesthesia, an indwelling 17-G cannula was inserted into a large forearm vein and used solely for the infusion of propofol. After induction, two additional cannulas were placed in the radial artery of the contralateral forearm (for blood pressure monitoring and blood sampling), and in the right subclavian vein (for monitoring central venous pressure). After surgical site preparation, an external drainage system (Codman, Johnson & Johnson, The Braccans, London Road, Bracknell, Berkshire RG12 2AT, UK) was inserted into one of the lateral brain ventricles. The correct position of the drainage system was confirmed by the CSF outflow. The presence of red blood cells in any CSF sample led to the withdrawal of the patient from the study. The intraventricular drainage was maintained for the entire duration of the surgery.

Three groups of patients were investigated: group I, 17 patients from whom CSF and blood samples were taken at the moment of drainage insertion. The data obtained for this group were used to investigate the relationship between the unbound propofol concentration in plasma and the total concentration of the drug in CSF; group II, 18 patients from whom CSF samples were taken at the moment of drainage insertion. The samples were used to confirm the presence of bound propofol in CSF and for the study of the relationship between bound and unbound CSF propofol; group III, eight patients from whom CSF and blood samples were taken at the moment of drainage insertion and then 30, 60, 90, 120 and 150 min after the first sample. The data were used to identify changes in free and bound CSF propofol concentrations during anaesthesia.

The composition of the groups, demographic data and mean time of drainage insertion are shown in Table 1.

Blood samples (5 ml) were taken from the radial artery using heparinized syringes. CSF samples (2.5 ml) were collected from the intraventricular drainage into syringes.

The CSF used for *in vitro* experiment was collected from a patient with an intraventricular drainage inserted to reduce intracranial pressure. This patient was not receiving propofol.

Table 1 Demographic data and mean times of the intraventricular drainage insertion.

Group	Patients	Sex		Mean age (years)	Mean body weight (kg)	Mean time of the drainage insertion (min)
		Men	Women			
I	17	10	7	50; SD 13; SEM 3	81; SD 12; SEM 3	105; SD 26; SEM 4
II	18	12	6	47; SD 16; SEM 4	77; SD 13; SEM 3	107; SD 18; SEM 5
III	8	5	3	45; SD 12; SEM 4	75; SD 13; SEM 5	105; SD 30; SEM 6

Drugs

The following drugs were used: propofol (2,6-diisopropylphenol) in soybean oil emulsion for infusions (Diprivan; AstraZeneca, Caponago, Italy); diazepam (Relanium; Polfa, Warsaw, Poland); fentanyl (Fentanyl; Polfa); *cis*-atracurium (Nimbex; Glaxo Wellcome, Dartford, UK); neostigmine bromide (Polstigminum; Pliva, Krakow, Poland); and atropine (Atropinum sulphuricum; Polfa).

Reagents and solutions

Standard reagents were obtained from the Polish Factory of Chemical Reagents-POCH (Gliwice, Poland) and were of analytical grade. A mixture composed of 75% methanol and 25% deionized Milli-Q water was used as the mobile phase. Propofol was obtained from AstraZeneca. Stock solutions of thymol and propofol in methanol (1 mg ml^{-1}) were each prepared and stored at 4°C . Tetramethylammonium hydroxide (TMAH; 25% in methanol; Aldrich Co, D-89555 Steinheim, Germany) was diluted with 2-propanol (3 : 37). The solution simulating CSF ('artificial CSF') and containing $200 \mu\text{g ml}^{-1}$ of albumin in a 1 : 1 mixture of Ringer and polyelectrolyte solutions (both Polfa) was prepared from human serum albumin (ZLB, Berno, Switzerland).

Sample preparation and propofol assay

Unbound propofol was isolated by ultrafiltration on Amicon MPS (Millipore, Bedford, MA, USA) units, utilizing the YM-10 membranes (product no. 40424; Millipore) of 10 kDa molecular mass cut-off. The ultrafiltration units were centrifuged in a constant rotor angle centrifuge MPW-341 (Mechanika Precyzyjna, Warsaw, Poland). One millilitre of each CSF or plasma sample was put into a sample compartment of the ultrafiltration unit. After the attachment of an ultrafiltrate collection container, the unit was centrifuged at 804 g until 400 μl of ultrafiltrate were obtained.

Propofol was assayed by high-performance liquid chromatography (HPLC) with fluorescence detection, according to previously published methods [13–16]. Briefly, to each sample of plasma (1 ml), CSF (400 μl), CSF ultrafiltrate (400 μl) or plasma ultrafiltrate (400 μl), thymol, dihydrogen sodium phosphate (1 ml of 0.1 M NaH_2PO_4), and cyclohexane (5 ml for plasma and 3 ml for CSF and both ultrafiltrates) were added. The mixtures were vigorously shaken for 10 min at 200 oscillations per minute. After centrifugation (1157 g for 5 min), an aliquot of the cyclohexane layer (4 ml or 2 ml, respectively) was transferred to a clean tube containing TMAH solution (20 μl or 10 μl , respectively). The solvent was evaporated to dryness in a stream of nitrogen. The residue

was reconstituted in mobile phase and injected onto the chromatographic column. The lower limit of propofol detection in plasma was 43 ng ml^{-1} with coefficient of variation ($n = 3$) of 2.8% at 150 ng ml^{-1} , 2.3% at 750 ng ml^{-1} and 0.9% at 1500 ng ml^{-1} . The lower limit of propofol detection in CSF and ultrafiltrates of plasma and CSF was 1.1 ng ml^{-1} with coefficient of variation ($n = 3$) of 11.1% at 5 ng ml^{-1} , 12.1% at 20 ng ml^{-1} and 9.8% at 40 ng ml^{-1} .

Statistical analysis

The data are expressed as mean value with 95% confidence interval (CI), standard deviation (SD) and standard error mean (SEM) where applicable. Statistical analysis was performed using Student's *t*-test for dependent samples. Differences were considered significant at $P < 0.05$.

Results

In patient group I (Figure 1) the mean value for total plasma propofol concentration was 6113 (CI 4971, 7255; SD 2221; SEM 539) ng ml^{-1} ; that for free propofol concentration in plasma was 63 (CI 42, 84; SD 40; SEM 9.8) ng ml^{-1} , and that for total propofol concentration in CSF was 96 (CI 76, 116; SD 39; SEM 9.5) ng ml^{-1} . The total propofol concentration in CSF was significantly higher than that of its unbound form in plasma ($P < 0.05$). The proportion of propofol present in plasma in the free form was 1.06%. The mean ratio of total propofol concentration in CSF to total plasma propofol concentration was 1.80%. The mean ratio of total pro-

Figure 1 Propofol concentrations in plasma and CSF ($\pm$ SEM) in patients from group I ($n = 17$). Total propofol concentration in plasma ($\blacklozenge$); free propofol concentration in plasma ($\circ$); and total propofol concentration in CSF (∇).

Figure 2 Concentrations of total and free propofol in CSF ($\pm$ SEM) in patients from group II ($n = 18$). Total propofol concentration in CSF (∇); and free propofol concentration in CSF ($\circ$).

propofol concentration in CSF to the free drug concentration in plasma was 2.05 (CI 1.405, 2.70; SD 1.26; SEM 0.31).

In patient group II (Figure 2), propofol was found in the CSF in both free and bound form. As in plasma, the concentration of the free form in CSF was significantly lower than its total concentration ($P < 0.001$), but the percentage [mean 31 (CI 26, 37; SD 11; SEM 3)%] of the free form was not as low as in plasma.

The results of the experiments carried out in order to determine the relationship between total and free propofol concentration in CSF during a continuous infusion of propofol (group III patients) are presented in Figure 3a,b. Haemodynamic parameters (heart rate and blood pressure) as well as end-tidal CO_2 , in each patient did not change significantly throughout surgery. Figure 3c illustrates the change in the percentage free propofol in the CSF during anaesthesia.

Figure 4 shows the percentage of unbound propofol *vs.* total propofol concentration in CSF samples spiked with the drug *in vitro* at concentrations typically seen in the CSF of anaesthetized patients.

Table 2 shows the concentrations of albumin, and free and bound propofol in the artificial CSF-propofol system, and the equilibrium constants assuming monomolecular propofol binding by albumin.

Discussion

The results showed significant differences between total propofol concentration and free drug concentration in plasma and total propofol concentration in CSF. The

Figure 3 Total propofol concentration, free propofol concentration, and free propofol percentage in CSF ($\pm$ SEM) during anaesthesia *vs.* sampling time in patients from group III ($n = 8$).

Figure 4 Percent of unbound drug ($\pm$ SEM) in propofol-spiked CSF *vs.* total drug concentration ($n = 3$).

Table 2 Albumin, total and free propofol concentrations, percentage of unbound drug and the drug-albumin association constants (K) in an artificial CSF-propofol system.

Concentration of Albumin, total, (mg L ⁻¹)	Propofol, total (ng mL ⁻¹)	Propofol, free (ng mL ⁻¹)	Albumin, total* (μM)	Propofol, total (μM)	Propofol, free (μM)	Unbound propofol (%)	K (M ⁻¹)
200	40	20.2	2.933	0.22	0.11	50.5	3.47 × 10 ⁵
200	75	37.6	2.933	0.42	0.21	50.1	3.65 × 10 ⁵

*Assuming m wt = 68 000.

difference between total and unbound propofol concentration in plasma is not novel and was reported earlier [17, 18]. Similarly, the difference between total propofol concentration in plasma and in CSF has also been reported previously [6–8, 19]. A new finding is the significantly higher concentration of total propofol in the CSF than its unbound concentration in plasma.

CSF contains molecules (e.g. proteins) and cells that have the ability to bind propofol. Such binding may lead to the higher total propofol concentration in CSF and, as claimed by Dutta *et al.* [9], to the shortening of propofol transit time into CSF. We found that propofol is present in CSF in both forms, and the concentration of the free drug is significantly lower than its total concentration. However, the percentage of free propofol is not as low as that in plasma (1–3% [17, 18]). The much higher content of unbound propofol observed in CSF (in comparison with plasma) probably results from a lower protein content of CSF [20]. The binding of propofol in the CSF provides an explanation of the higher total propofol concentration in CSF compared with that of its free form in plasma. However, the present results do not exclude the possibility of propofol transport in bound form, which was also considered by Dutta *et al.* [9].

A knowledge of propofol concentration in the CNS may also be relevant to clinical practice. It is not possible to determine propofol concentration in the living human brain. There are a few reports describing total propofol concentration in the CSF and how it changes during the induction [6] and maintenance of anaesthesia [7]. In the present work the intraventricular drainage application and CSF sampling caused a decrease in total as well as free propofol concentration. The same effects in other patient groups were reported earlier for total propofol concentration in CSF during Total Intravenous Anaesthesia [19] and during TCI [7]. This decrease in both concentrations of the drug may be the result of replacing the CSF richer in propofol from ventriculous spaces, with CSF containing smaller anaesthetic concentrations from more distant CNS regions – the so-called ‘mixing effect’. The CSF propofol concentration gradient may result from the mass transfer resistance of the drug (a well-known phenomenon characteristic for almost stagnant liquid media) after

its transition through one of the brain-blood-CSF barriers. The best example of the presence of concentration gradients in CSF is that of a lower protein content and higher glucose concentration in the CSF from brain ventricles in comparison with the CSF from subarachnoid cisterns [20, 21]. In addition, the volumes of CSF in the brain and spinal cord regions are comparable, indicating that in the presence of a CSF anaesthetic concentration gradient, the mixing effect, caused by CSF sampling, must result in change in the drug concentration.

The higher propofol concentration at the time of the drainage application can be also explained by higher initial CSF propofol concentrations resulting from the higher anaesthetic infusion rate during the induction of anaesthesia. The possible occurrence of similar transient drug concentration peaks in the effect site has been discussed for alfentanil, fentanyl, and sufentanil [22].

The pronounced changes in propofol concentration in CSF were not accompanied by any significant changes in haemodynamic parameters (heart rate and blood pressure) and in end-tidal CO₂ during surgery.

The observed decreases in total and free propofol concentration in CSF occurred almost in parallel, what resulted in an almost constant unbound propofol percentage during anaesthesia (Figure 3c). The data from our *in vitro* experiments also showed that the percentage of unbound drug was also constant over a range of total drug concentrations. The two values for the binding constant were almost the same. Thus, the results of our *in vitro* experiments confirmed the constancy of the percentage of unbound drug found *in vivo* during anaesthesia.

In conclusion, it was found that the concentration of propofol in the CSF was not equal to the unbound drug concentration of propofol in plasma. Thus the unbound drug concentration in plasma cannot be directly related to its concentration in the brain. Propofol binding in CSF may be an additional mechanism regulating the transport of the drug from blood into CSF.

A clear correlation was demonstrated between unbound propofol concentration in plasma, total drug concentration in the CSF, and unbound drug concentrations in the CSF. Thus, despite a lack of knowledge of the absolute concentration of propofol in the brain, it is

possible to estimate values of the drug concentration in CSF (total and unbound) from those in blood.

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