Disposition and pharmacodynamics of propofol during isovolaemic haemorrhage followed by crystalloid resuscitation in humans

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Aims

The purpose of this study was to estimate the changes in unbound propofol concentration and pharmacodynamics of propofol during isovolaemic haemorrhage followed by crystalloid resuscitation.

Methods

Ten patients undergoing elective surgery were enrolled in this study. Anaesthesia was maintained by 60% nitrous oxide in oxygen, fentanyl 10–20 µg kg⁻¹ and an infusion of propofol at 8 mg kg⁻¹ h⁻¹ until the end of the operation. Radial arterial samples were collected for measurement of propofol concentration just before the start of the operation, and at the point when blood loss was > 10 ml kg⁻¹, 20 ml kg⁻¹ and 30 ml kg⁻¹. Cardiac output (CO), haemoglobin values and plasma concentrations of albumin were also determined. Patients were resuscitated with lactated Ringer's solution to maintain a mean arterial blood pressure (± 20% of prehaemorrhage). Bispectral index (BIS) was measured continuously.

Results

Mean blood pressure, heart rate and CO were well maintained during the operation in all patients. Haemoglobin values and plasma albumin concentrations decreased significantly during surgery. There were no significant differences in total propofol concentrations across the time points. The unbound propofol concentration was increased from 0.10 ± 0.040 µg ml⁻¹ to 0.17 ± 0.041 µg ml⁻¹ after the haemorrhage of 30 ml kg⁻¹ ($P < 0.05$). BIS was significantly decreased from 47 ± 5.9 to 39 ± 3.7 ($P < 0.05$) after the haemorrhage of 30 ml kg⁻¹.

Conclusions

The hypnotic potency of propofol is increased during isovolaemic haemorrhage in crystalloid resuscitated patients even if CO is maintained.

Introduction

Propofol has been widely used for anaesthesia during surgical procedures and for sedation of patients. Propofol is a short-acting drug with a large volume of distribution, high total body clearance [1] and is highly bound to plasma albumin, erythrocytes and lipoproteins [2, 3] (unbound fraction of less than 3%) [4]. Since the hepatic

extraction ratio of propofol is high [4], hepatic metabolism is considered to be the primary elimination pathway and drug metabolism thus depends on liver blood flow. Recently, we found that human kidneys play an important role in the elimination of propofol [5, 6].

It has been reported that haemorrhagic shock alters the pharmacodynamics of propofol [7, 8]. Haemor-

rhagic shock followed by crystalloid resuscitation did not alter the pharmacokinetics but did increase the potency of propofol [7]. A proposed explanation for this increase in potency is that haemorrhagic shock leads to an increase in circulating beta endorphins [9] that act in a synergistic fashion with propofol. Other potential sources of increased end-organ sensitivity to propofol include the increase in unbound propofol. This can be achieved through competitive displacement interactions with other drugs or endogenous substances; it may also be achieved by decreases in the concentration of proteins. However, no investigators have demonstrated an increase in unbound propofol during haemorrhage.

Recently, we reported that a significant increase in the concentration of unbound propofol (a two-fold increase in the unbound fraction) occurred without an alteration in the total propofol concentration in blood during cardiopulmonary bypass [4]. The increase in the unbound fraction was caused mainly by a lower concentration of plasma proteins. As propofol is a drug with a high clearance and is extensively bound to plasma protein, the increase in unbound fraction is clinically important. Yoshitani *et al.* reported that the burst suppression ratio increased during cardiopulmonary bypass [10]. The burst suppression ratio is an accepted measure of the effect of propofol [11]. The increase of propofol hypnotic potency during haemorrhage might be explained in the same way. The possibility cannot be excluded that the increase in unbound propofol without an alteration in the total propofol concentration in blood occurs as a result of the loss of plasma protein accompanying haemorrhage, especially when followed by crystalloid resuscitation. The purpose of this study was to estimate the changes in unbound propofol concentration and pharmacodynamics of propofol during isovolaemic haemorrhage followed by crystalloid resuscitation.

Methods

Subjects

The study was approved by the Committee on Medical Ethics at Gunma University Hospital. Informed consent was obtained from 10 patients undergoing elective surgery (ASA I-II; four total gastrectomy, four total esophagectomy and three total hip replacement; seven men and three women; age range 48–67 years; weight range 51–62 kg; height range 160–173 cm). Routine clinical laboratory tests indicated normal renal and hepatic function in all patients. Individuals who had a known allergy to eggs or propofol were excluded from the study. Individuals who had significant haemodynamic instability during surgery or lower blood loss than 30 ml kg⁻¹ were also excluded from the study.

Sampling procedure

Before induction of anaesthesia, routine monitoring was established, including pulse oximetry, ECG and noninvasive blood pressure measurement. BIS (version 3.12; Aspect Medical Systems, Natick, MA, USA) was measured continuously. Anaesthesia was induced with propofol 2 mg kg⁻¹ and vecuronium 0.2 mg kg⁻¹ and then maintained by 60% nitrous oxide in oxygen, fentanyl 10–20 µg kg⁻¹ and an infusion of propofol at 8 mg kg⁻¹ h⁻¹ until the end of the operation. Radial arterial samples were collected from a cannula inserted in the radial artery for measurement of propofol concentration just before the start of the operation (Baseline), and at the point when blood loss was >10, 20 and 30 ml kg⁻¹. The blood loss was calculated from the sum of the quantity absorbed by gauze and suction. Haemoglobin values and plasma concentrations of albumin were also determined because propofol is bound to erythrocytes and plasma albumin [2]. Injection of 10 mg indocyanine green was performed for the measurement of cardiac output (CO) at the same point. Patients were resuscitated with lactated Ringer's solution to maintain a mean arterial blood pressure (±20% of prehemorrhage). Three-fold of the volume of blood loss was replaced by lactate Ringer. None of the patients received albumin solution.

Measurement of plasma unbound fraction by equilibrium dialysis

The unbound fraction of propofol to arterial plasma protein was estimated using equilibrium dialysis. Plasma samples (1 ml each) were dialyzed against an isotonic buffer containing 0.067 M sodium phosphate and 0.05 M sodium chloride (pH 7.4) for 10 h at 37°C using a dialysis membrane with a molecular weight cut-off of 6000 (VB-8, Sanplatec, Japan). After dialysis, the propofol concentrations in the plasma and the dialyzed fractions were analyzed separately. In the preliminary experiments, propofol recovery after dialysis (sum of concentration in dialysate and plasma after dialysis) was more than 80%, and the reproducibility of measuring the plasma unbound fraction of propofol was confirmed with a coefficient of variation of less than 9.4%.

Analytical procedure

The dilution curve of indocyanine green was simultaneously recorded at the nasal wing using a specially designed optical sensor that sandwiched the nasal wing between the light-emitting diodes and a photodiode measuring the intensity of the light transmitted through the nasal wing. CO, blood volume and the indocyanine green clearance slope (K) were measured by a pulse dye densitometer (R470; Nihon-kohden, Tokyo, Japan) [12].

Propofol concentrations in whole blood and plasma from artery, and dialysate and retentate after dialysis were measured using high performance liquid chromatography (HPLC) as reported previously [4]. Briefly, each sample (0.5 ml [0.1 ml whole blood plus 0.4 ml distilled water]) with internal standard (thymol in methanol: 20 µl, 5 µg ml⁻¹) was buffered with 1 ml of 0.1 phosphate buffer (pH 7.4) and extracted using 5 ml of n-hexane. After centrifugation, 30 µl of tetra-n-butyl-ammonium hydroxide (TBAH) solution (0.5 M TBAH : methanol : 2-propanol = 0.75 : 2.27 : 37.0) was added to 4 ml of the organic phase, and the solution was evaporated to dryness. The residue was re-dissolved in methanol (200 µl), and an aliquot (10 µl) was injected into a HPLC system (Waters 2690, Waters, U.S.A.). The column (WAT054275, 5 µm ODS, 4.6 × 250 mm I.D., Waters, U.S.A) was maintained at 30°C. The mobile phase comprising methanol-water (84 : 16, v : v) was pumped at a flow rate of 1.0 ml min⁻¹. Propofol and thymol were detected using a fluorescence detector (Waters 474, Waters, U.S.A., excitation: 276 nm, emission: 297 nm). The limit of quantification was 4 ng ml⁻¹ and the reproducibility of the measurement was confirmed with a coefficient of variation of 3.3%, 2.1% and 0.6% at 10 ng ml⁻¹, 20 ng ml⁻¹ and 50 ng ml⁻¹, respectively.

Calculation of pharmacokinetic parameters of propofol

The unbound fraction of propofol in plasma (fu) and blood (fB) and blood unbound concentration (Cu) were calculated according to the following equations:

$$fu(\%) = \frac{\text{concentration in buffer fraction}}{\text{concentration in plasma fraction}} \times 100$$

$$fB(\%) = fu \times Cp/Ca$$

$$Cu(\mu\text{g ml}^{-1}) = fB \times Ca$$

$$CRBC = Cp + (Ca - Cp) \times 100/Ht$$

in which Ca and Cp represents total propofol concentration in blood and plasma, respectively. Cu represents the unbound propofol concentration.

Statistical analysis

Data are expressed as mean ± SD. Analysis of variance for repeated measurements was used to detect significant changes and 95% confidence intervals (CI) of the differences were calculated. When significance was found, the Scheffe test was used as a *post hoc* comparison procedure. A *P* value <0.05 was considered statistically significant.

Results

The mean duration of the operation was 7.8 ± 4.2 h. The mean blood loss was 1656 ± 434 ml. Haemodynamic changes are shown in Table 1. Mean blood pressure, heart rate and CO were well maintained during the operation in all patients. There were no significant differences in haemodynamic values across the time points. After the haemorrhage of 30 ml kg⁻¹, haemoglobin values and plasma albumin concentrations decreased from 12.6 ± 0.94 to 8.5 ± 0.80 g dl⁻¹ (*P* < 0.05) and 3.96 ± 0.40 to 2.31 ± 0.48 g dl⁻¹ (*P* < 0.05), respectively. The unbound fraction of propofol was significantly increased from 0.023 ± 0.004 to 0.037 ± 0.008 (95% CI of the difference; 0.011, 0.020) after the haemorrhage of 30 ml kg⁻¹ (*F* = 10.333, *P* < 0.05) (Figure 1). There were no significant differences in total propofol concentrations across the time points (Figure 1). The unbound propofol concentration was increased from 0.10 ± 0.040 µg ml⁻¹ to 0.17 ± 0.041 µg ml⁻¹ (95% CI of the difference; 0.037, 0.082) after the haemorrhage of 30 ml kg⁻¹ (*F* = 4.377, *P* < 0.05) (Figure 1). The ratio of propofol concentration in erythrocytes to that in blood increased significantly after the haemorrhage of 30 ml kg⁻¹ (*P* < 0.05) (Figure 1). BIS was significantly decreased from 47 ± 5.9 to 39 ± 3.7 (95% CI of the difference; 5.0, 15.2) (*F* = 5.847, *P* < 0.05) and the burst suppression ratio (BSR) was significantly increased after haemorrhage of 30 ml kg⁻¹ (Figure 2).

Discussion

This study examined the effect of haemorrhage on the pharmacokinetics of propofol in humans. To the best of

Table 1

Haemodynamic data (mean (SD)) during surgery

	Baseline	10 ml kg ⁻¹	Blood loss 20 ml kg ⁻¹	30 ml kg ⁻¹
Heart rate (beats min ⁻¹)	72 (12)	74 (13)	77 (19)	76 (21)
Mean arterial pressure (mm Hg)	73 (15)	72 (18)	72 (21)	75 (19)
Cardiac output (l min ⁻¹)	4.7 (1.9)	4.5 (1.8)	4.9 (2.2)	5.0 (2.3)

Figure 1

The changes of total and unbound concentration of propofol in blood, unbound fraction and ratio of propofol concentration in erythrocytes to that in blood. * $P < 0.05$; a significant difference is represented in comparison with the value of baseline

our knowledge, this is the first study to describe the increase in unbound propofol without an alteration in the total propofol concentration in blood during isovolaemic haemorrhage with crystalloid resuscitation in humans.

Figure 2

The changes of BIS value and BSR during surgery. BIS was significantly decreased (○) and burst suppression ratio (BSR) (●) was significantly increased after the haemorrhage of 30 ml kg⁻¹ * $P < 0.05$; a significant difference is represented in comparison with the value of baseline

It has been reported that haemorrhagic shock increases the potency of propofol [7, 8, 13]. A first explanation for the increased propofol potency is that haemorrhagic shock leads to an increase in circulating beta endorphins. Beta endorphins have been shown to increase in response to haemorrhage [9]. Because endorphins may stimulate the γ -aminobutyric acid (GABA) system [14] and because it is proposed that propofol produces anaesthesia by acting on GABAA receptors [15], an interaction between endorphins and propofol might be hypothesized as a possible mechanism for the increased end organ sensitivity. However, Depaepe *et al.* reported that endorphin antagonism with naloxone does not influence end-organ sensitivity during haemorrhagic shock in the rat [16]. Furthermore, haemorrhage has little effect on intrinsic brain sensitivity to etomidate [17] which acts on GABAA receptors similarly to propofol. Therefore, the increase in potency of propofol cannot be explained solely by the circulating beta endorphins.

Recently, we found that a significant increase in the concentration of unbound propofol (a two-fold increase in the unbound fraction) occurred without an alteration in the total propofol concentration in blood during cardiopulmonary bypass [4]. It is now widely accepted that the pharmacological effects of a drug are elicited by the unbound fraction in the blood. Only those drug molecules that are not bound to plasma protein are able to pass through the blood vessel wall and reach their target sites within the tissue. For drugs that are non-restrictively cleared and administered intravenously, an increase in the unbound fraction could not affect total

body clearance since such drugs are extracted by the eliminating organ so efficiently that protein binding does not limit their removal. Thus, the total concentration at steady state is unchanged and an increase in the unbound fraction leads to an immediate and sustained increase in the unbound concentration. This is of clinical significance for highly protein-bound drugs with narrow therapeutic indices [18, 19] such as propofol. In the current study, we demonstrated that the unbound concentration of propofol was increased during haemorrhage. The increased unbound blood concentration of propofol would be expected to lead to a similar increase at its target site in the brain. In fact, the BIS value was significantly decreased and burst suppression ratio was significantly increased after the haemorrhage of 30 ml kg⁻¹ in the current study.

The increase in unbound fraction is clinically important for only a limited number of high clearance drugs that are highly protein bound such as propofol [4]. For drugs that are restrictively cleared, regardless of the route of administration, an increase in the unbound fraction leads to accelerated total body clearance, thereby reducing the total concentration of drug. The unbound concentration at steady state is unchanged since an increase in the unbound concentration gradually returns to the control value after redistribution [18]. Thus, the ultimate effect of changes in protein binding is only transient [18]. Haemorrhage has little effect on intrinsic brain sensitivity to etomidate [17]. In contrast with etomidate, propofol is a high clearance drug which is extensively bound to plasma protein [4]. The fact that the brain becomes more sensitive to propofol during haemorrhage might be explained by its high clearance and high protein binding.

In contrast to the current study, Depaepe *et al.* reported that protein binding was similar in hypovolaemic and control groups in an animal study [13]. Crystalloid resuscitation was probably the factor responsible for the differences between the findings from the two studies. In the Depaepe *et al.* study, hypovolaemia was induced by removing 30% of the initial blood volume but not followed by crystalloid resuscitation. Therefore, plasma albumin concentration was slightly higher in the control group than in the hypovolaemic group, but this difference was not significant. In clinical anaesthesia, however, blood loss is resuscitated by crystalloid administration to maintain CO. Three- or fourfold of the volume of blood loss must be replaced by lactate Ringer [20]. This induces a decrease in plasma albumin concentration by dilution. In the current study, blood loss was well resuscitated with lactated Ringer's solution. We demonstrated here a significant decrease in plasma

albumin concentration during haemorrhage and a significant increase in unbound propofol in blood. It is considered that the increase in the hypnotic potency of propofol is partially explained by changes in propofol kinetics shown in this study especially followed by crystalloid resuscitation.

Johnson *et al.* reported that the change in pharmacokinetics of propofol induced by haemorrhagic shock could be reversed by resuscitation with lactate Ringer solution [7]. This finding is consistent with our results. Previous researchers investigating the influence of CO on propofol concentrations [21–23], have demonstrated an inverse relationship between CO and propofol concentration. In the current study, blood loss was well resuscitated with lactated Ringer's solution and CO was successfully maintained. Therefore, it is considered that total propofol concentrations were unchanged throughout the study.

In conclusion, an increase in unbound propofol without an alteration in the total propofol concentration in blood occurred in conjunction with haemorrhage. These results may explain why propofol pharmacokinetics did not alter while the haemorrhage-induced increase in propofol hypnotic potency could not be reversed with crystalloid resuscitation as reported previously [7].

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