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ORIGINAL ARTICLE

The influence of haemorrhagic shock on the pharmacokinetic and pharmacodynamic effects of remimazolam in a swine model

A laboratory study

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BACKGROUND Haemorrhagic shock enhances the potency of several intravenous anaesthetics.

OBJECTIVE To assess the influence of haemorrhagic shock on the pharmacokinetic and pharmacodynamic effects of remimazolam, a new short-acting benzodiazepine.

DESIGN An animal observational study.

SETTING An animal laboratory in Hamamatsu University School of Medicine, Hamamatsu, Japan, from 3 April to 7 June 2021.

ANIMALS Ten pigs, 24.5 ± 0.5 (mean ± standard deviation) kg.

INTERVENTIONS Pigs were anaesthetised with isoflurane, and raw electroencephalographic waveforms, bispectral index (BIS) and 95% spectral edge frequency (SEF) were recorded throughout the study. After isoflurane was stopped, remimazolam was administered at a rate of 150 mg h^{-1} for 10 min and arterial blood was collected 16 times until 180 min to measure the remimazolam concentration (baseline condition). After the baseline measurements, haemorrhagic shock was induced by 750 ml bleeding and maintained for 40 min. The same dose of remimazolam was administered again (4 h after the first remimazolam infusion) and blood samples were collected.

MAIN OUTCOME MEASURES Pharmacokinetic variables were quantified using a three-compartment model and the pharmacodynamic variables were estimated using an inhibitory sigmoid maximal effect model.

RESULTS The peak remimazolam concentration increased from 1.0 ± 0.3 to 1.5 ± 0.4 μg ml^{-1}. Haemorrhagic shock decreased the central compartment volume, elimination clearance, and fast distribution clearance by 30 to 50%. The effect-site concentration producing 50% of the maximal BIS effect was 0.10 ± 0.09 μg ml^{-1} at baseline and 0.11 ± 0.09 μg ml^{-1} during haemorrhagic shock ($P = 0.78$), and that of SEF was 0.09 ± 0.03 and 0.11 ± 0.04 μg ml^{-1}, respectively ($P = 0.28$).

CONCLUSION Haemorrhagic shock alters the pharmacokinetics of remimazolam, but does not enhance the end-organ sensitivity. Because the impact of haemorrhagic shock is small, remimazolam might be a suitable sedative/hypnotic for the management of patients who have massive bleeding.

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KEY POINTS

- The influence of haemorrhagic shock on the pharmacokinetic and pharmacodynamic effects of remimazolam, a new ultra-short acting benzodiazepine, is unclear.
- Haemorrhagic shock alters the pharmacokinetics of remimazolam by increasing the peak concentration and duration of EEG effects 1.5-fold, but does not change the end-organ sensitivity.
- The impact of haemorrhagic shock on the hypnotic effect of remimazolam is limited; therefore, remimazolam might be a more suitable sedative/hypnotic than propofol for the management of patients who have significant blood loss.

Introduction

Haemorrhagic shock enhances the potency of several intravenous anaesthetics.[1–6] It also increases drug concentrations when associated with a decrease in the distribution volume and clearance, which further increases the end-organ sensitivity of some intravenous anaesthetics.[1–6] These pharmacokinetic and pharmacodynamic changes require a reduction in the administered dose depending on the individual intravenous anaesthetic used,[7,8] complicating the appropriate anaesthetic management of patients who have significant blood loss before and during surgery.[7,8] In this regard, propofol is a poor choice.[7,8] Animal haemorrhagic shock studies have demonstrated the potentiated hypotensive effect of propofol,[4] and that plasma concentrations and end-organ sensitivity were increased 2.5-fold and 2.7-fold, respectively, compared with normal conditions.[6] Fluid resuscitation reversed these pharmacokinetic changes, but the increased end-organ sensitivity remained[9,10] because of the increased amount of unbound propofol.[10]

Remimazolam, an ultra-short-acting benzodiazepine, is a new sedative/hypnotic used for the induction and maintenance of general anaesthesia in Japan.[11] Remimazolam is rapidly metabolised by liver carboxylesterase to an inactive metabolite,[12] and is therefore superior to midazolam, which can prolong recovery from sedation because of its active metabolite (α-hydroxymidazolam).[12] Therefore, during haemorrhagic shock, the effect of midazolam can be prolonged because of the alterations to the pharmacokinetics of midazolam and α-hydroxymidazolam.[13] Remimazolam induces minimum cardiovascular depression and can thus be safely used for the induction and maintenance of anaesthesia in patients who are haemodynamically unstable[14–16] and at high risk of complications, such as those with American Society of Anesthesiologists physical status III.[17]

On the basis of these pharmacological advantages, remimazolam might be a useful sedative/hypnotic for patients suffering from haemorrhagic shock in emergency and ICUs, and also those expecting to develop massive haemorrhage during surgery. At present, no studies have reported how haemorrhagic shock might alter the effects of remimazolam.

We conducted this study in swine to examine the influence of haemorrhagic shock on the pharmacokinetic and pharmacodynamic effects of remimazolam. We hypothesised that haemorrhagic shock increased the remimazolam concentration, related to pharmacokinetic changes, but did not change the end-organ sensitivity.

Materials and methods

Ethics

Ethical approval for this study (approval number 2020071) was provided by the Ethics Committee of the Animal Research Division at Hamamatsu University School of Medicine, Hamamatsu, Japan on 4 March 2021.

Animal preparation

All the experiments in this study were conducted on 10 swine in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. The pigs comprised two males and eight females, body weight (mean ± SD) 24.5 ± 0.5 kg, range 23.8 to 25.5 kg and approximately 2 months old. All animals were sheltered with unrestricted water and food before experiments. General anaesthesia was induced by the inhalation of 5% isoflurane and oxygen using an animal face mask, and a tracheostomy was performed. Anaesthesia was maintained with 2.5% isoflurane (approximately 1.2 minimum alveolar anaesthetic concentration[18]) with an oxygen-air mixture (fraction of inspiratory oxygen = 0.6) through mechanical ventilators. An IntelliVue G5-M1019A (Philips Medical Systems, Eindhoven, the Netherlands) was used to analyse the exhaled gases. The end-tidal carbon dioxide partial pressure was confirmed as 35 to 45 mmHg during the animal preparation period, and the ventilator setting was maintained throughout the experiment. Three cutaneous electrodes were used to monitor electrocardiographic lead II. A 14-gauge double-lumen catheter and 5-F pulmonary artery catheter (Nihon Kohden, Tokyo, Japan) were inserted via the right jugular vein, and 16-gauge catheters were inserted into the bilateral femoral arteries for the continuous measurement of arterial blood pressure and collecting blood samples. All processes from tracheostomy to catheter insertions were performed under local anaesthetic or general anaesthesia. An isotonic saline solution was infused at 100 ml h^{-1} for maintenance. An electric heater and air conditioning were used to maintain the body temperature at 37.0°C to 38.5°C throughout the study. At the end of the animal preparations, electroencephalogram (EEG) monitoring was initiated using an Aspect A-1000 instrument with

software version 3.0 (Aspect Medical Systems, Natick, Massachusetts, USA) using five cutaneous needle electrodes placed on the bilateral frontal and occipital regions, with the centre of the frontal region as the ground. Low-pass and high-pass filters were set at 0.2 and 70 Hz, respectively. Raw EEG waveform, bispectral index (BIS) and spectral edge frequency (SEF) values were collected electronically at two second intervals until the end of the experiment.

Experimental protocol

After the animal preparation was completed, the inhalation of isoflurane was terminated. After confirming that the end-tidal isoflurane (Fig. 1) concentration had decreased to less than 0.2% and EEG variables had returned to awake conditions, remimazolam was infused at a rate of 150 mg h^{-1} for 10 min (total of 25 mg to a 25 kg pig) via the right jugular vein (baseline condition). The remimazolam dose (1 mg kg^{-1}) was based on our pilot studies, in which induction of sleep in the pigs was confirmed. Isoflurane was washed out before remimazolam infusion because isoflurane masks the effect of remimazolam on EEG making the pharmacodynamic analysis difficult. Arterial blood samples (2 ml) were collected at 4, 8, 10, 11, 12, 13, 14, 15, 17.5, 20, 25, 30, 40, 60, 120 and 180 min after the start of remimazolam infusion. The inhalation of isoflurane was restarted at 120 min to induce sleep in the animals. After blood sampling at 180 min, arterial blood was withdrawn to a mean arterial pressure (MAP) of 50 mmHg and removed or re-infused to maintain a MAP of 50 mmHg for 20 min (isobaric haemorrhagic model). Maximal blood loss amount was set to 750 ml (approximate 43% of the estimated circulatory volume in a 25 kg pig) to avoid animal death. Then, haemorrhagic shock was maintained for 40 min, and the inhalation of isoflurane was terminated at 20 min (halfway through the maintenance of haemorrhagic shock). After confirming that the end-tidal isoflurane concentration had decreased to less than 0.2% and EEG variables had returned to the awake condition, remimazolam was infused again (240 min after the first remimazolam infusion) at a rate of 150 mg h^{-1} for 10 min and arterial blood samples were collected at 4, 8, 10, 11, 12, 13, 14, 15, 17.5, 20, 25, 30, 40, 60, 120 and 180 min after the start of remimazolam infusion similar to that for the baseline condition. The inhalation of isoflurane was restarted after the sampling at 120 min. Heart rate (HR), MAP, mean pulmonary arterial pressure (MPA), central venous pressure (CVP) and cardiac output (CO) at baseline and after haemorrhagic shock were recorded just before and after remimazolam infusion and metabolic variables were measured just before remimazolam infusion.

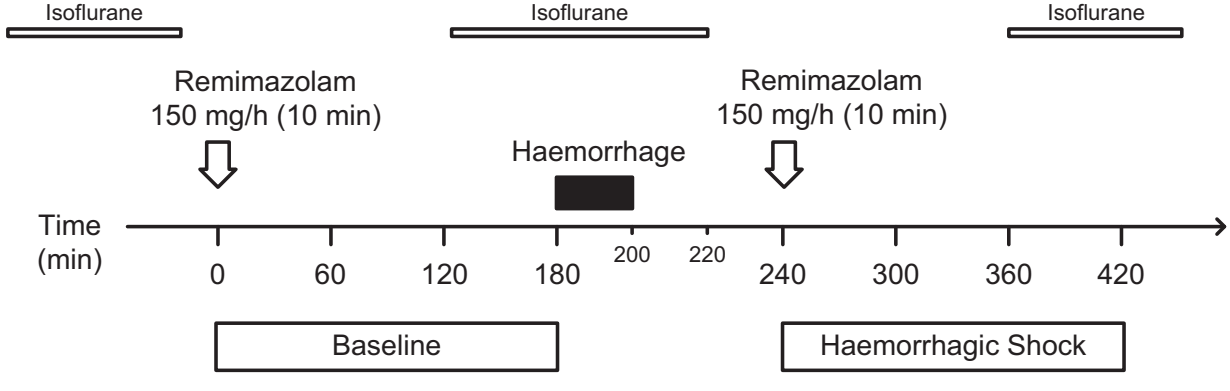
Remimazolam assay

Arterial blood samples were immediately centrifuged, and plasma was stored at −80°C until sample pretreatment. The detailed methods of sample pretreatment were reported previously.[19] A certain volume of D4-remimazolam was added as internal standard to each plasma sample and used for internal standard calibration to measure the remimazolam concentration precisely. Remimazolam plasma concentrations were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) with a lower limit of detection of 0.4 ng ml^{-1} and a linear range of 0.4 to 4500 ng ml^{-1}. The intra- and inter-assay accuracies and coefficients of variation were 96.5 and 1.3%, and 97.2 and 0.8%, respectively. Detailed explanations of chromatographic and mass spectrometric conditions were reported previously.[19]

Pharmacokinetic analysis

The pharmacokinetics of remimazolam were quantified using a three-compartment model using concentration versus time data, as described previously in our propofol study,[20] and the pharmacokinetic variables for each animal were calculated. The remimazolam concentration in the central compartment (C_1) was determined using the differential equation $dC_1/dt = -(k_{12}+k_{13}+k_{10})C_1 + k_{21}C_2V_2/V_1 + k_{31}C_3V_3/V_1 + R$, in which C_2 and

Fig. 1 Time course of the experiment. Time zero indicates the start of the 150 mg h^{-1} remimazolam infusion at baseline. Remimazolam was infused again at 240 min during haemorrhagic shock.



V_2 are the concentration and distribution volume in the rapid peripheral compartment, C_3 and V_3 are those in the slow peripheral compartment, k_{ij} is the rate constant for transfer from the i-compartment to the j-compartment, k_{10} is the elimination constant from the central compartment to outside the body and R is the infusion rate during remimazolam infusion. These pharmacokinetic variables were fitted to the measured remimazolam concentration versus time data by least-squares regression using the Solver tool in Microsoft Excel (Microsoft Excel 2019; Microsoft Corporation, Redmond, Washington, USA).

Pharmacodynamic analysis

We previously reported the influence of haemorrhagic shock,[20–22] β-blockers,[23,24] endotoxaemia[25] and intracranial space occupying lesions[26] on the EEG effect of isoflurane or propofol, which was characterised by examining its effect on the BIS or SEF. The same pharmacodynamic analysis was used in the present study. The BIS and SEF were related to the effect-site concentration (Ce) derived from the first-order decay of the remimazolam concentration (Cp): $dCe/dt = k_{e0}(Cp - Ce)$, where k_{e0} is the elimination constant from the effect site, which determines the equilibration between Cp and Ce. The k_{e0} value was calculated for each animal using nonlinear least-squares fitting, and the optimisation of k_{e0} was accomplished using the Solver tool in Microsoft Excel by minimising the area bounded by the hysteresis loop plotted between the BIS or SEF values every 2 min and the Cp values at the respective times. The following inhibitory sigmoid E_{max} equation (Hill equation[27]) was used to model the relationship parametrically for an individual animal. $E = E_0 - (E_0 - E_{max}) \times [Ce^{\gamma}/(Ce^{\gamma} + EC_{50}{}^{\gamma})]$, in which E is the predicted effect, E_0 is the baseline effect, E_{max} is the maximal effect, EC_{50} is the effect-site concentration that produces 50% of the maximal effect and γ is a measure of the curve steepness. The values in the model were estimated using nonlinear least square fitting in Microsoft Excel, through optimisation with the Solver tool to minimise the sum of squares between the estimated and measured BIS and SEF values. The coefficient of determination (R^2) was calculated as described previously.[21,22]

Statistical analysis

Data are shown as mean ± SD. Haemodynamic and metabolic variables, and pharmacokinetic and pharmacodynamic variables for each state were analysed using paired *t*-tests. *P* values less than 0.05 were considered statistically significant.

Results

The total blood volume withdrawn reached 750 ml (maximum volume) in all pigs after maintenance of the isobaric haemorrhagic model (MAP = 50 mmHg) for 20 min. One pig died after 750 ml of bleeding and before remimazolam infusion, leaving nine pigs for analysis. Haemorrhagic shock increased the HR and lactate level ($P < 0.0001$ and 0.0009), and decreased the MAP, CVP, CO, $PaCO_2$ and base excess ($P = 0.0020$, 0.0044, <0.0001, <0.0001 and 0.0002, respectively) (Table 1). Remimazolam infusion decreased the MAP in both conditions ($P = 0.0002$ and 0.0163) and the HR and MPA during haemorrhagic shock ($P = 0.0404$ and 0.0140).

Figure 2 shows the changes in the mean remimazolam concentrations in each condition. At all sampling timepoints, remimazolam concentrations during haemorrhagic shock were significantly higher than those at baseline, and the peak concentration (10 min) increased from 1.0 ± 0.3 to 1.5 ± 0.4 μg ml^{-1} ($P = 0.0001$). Pharmacokinetic analysis (Table 2) demonstrated that haemorrhagic shock decreased the distribution volume of the central compartment ($P = 0.0010$), elimination clearance ($P < 0.0001$) and fast distribution clearance ($P = 0.0123$), compared with baseline. The distribution volume of the rapid peripheral compartment ($P = 0.3182$), slow peripheral compartment ($P = 0.4854$) and slow distribution clearance ($P = 0.9704$), did not change after haemorrhage occurred.

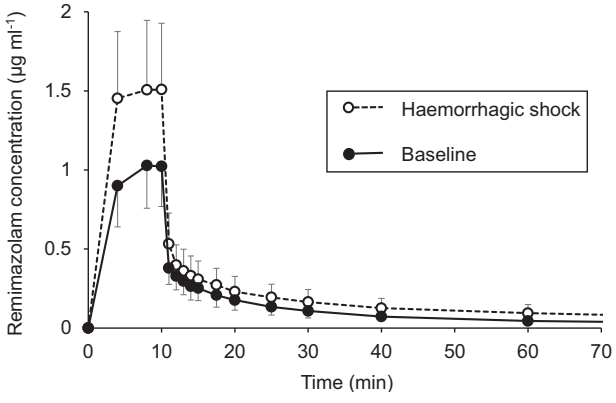
Individual BIS and SEF responses during and after remimazolam infusion are shown in Fig. 3. The peak depression of BIS and SEF at 10 min after the start of remimazolam infusion did not differ between baseline and haemorrhagic shock (BIS; 72 ± 6 *versus* 74 ± 8, and SEF; 15.6 ± 1.2 *versus* 16.0 ± 1.3, respectively) ($P = 0.30$ and 0.52), but the times to return to pre-infusion EEG values during haemorrhagic shock were longer than those at baseline (BIS; 102.9 ± 31.3 *versus* 68.9 ± 15.6 min and SEF; 77.9 ± 28.4 *versus* 56.6 ± 16.8 min, respectively) ($P = 0.0157$ and 0.029). Individual relationships between the effect-site concentration and predicted effect, and a summary of the pharmacodynamic variables are shown in Fig. 4 and Table 3. The effect-site concentration producing 50% of the maximal BIS effect was 0.10 ± 0.09 μg ml^{-1} at baseline and 0.11 ± 0.09 μg ml^{-1} during

Table 1 Haemodynamic variables before and immediately after remimazolam infusion, and metabolic variables before remimazolam infusion in each state.

	Baseline		Haemorrhagic shock	
	Before infusion	After infusion	Before infusion	After infusion
HR (beats min^{-1})	166 ± 34	143 ± 28	239 ± 26*	217 ± 36**
MAP (mmHg)	99 ± 18	78 ± 14**	72 ± 14*	60 ± 13**
MPA (mmHg)	18 ± 3	17 ± 2	17 ± 2	15 ± 2**
CVP (mmHg)	2 ± 2	2 ± 2	−1 ± 1*	−1 ± 2
CO (l min^{-1})	3.1 ± 0.5	3.0 ± 0.5	2.0 ± 0.5*	2.0 ± 0.5
pH	7.49 ± 0.03		7.46 ± 0.04	
$PaCO_2$ (mmHg)	37.4 ± 4.4		32.4 ± 4.7*	
PaO_2 (mmHg)	174 ± 46		167 ± 40	
Base excess (mmol l^{-1})	5.0 ± 1.1		-0.3 ± 3.3*	
Lactate (mmol l^{-1})	2.1 ± 0.6		4.4 ± 1.8*	
Haematocrit (%)	30.1 ± 2.9		28.9 ± 2.4	

Data are expressed as mean ± SD. CO, cardiac output; CVP, central venous pressure; HR, heart rate; MAP, mean arterial blood pressure; MPA, mean pulmonary arterial pressure. * $P < 0.05$ versus before remimazolam infusion in baseline condition. ** $P < 0.05$ versus before remimazolam infusion in each state.

Fig. 2 Mean remimazolam concentration versus time during and after 150 mg h^{-1} of remimazolam for 10 min. Solid circles = mean plasma concentration during baseline condition. Open circles = mean plasma concentration during haemorrhagic shock. At each sampling timepoint, the remimazolam concentration during haemorrhagic shock was significantly higher than that at baseline.



haemorrhagic shock; $P = 0.7761$, mean difference 0.015; 95% CI for the difference −0.086 to 0.116, and the effect-site concentration producing 50% of the maximal SEF effect was 0.09 ± 0.03 μg ml^{-1} at baseline and 0.11 ± 0.04 μg ml^{-1} during haemorrhagic shock ($P = 0.2807$, mean difference 0.016; 95% CI for the difference −0.011 to 0.042). The EC_{50} values were similar for the BIS and SEF, and haemorrhagic shock did not alter the EC_{50} value of either EEG index.

Figure 5 shows individual relative beta ratio responses calculated from the raw EEG. The initial value before remimazolam infusion (-0.61 ± 0.12 at baseline versus -0.65 ± 0.15 during haemorrhagic shock, $P = 0.1376$) decreased immediately after remimazolam infusion and the peak depression was observed 6 min after the initiation of remimazolam infusion (-0.96 ± 0.06 at baseline versus -0.97 ± 0.11 during haemorrhagic shock, $P = 0.7380$). The mean changes in the relative beta ratio were similar to those for the BIS and SEF; however, in most animals, the relative beta ratio decreased again from 20 to 40 min after the start of remimazolam infusion and in some animals, the depression remained and did not return to the pre-infusion value, suggesting the relative beta ratio did not correlate with the effect-site remimazolam concentration.

Discussion

Our study investigated the influence of haemorrhagic shock on the pharmacokinetic and pharmacodynamic effects of remimazolam using a porcine haemorrhagic shock model. This study included animals of similar weights and ages that were bred under similar conditions, with little variation in the pharmacokinetic and pharmacodynamic profiles and EEG responses between animals. Our findings indicated that the peak remimazolam concentration increased 1.5-fold and the recovery of EEG effect was prolonged 1.5-fold during haemorrhagic shock, and were associated with a decrease in the distribution volume of central compartment, elimination clearance and fast distribution clearance. These pharmacokinetic changes did not enhance the peak EEG effect. Furthermore, the pharmacodynamic analysis indicated haemorrhagic shock did not change the end-organ sensitivity of remimazolam.

All pigs had 43% of their estimated blood volume withdrawn to induce severe haemorrhagic shock and one pig died after the completion of haemorrhage. Despite the massive haemorrhage, the impact of 1 mg kg^{-1} (five-fold that required for the loss of consciousness in humans[17]) of remimazolam on MAP was similar to that before haemorrhage (decreased by 21 ± 8 at baseline versus 15 ± 16% during haemorrhagic shock, $P = 0.300$), and the CO was not reduced after remimazolam infusion (from 2.0 ± 0.5 to 2.1 ± 0.5 l min^{-1}), suggesting that remimazolam-induced cardiovascular depression was minimal, even during severe hypovolaemia.

Compared with previous haemorrhagic shock studies using pigs,[5,6] our pharmacokinetic analysis indicated the distribution volume was larger than remifentanil[5] but smaller than propofol,[6] and that clearance was similar to remifentanil,[5] suggesting the rapid onset and offset of remimazolam. Haemorrhagic shock decreased the distribution volume of the central compartment, elimination clearance and fast distribution clearance of remimazolam to 70, 63 and 48%, respectively. We speculate that the decrease in clearance was caused by a decrease in the CO (from 3.1 ± 0.5 to 2.0 ± 0.5 l min^{-1}), which subsequently reduced hepatic clearance. The liver is the main site of remimazolam metabolism.[28]

On the basis of a simulation using our pharmacological findings, a reduction of the remimazolam dose to

Table 2 Pharmacokinetic variables in each state.

	Baseline	Haemorrhagic shock	*P*
Central compartment: V_1 (ml kg^{-1})	99 ± 36	69 ± 20	0.0010
Rapid peripheral compartment: V_2 (ml kg^{-1})	395 ± 280	304 ± 258	0.3182
Slow peripheral compartment: V_3 (ml kg^{-1})	684 ± 548	882 ± 507	0.4854
Elimination clearance: Cl_1 (ml kg^{-1} min^{-1})	64 ± 18	40 ± 15	< 0.0001
Fast distribution clearance: Cl_2 (ml kg^{-1} min^{-1})	58 ± 33	28 ± 16	0.0123
Slow distribution clearance: Cl_3 (ml kg^{-1} min^{-1})	18 ± 12	18 ± 13	0.9704

Data are expressed as mean ± SD.

Fig. 3 Individual bispectral index and 95% spectral edge frequency changes versus time profiles at baseline and during haemorrhagic shock. The bold line in each figure represents the mean change.

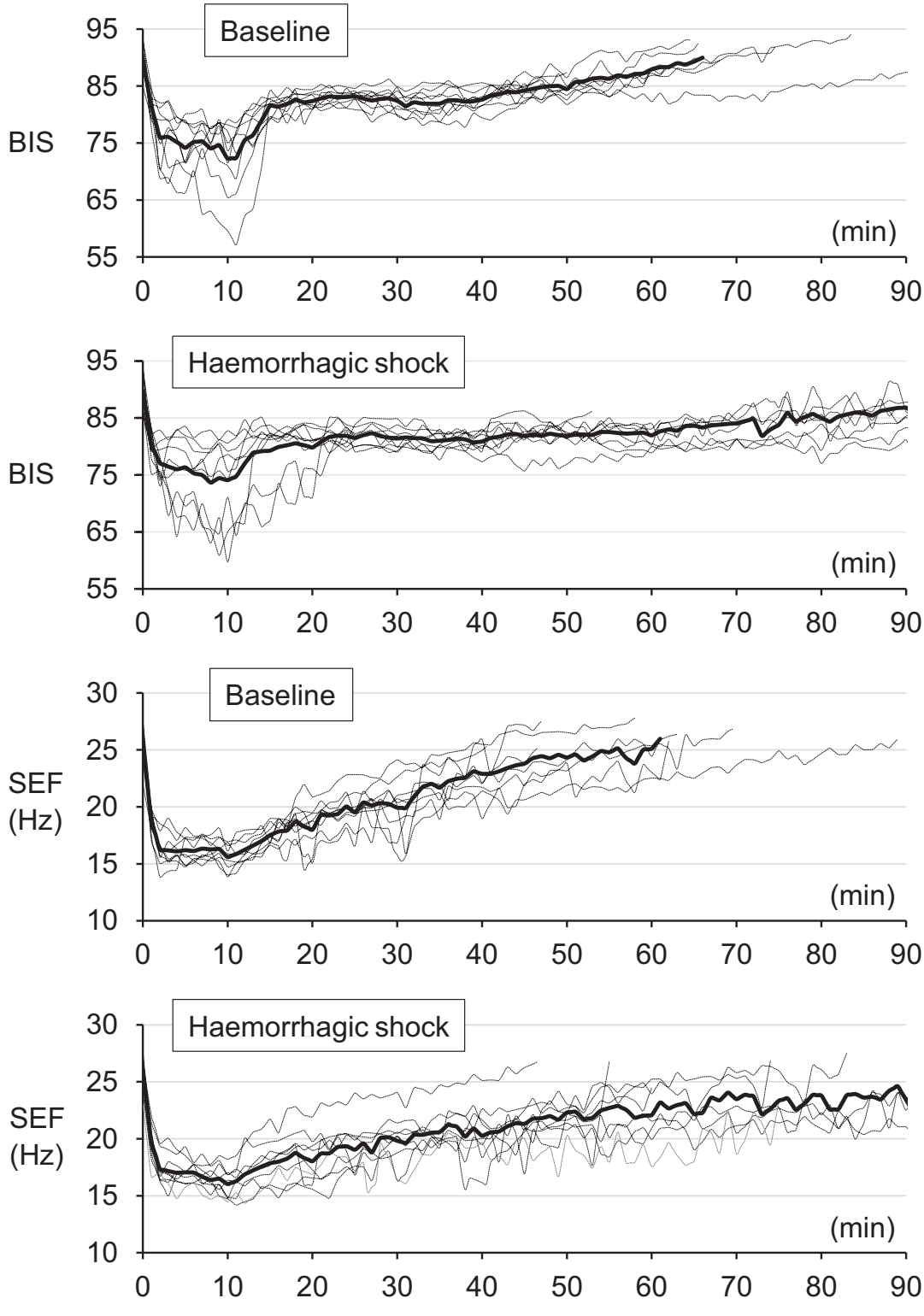


Fig. 4 Individual relationships between the predicted bispectral index or 95% spectral edge frequency and effect-site remimazolam concentration (Ce) at baseline and during haemorrhagic shock. The bold line in each figure represents the mean change.

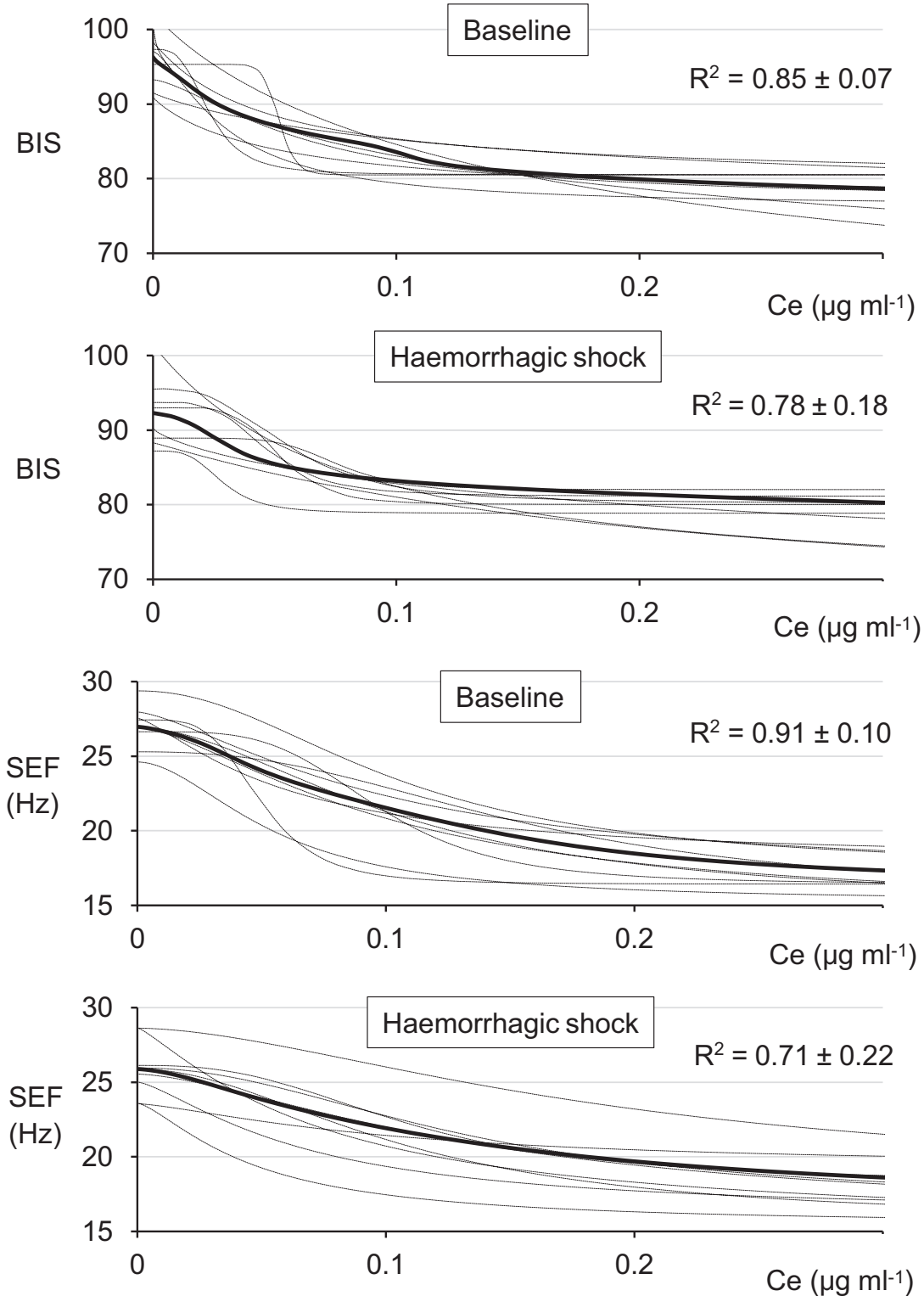


Table 3 Pharmacodynamic variables in each state.

	Baseline	Haemorrhagic shock	*P*
Bispectral index (BIS)			
k_{e0} (min^{-1})	0.53 ± 0.17	0.84 ± 0.71	0.2974
E_0	96 ± 4	92 ± 5	0.0072
E_{max}	73 ± 11	76 ± 7	0.4347
γ	2.9 ± 4.8	3.1 ± 2.0	0.9776
EC_{50} ($\mu g\,ml^{-1}$)	0.10 ± 0.09	0.11 ± 0.09	0.7761
95% spectral edge frequency (SEF95)			
k_{e0} (min^{-1})	0.84 ± 0.36	0.51 ± 0.35	0.0890
E_0	27.0 ± 1.4	25.9 ± 1.8	0.0724
E_{max}	16.2 ± 1.1	16.8 ± 1.4	0.2120
γ	2.3 ± 1.1	1.7 ± 0.5	0.1964
EC_{50} ($\mu g\,ml^{-1}$)	0.09 ± 0.03	0.11 ± 0.04	0.2807

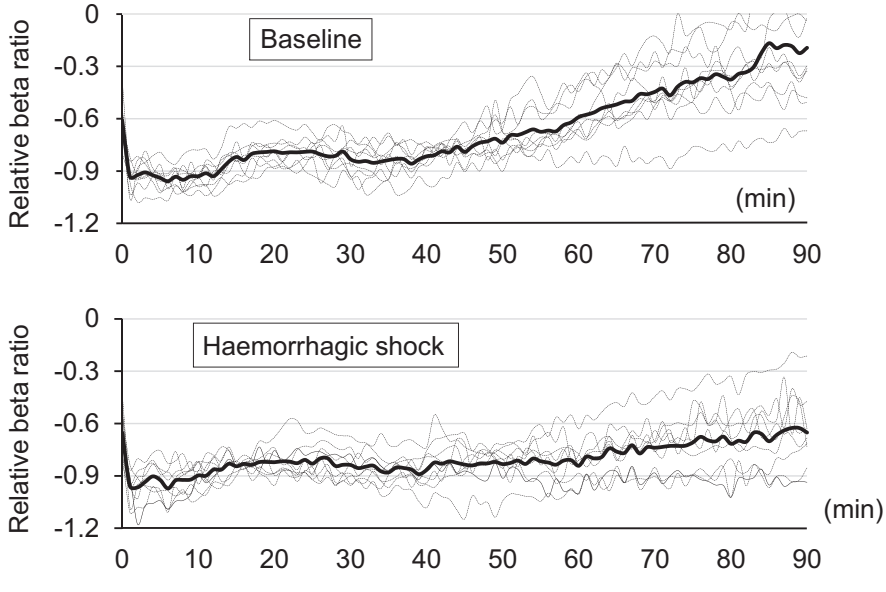
Data are expressed as mean ± SD. γ, measure of curve steepness; E_0, baseline effect level; EC_{50}, effect-site concentration producing a 50% of the maximal effect; E_{max}, maximal effect level; k_{e0}, elimination constant from the effect site.

two-thirds (67%) during severe haemorrhagic shock was sufficient to obtain a potency equivalent to normal conditions. Johnson *et al.*[6] reported that the propofol dose had to be reduced to 20% or less during severe haemorrhagic shock because of pharmacokinetic and pharmacodynamic change, and even after fluid resuscitation, it needed to be reduced to 50% because of persistent pharmacodynamic disturbance.[9] Furthermore, the remifentanil dose had to be reduced to 50% because of pharmacokinetic changes.[5] The requirement to reduce the dose of remimazolam might be lower than that of propofol and remifentanil after massive blood loss.

The peak EEG effect was similar between each condition despite a 1.5-fold increase in the remimazolam concentration during haemorrhagic shock. These findings might be consistent with previous reports using benzodiazepines. Miyake *et al.*[29] reported no differences in the BIS, SEF and relative beta ratio (log ratio power 30 to 47 Hz to power 11 to 20 Hz) between patients receiving midazolam 0.2 and 0.3 $mg\,kg^{-1}$ for the induction of general anaesthesia although plasma and effect-site concentrations were significantly higher after 0.3 than 0.2 $mg\,kg^{-1}$. Upton *et al.*[30] reported that increasing the dose of remimazolam in sheep did not produce corresponding increases in maximum EEG alpha power, which can be used as a suitable measure of the sedative effects of remimazolam in sheep. Midazolam[31,32] and remimazolam[33] increased the EEG activity between 13 to 30 Hz with increasing concentration, possibly resulting in a ceiling effect for the BIS and SEF, even if the effect-site concentration was increased during haemorrhagic shock.

Previous reports suggested the EEG alpha power[30] and relative beta ratio[33] are suitable for monitoring the depth of sedation during remimazolam administration. On the basis of our raw EEG analysis, the power in the alpha band in EEG showed no correlation with the effect-site remimazolam concentration. We calculated the relative beta ratio from the raw EEG (Fig. 5). The mean changes in the relative beta ratio were similar to those for the BIS and SEF, but the relative beta ratio did not correlate with the effect-site remimazolam concentration in most animals. We expect that the relative beta ratio might reflect the enhanced effect of remimazolam during haemorrhagic shock, but the

Fig. 5 Individual relative beta ratio changes versus time profiles at baseline and during haemorrhagic shock. The bold line in each figure represents the mean change.



peak effect did not differ between either condition, similar to the BIS and SEF. Therefore, in our study, the relative beta ratio was less suitable for evaluating the hypnotic effect of remimazolam compared with the BIS and SEF.

Our study had several limitations. First, haemorrhagic shock was not resuscitated by fluid and/or blood transfusion before remimazolam infusion. Furthermore, the influence of haemorrhagic shock on the effect of remimazolam was examined by the re-infusion of remimazolam 240 min after the first infusion at baseline conditions. We measured the plasma remimazolam concentration just before the second infusion in four animals and confirmed the blood concentration was $0.00041 \pm 0.00035\ \mu g\ ml^{-1}$ $(0.41 \pm 0.35\ ng\ ml^{-1})$. The first infused remimazolam is probably almost eliminated, but our pharmacokinetic and pharmacodynamic findings during haemorrhagic shock might differ when remimazolam was administered as a first infusion. In addition, as mentioned above, we used the BIS and SEF to evaluate the pharmacodynamic effect of remimazolam because the alpha power and relative beta ratio were not superior to BIS and SEF in the present study. However, it was unclear whether the BIS and SEF were appropriate for evaluating the pharmacodynamic effect of remimazolam, and of note, the BIS has no reliable validation in the swine study. Finally, the study used a small convenience sample, and our results may only be considered as exploratory. Further clinical studies are required to validate our findings.

In conclusion, haemorrhagic shock increased remimazolam concentration, and was associated with pharmacokinetic changes, but it did not change the end-organ sensitivity. Our findings indicate that the impact of haemorrhagic shock on the pharmacological effect of remimazolam is small, and that remimazolam might be a more suitable sedative/hypnotic than propofol and midazolam for the management of patients who have massive bleeding.

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Conflicts of interest: none.

Presentation: none.

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