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ORIGINAL ARTICLE

Electroencephalographic response following midazolam-induced general anesthesia: relationship to plasma and effect-site midazolam concentrations

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Abstract
Purpose To examine the relationships between effect-site concentrations and electroencephalographic parameters after the induction of general anesthesia with midazolam.
Methods Twenty-four patients with American Society of Anesthesiologists status I or II were randomly allocated to receive either an intravenous (i.v.) bolus of midazolam 0.2 mg kg^{-1} (small-dose group, $n = 12$) or 0.3 mg kg^{-1} (large-dose group, $n = 12$) for induction of general anesthesia in a double-blind experimental design. The bispectral index (BIS), 95% spectral edge frequency (SEF95), spectral power density, and plasma concentrations of midazolam were measured for 60 min following the induction of general anesthesia.
Results Plasma and simulated effect-site concentrations of midazolam were significantly higher in the large-dose group than in the small-dose group ($P = 0.005$ and <0.001, respectively). There was a correlation between the relative beta ratio and BIS ($r^2 = 0.30$, $P < 0.001$; $n = 168$); however, effect-site concentrations of midazolam showed no association with BIS, relative beta ratio, or SEF95 ($r^2 = 0.07$, 0.11 and 0.01, respectively; $n = 168$). The electroencephalographic spectral power density in the beta-band ($\geq$13 and $<$30 Hz) was significantly increased after induction and was significantly larger in the large-dose group than in the small-dose group ($P = 0.009$).
Conclusion Following the induction of general anesthesia with i.v. midazolam 0.2 or 0.3 mg kg^{-1}, the BIS was positively correlated with the relative beta ratio. Despite a rapid decrease in the plasma and effect-site concentrations of midazolam, the average BIS remained >60 for 60 min after induction, reflecting an increased power of the electroencephalographic high-frequency band.

Keywords Bispectral index · Electroencephalogram · Midazolam · Relative beta ratio · Spectral edge frequency

Introduction

Midazolam is used for sedation and general anesthesia [1]. During anesthesia, monitoring of the depth of anesthesia is required in order to maintain an adequate level of anesthesia and prevent intraoperative awareness. The bispectral index (BIS) is a processed electroencephalogram (EEG) and a reliable parameter for indicating the depth of anesthesia as well as the levels of sedation used to maintain this depth of anesthesia [2, 3]. Although the pharmacokinetics of midazolam has been extensively studied [4–8], little is known about the electroencephalographic effects of higher doses of midazolam used for the induction of general anesthesia relative to those used for sedation [9], and their relationships with plasma and effect site concentrations. We have measured the plasma concentrations of midazolam following bolus administration of two different doses, simulated the effect-site concentrations, and examined the relationships between electroencephalographic parameters,

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such as BIS, relative beta ratio, and 95% spectral edge frequency (SEF95).

Materials and methods

Study protocol

This study protocol was approved by the institutional ethics committee of Osaka City University Hospital (Osaka, Japan), and informed consent was obtained from all patients. Twenty-four patients with American Society of Anesthesiologists (ASA) physical status I or II who were undergoing elective orthopedic surgery were randomized to receive either an intravenous (i.v.) bolus of midazolam 0.2 mg kg^{-1} (small-dose group, $n = 12$) or 0.3 mg kg^{-1} (large-dose group, $n = 12$) for the induction of anesthesia. The dose of midazolam was chosen based on previous reports and the fact that a dose <0.2 mg kg^{-1} is insufficient to produce total loss of consciousness for the induction of general anesthesia [1, 4, 5, 10]. Random allocation to the groups was conducted using a computer-generated allocation table. Midazolam was dissolved in saline in a syringe to a total volume of 20 ml, and the concentration of midazolam was unknown to the anesthesiologists. The allocation of patients and preparation of the midazolam solution was performed by one of the authors (Y.I.). Patients with an abnormal electrocardiogram (ECG), cardiovascular, respiratory, or psychological diseases, or a predicted difficulty in tracheal intubation were excluded. Other exclusion criteria included old age (>70 years), a regular use of hypnotic medication, drug or alcohol abuse, morbid obesity with a body mass index >30 kg m^{-2}, and current use of known cytochrome P450 3A (CYP3A)-inducing or -inhibiting drugs [11].

Premedication was not used. Once the patient arrived at the operating room, an i.v. catheter was inserted and lactated Ringer's solution 500 ml was infused rapidly for fluid loading. Patients were monitored with a three-lead ECG for pulse oximeter oxygen saturation (SpO_2) and non-invasive arterial pressure measurements at 1-min intervals. Once the EEG and baseline hemodynamic measurements were being recorded, continuous infusion of remifentanil was started at 0.1 μg kg^{-1} min^{-1} at 5 min before the induction of general anesthesia. Anesthesia was performed by a staff anesthesiologist in our department who was blinded to group allocation. After the administration of i.v. midazolam, the name of the patient was repeatedly called out and the shoulders shaken. Following confirmation of the loss of consciousness, vecuronium bromide (0.1 mg kg^{-1}) was administered, the trachea was intubated 5 min after induction, and the lungs were mechanically ventilated to maintain an end-tidal carbon dioxide tension between 35 and 40 mmHg. An intraarterial catheter was inserted into the radial artery for continuous blood pressure monitoring and blood sampling. Infusion of remifentanil was stopped after tracheal intubation. The surgery did not start until the blood had been sampled at 60 min after the induction of anesthesia.

After obtaining the blood sample at 60 min, our study was ended; 3 μg kg^{-1} of fentanyl and sevoflurane were then administrated and the surgery started. An end-tidal sevoflurane concentration was maintained at ≥2.0% and the BIS was <50 during surgery. At the end of the surgical procedure, the neuromuscular blockade was reversed, the endotracheal tube was removed, and the patients were transferred to the recovery room. Patients were interviewed by a blinded observer as soon as they were oriented to time, place, and person, at 2–4 h post-surgery, and the next day in the ward. A number of questions were asked following the format of a standardized interview [12]. These included "What was the last thing you remember before you went to sleep for your surgery?" "What was the first thing you remember after surgery?" "Can you remember anything in between these two periods?" "Did you have any dreams during anesthesia?"

Analysis of EEG

The EEG data were continuously observed on a monitor (BIS XP version 4.0; Aspect Medical Systems, Newton, MA) using a BIS Quatro sensor (Aspect Medical Systems). All binary data packets containing raw wave data, BIS, and SEF95 were recorded on a personal computer (LB500/J2; NEC Corp, Tokyo, Japan). The BIS smoothing rate was set at 15 s. Recorded EEG signals were subjected to fast Fourier transformation with 2-s epochs, and the spectral power densities in frequency bins with a 0.5-Hz band width were calculated using a special computer software program (Bispectrum Analyzer) developed by our group [13]. The spectral power analysis was performed on the EEG waves obtained for a period of 1 min commencing with each time point of blood sampling. The relative beta ratio at the same time point was calculated following the equation [14]: $\log_{10}$ [spectral power (30–47 Hz)/spectral power (11–20 Hz)]. The EEG analysis was performed by one of the authors (W.M.) who was not included in the anesthesia and blinded to group allocation.

Measurement of plasma concentration and simulation of the effect-site concentration of midazolam

Blood samples were collected at 5, 10, 15, 20, 30, 45, and 60 min after the induction of anesthesia from the indwelling arterial catheter and immediately centrifuged; the plasma was stored at −70°C until analysis. Concentrations of total

(protein-bound and unbound) midazolam were measured by one of the authors (Y.O.), who was blinded to group allocation, using high-performance liquid chromatography-mass spectrometry (LC–MS) based on a method reported previously [4]. Briefly, the serum was pretreated with β-glucuronidase, and both the protein-bound and unbound midazolam was extracted with ethyl acetate from 300 μl plasma and evaporated to dryness under reduced pressure. The residue was reconstituted with a mobile phase consisting of ammonium acetate (pH 4.5) and acetonitrile (51/49, v/v), injected onto a high-performance LC apparatus equipped with a C18-column (ODS-100Z; 5.0 × 2.8 mm; Tosoh, Tokyo, Japan), and eluted at a flow rate of 0.2 ml min^{-1}. Analyses were conducted using a tandem mass spectrometer (4000 Qtrap; Applied Biosystems, Foster City, CA) equipped with an electrospray ionization interface that was operated in the positive ionization mode using diazepam as an internal standard. The lower limit of quantitation was 1 ng ml^{-1}. The coefficients of within-day and between-day variance were 4.8 and 5.5%, respectively, at 100 ng ml^{-1}. The pharmacokinetics of midazolam was calculated using the WinNonlin professional software package ver. 5.2 (Pharsight Corp, Mountain View, CA). The effect-site concentration (C_e) of midazolam was calculated according to the following equation:

$$\frac{dC_e}{dt} = k_{e0} \times (C_p - C_e)$$

where k_{e0} is a blood-effect site equilibration constant and C_p is the measured plasma concentration of midazolam at each point in both groups. In this study, k_{e0} was assumed to be 0.144 min^{-1} based on a previous study [15], which is also used in a target-controlled infusion program.[1]

Statistics

Data are expressed as the mean ± standard deviation (SD). Statistical analyses were performed using Sigma Stat ver. 3.0 (Systat Software, San Jose, CA). The number of patients was determined by power analysis based on the findings of our preliminary study. In that study, average power in the beta-band from 5 to 60 min after the induction of anesthesia was 5.5 ± 1.5 μV^2. Assuming a type 1 error protection of 0.05 and a power of 0.80 for detecting a 30% difference in the power in the beta-band, 12 patients were required in each group. Categorical data were compared using chi-square tests. Age, weight, and height differences between the small-dose and the large-dose groups were examined using Student's t test. Plasma concentrations of midazolam in the two groups were compared by one-factor analysis of variance (ANOVA) with repeated measurements. The pharmacokinetic parameters of midazolam were compared by Student's t test.

The relationships between the effect-site concentration of midazolam and BIS, the relative beta ratio, and SEF95, and between BIS and the relative beta ratio were examined by linear regression. Subsequent calculation of the Pearson correlation coefficient was used to quantify the statistical significance. Differences in BIS, relative beta ratio, SEF95, and the spectral power density in the delta- (≥2 and <4 Hz), theta- (≥4 and <8 Hz), alpha- (≥8 and <13 Hz), and beta- (≥13 and <30 Hz) bands throughout the experiments were examined using ANOVA for repeated measurements. We subsequently examined the differences of these parameters within the same study group at baseline and 5–60 min after the induction of anesthesia and between the small-dose and large-dose groups at the same time points using the Scheffé test, taking into account the number of measurements. $P < 0.05$ was considered to be statistically significant.

Results

All patients completed the study period. No differences were found in patient demographic data between the two groups (Table 1). After the induction of anesthesia, all patients lost consciousness and were non-responsive to their name being called and their shoulders shaken, with an observer's assessment of alertness/sedation (OAA/S) score of 1 within 2 min. No patient developed a mean arterial pressure <70 mmHg or a heart rate of <60 bpm and, therefore, the administration of vasopressors or atropine during the study period was not required.

The plasma concentration of midazolam decreased in a dual-exponential manner, and it was approximately 50% higher in the large-dose group than in the small-dose group ($P = 0.005$; Fig. 1a), although no differences were found

Table 1 Demographic data

Demographic parameters	Small-dose group ($n = 12$)	Large-dose group ($n = 12$)
Age (years)	58 ± 11	55 ± 10
Weight (kg)	53 ± 5	56 ± 10
Height (cm)	153 ± 7	157 ± 10
Sex (male/female)	4/8	3/9
ASA stage I/II (n)	3/9	0/12

ASA, American Society of Anesthesiologists

Data are expressed as mean ± standard deviation

There are no significant differences between the two groups

[1] STANPUMP; developed by Dr. SL Shafer. This computer software package is presently not available on the web.

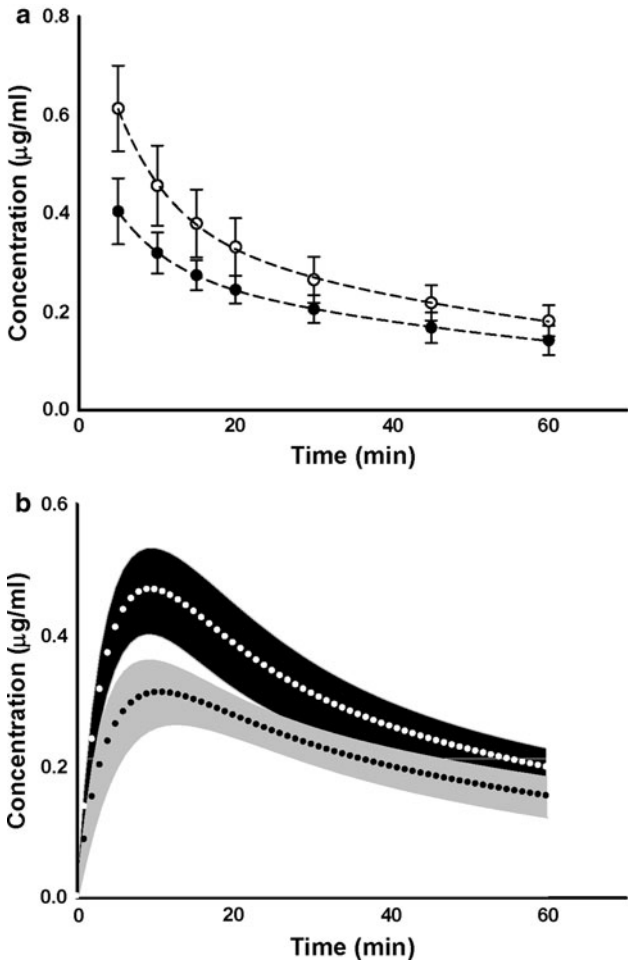

Fig. 1 Plasma and effect-site concentrations of midazolam. Plasma (**a**) and simulated effect-site concentrations (**b**) of midazolam following intravenous administration of 0.2 mg kg^{-1} (small-dose group, *filled circle*) and 0.3 mg kg^{-1} (large-dose group, *open circle*). **a** Plasma concentrations are expressed as the mean ± standard deviation (SD) of 12 measurements. Simulated plasma concentration–time curves (AUC) were fitted to a two-compartment model (*dashed line*). **b** Effect-site concentrations of midazolam were simulated on the basis of the pharmacokinetic parameters calculated from the measured plasma concentrations and blood-effect site equilibration constant of 0.144 min^{-1} (*dotted line*) and expressed as the mean ± SD in the small-dose group (*filled circle* and *gray-shadowed area*) and in the large-dose group (*open circle* and *black-shadowed area*) ($n = 12$ in each group). Both the plasma and effect-site concentrations of midazolam were significantly higher in the large-dose group than in the small-dose group ($P = 0.005$ and 0.001, respectively)

in the pharmacokinetic parameters between the two groups, with the exception of the area under the plasma concentration–time curve (AUC) ($P = 0.04$; Table 2). Effect-site concentrations (C_e) simulated on the basis of the pharmacokinetic parameters and blood-effect site equilibration constant (k_{e0}) were also significantly higher in the large-dose group than in the small-dose group ($P < 0.001$; Fig. 1b).

Table 2 Pharmacokinetic parameters

Pharmacokinetic parameters	Small-dose group ($n = 12$)	Large-dose group ($n = 12$)	P value
$AUC_{(0-\infty)}$ (ng ml^{-1} h)	470 ± 131	646 ± 244	0.04
Cl (ml min^{-1} kg^{-1})	7.6 ± 2.1	8.5 ± 2.2	0.3
V_{ss} (l kg^{-1})	0.6 ± 0.2	0.7 ± 0.1	0.2
$T_{1/2\alpha}$ (min)	5.2 ± 1.9	5.6 ± 3.2	0.7
$T_{1/2\beta}$ (min)	66.6 ± 32.3	68.9 ± 35.5	0.6
MRT (min)	89.8 ± 44.2	90.2 ± 45.6	0.9

$AUC_{(0-\infty)}$, Area under the plasma concentration–time curve from time 0 to infinity; Cl, systemic clearance; V_{ss}, volume of distribution at steady state; $T_{1/2\alpha}$, distribution half-time; $T_{1/2\beta}$, elimination half-time; MRT, mean residence time

After the induction of general anesthesia, the BIS and relative beta ratio decreased and remained between 60 and 70 and between −1.8 to −1.2, respectively; in addition, no differences were found at any time points between 5 and 60 min post-induction of anesthesia. The SEF95 did not change after the induction of anesthesia and remained between 15 and 25 Hz, with no observable differences at any of the time points (Fig. 2). There were no differences in BIS, relative beta ratio, or SEF95 between the two groups ($P = 0.2$, 0.4 and 0.9, respectively). The BIS, relative beta ratio, or SEF95 did not correlate with the effect-site concentrations of midazolam after the induction of general anesthesia ($r^2 = 0.07$, 0.11, and 0.01, respectively; Fig. 3a–c); there was a weak, but significant correlation between relative beta ratio and BIS ($r^2 = 0.30$, $P < 0.001$, $n = 168$; Fig. 3d). The levels of the electromyogram decreased after induction, dropping to <35 dB at the time of tracheal intubation and to <30 dB thereafter until the end of the study (data not shown).

There were significant differences in the EEG spectral power density in the alpha- and beta-bands ($P = 0.03$ and 0.009, respectively) whereas no differences were found in the delta- or theta-band ($P = 0.2$ and 0.05, respectively) between the two groups (Fig. 4). The spectral power density in the alpha-band was significantly increased 10 min after the induction of anesthesia compared with those at baseline only in the large-dose group ($P = 0.02$), despite no differences in the small-dose group. In the beta-band, the spectral power density was significantly increased 5 min after the induction of anesthesia compared with baseline in both the small-dose and large-dose groups ($P = 0.04$ for both). There were no within-group differences in the spectral power density in either the delta- or theta-band. A weak correlation between the effect-site concentration of midazolam and the spectral power density in the beta band was obtained at eight time points from 24 patients ($r^2 = 0.32$); this correlation was not observed with the other bands (data not shown).

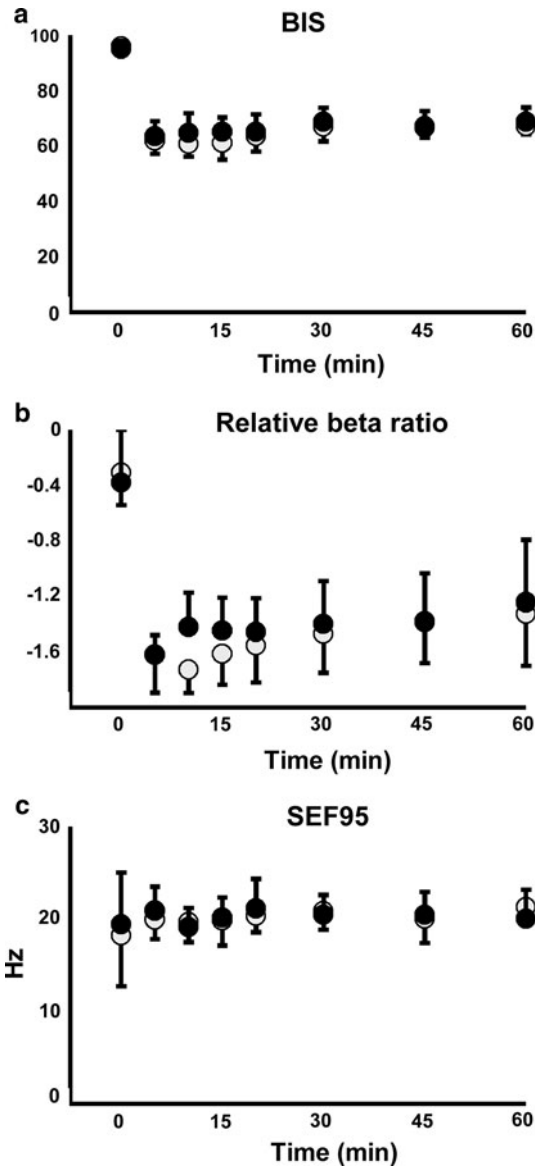

Fig. 2 Bispectral index (*BIS*) (**a**), relative beta ratio (**b**), and 95% spectral edge frequency (*SEF95*) (**c**) in patients receiving 0.2 mg kg^{-1} (small-dose group, *filled circle*) and 0.3 mg kg^{-1} (large-dose group, *open circle*) of intravenous (i.v.) midazolam. BIS, relative beta ratio, and SEF95 were calculated using the bispectral index monitor (BIS XP ver. 4.0) with a smoothing rate of 15 s. Data are expressed as the mean ± SD of 12 measurements. There were no differences in BIS, relative beta ratio, or SEF95 between the large-dose and small-dose groups ($P = 0.2$, 0.4 and 0.9, respectively). The BIS and relative beta ratio decreased significantly after the induction of anesthesia. There were no within-group differences for BIS or relative beta ratio at any of the time points from 5 to 60 min in either the small-dose or large-dose group. There were no within-group differences in SEF95 from baseline (time 0) to 60 min at any of the time points in either the small-dose or large-dose groups

Two patients (one male in the large-dose group, one female in the small-dose group) complained of dreams during anesthesia when questioned in the recovery room. The average BIS and SEF95 of these patients were not different from those of the remaining patients. Incidentally, their dreams were pleasant ones and not related to anesthesia or surgery. No patients complained of intraoperative awareness or of hearing the physicians' voices.

Discussion

Despite significant differences in both the plasma and effect-site concentrations of midazolam, there were no differences in the BIS, relative beta ratio, or SEF95 between patients receiving midazolam 0.2 and 0.3 mg kg^{-1}, suggesting that the effect of these two doses of midazolam on the EEG would be similar. These results are consistent with those reported earlier showing that BIS decreased only to 70 by the end of continuous infusion of midazolam at 0.03 mg kg^{-1} min^{-1} for 10 min [9] and that the maximum effect of midazolam on the BIS is approximately 70 [6]. These findings suggest that BIS does not decrease further even if its plasma concentration increases to levels higher than that required for sedation. Although contaminating electromyogram activity can be interpreted as the high-frequency, low-amplitude fraction of the EEG, thereby indicating false increases in BIS, particularly during light sedation [2], its effect seems to be negligible since muscle relaxants were used from the beginning of our study, and the levels of electromyogram were low.

We found a weak but significant correlation between the BIS and relative beta ratio, suggesting that the BIS is related to electroencephalographic beta activity. Poorer correlations between BIS and relative beta ratio in our study would result from the narrower range of these parameters in comparison to those that occur during anesthesia with other agents, such as isoflurane [14]. In contrast to the unchanged BIS, relative beta ratio, and SEF95 within 60 min after the induction of anesthesia, the spectral power density in the beta-band was highest at 5 min, decreasing thereafter but remaining higher than the baseline levels at 60 min. This change in the beta-band is common in patients receiving either 0.2 or 0.3 mg kg^{-1} of midazolam, suggesting that midazolam increases the power of the beta waves, resulting in an average BIS >60. Previous studies have also shown midazolam induces a plasma concentration-dependent increase in electroencephalographic beta activity [7].

Basic changes in the EEG that are commonly observed during anesthesia with intravenous and volatile agents, such as barbiturates, propofol, isoflurane, and sevoflurane, are characterized by an initial increase in high-frequency (>20 Hz) activity, followed by a decrease in high-frequency activity and an increase in middle-frequency (10–20 Hz) activity, and finally by a decrease in middle-frequency activity and an increase in low-frequency (0.5–5 Hz)

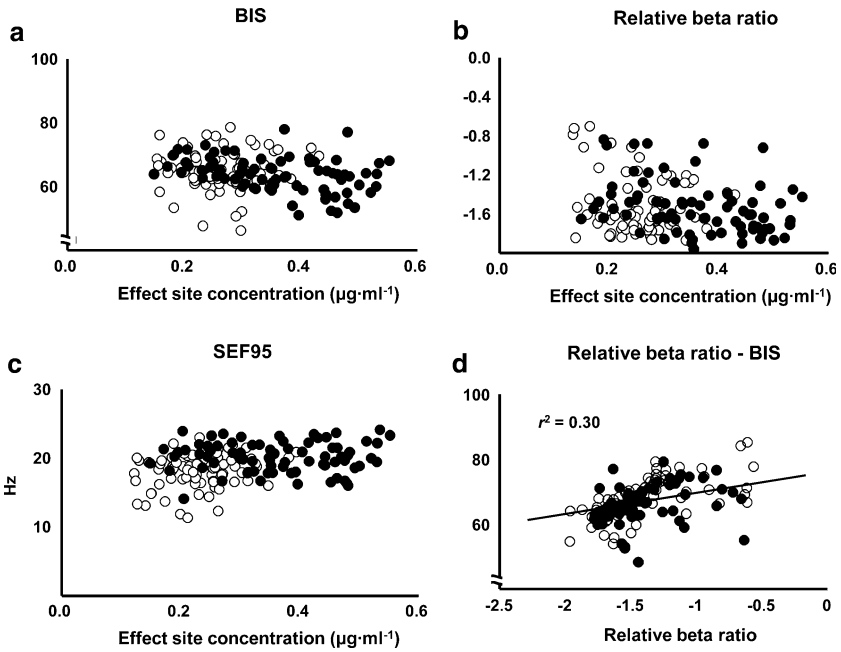

Fig. 3 Correlations between the effect-site concentration of midazolam and the BIS (**a**), relative beta ratio (**b**), and SEF95 (**c**). **d** Correlations between relative beta ratio and BIS. All measurements were obtained at seven time points (5–60 min) on patients in the small-dose group (*closed circle*) and large-dose group (*open circle*) ($n = 168$). There were no relationships between the effect-site concentration of midazolam and BIS, relative beta ratio or SEF95 ($r^2 = 0.07$, 0.11 and 0.01, respectively). BIS was weakly correlated with relative beta ratio ($r^2 = 0.30$, $P < 0.001$)

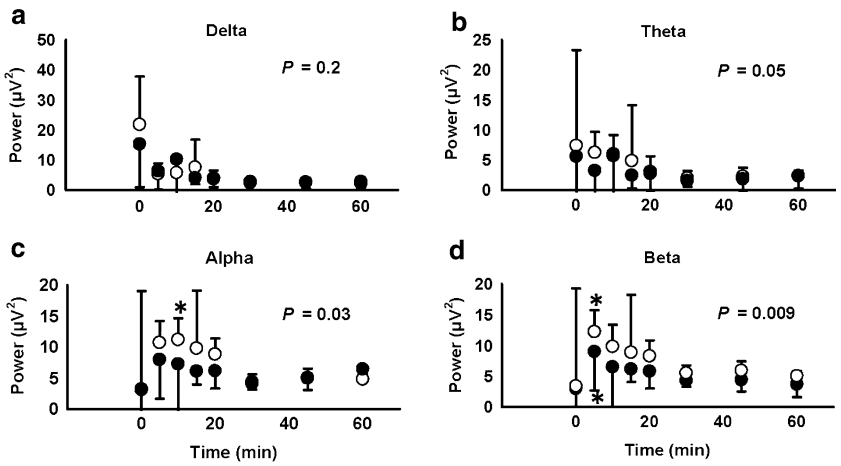

Fig. 4 Spectral power density in each electroencephalographic band. Spectral power density in the delta- (≥2 and <4 Hz) (**a**), theta- (≥4 and <8 Hz) (**b**), alpha- (≥8 and 13< Hz) (**c**), and beta- (≥13 and <30 Hz) (**d**) bands in patients receiving 0.2 mg kg^{-1} (small-dose group, *filled circle*) and 0.3 mg kg^{-1} (large-dose group, *open circle*) of i.v. midazolam. Data are expressed as the mean ± SD of 12 measurements. *$P < 0.05$ compared with baseline (time 0) within the same study group. Spectral power density in the alpha- and beta-bands was significantly larger in the large-dose group than in the small-dose group ($P = 0.03$ and 0.009, respectively). Power in the alpha-band was significantly larger at 10 min after the induction of anesthesia compared with baseline only in the large-dose group ($P = 0.02$). Power in the beta-band was significantly larger at 5 min after the induction of anesthesia compared with baseline in both the small-dose and large-dose groups ($P = 0.04$ for both)

activity [16, 17]. The burst suppression pattern is also induced during deep anesthesia with these anesthetics. In sharp contrast, following the induction of anesthesia with midazolam in our study, the EEG was characterized by an increased beta power, unchanged SEF95, average BIS >60, and a lack of burst suppression even at supra-sedation levels

of plasma and effect-site concentrations. Although the mechanism of these unique EEG changes by midazolam is not clear, one possible explanation may be provided by its effect site. Midazolam exclusively exerts its anesthetic effect through a benzodiazepine receptor, which is part of the γ-aminobutyric acid type-A ($GABA_A$) receptor, as shown by the complete reversal of its anesthetic effect by flumazenil, a specific benzodiazepine receptor antagonist [18]. On the other hand, other anesthetics, such as volatile agents, barbiturates, and propofol, have numerous effect sites in addition to the $GABA_A$ receptor, and their anesthetic effects are not reversed by flumazenil [18]. The similar EEG pattern produced by diazepam, another benzodiazepine, also supports this explanation [7]. Saturation at the benzodiazepine receptor site would account for the small differences in EEG between patients receiving midazolam 0.2 and 0.3 mg kg^{-1}, respectively, and the absence of burst suppression.

A prospective cohort study involving approximately 5,000 consecutive surgical patients revealed that awareness with recall is unlikely when the BIS value is <60 [19]. However, results of more recent studies suggest the absence of a definite relationship between the occurrence of intraoperative awareness and BIS values >60 [20]. In our study, the average BIS was >60 during the entire experimental period. Two patients reported dreams not related to surgery, while no patients complained of awareness. Despite the high BIS value, amnesia produced by midazolam, the absence of noxious stimuli (with the exception of tracheal intubation during the experimental period), and the maintenance of anesthesia with sevoflurane and fentanyl during surgery with a BIS below 50 would have been effective for preventing intraoperative awareness and its recall.

There are several limitations to our study. First, remifentanil was used for preventing an abrupt increase in blood pressure and heart rate induced by laryngoscopy and tracheal intubation. Although the infusion of remifentanil was discontinued after tracheal intubation, its effect on the EEG cannot be completely eliminated. In our preliminary study, however, the BIS was 96 ± 2, 64 ± 3, 60 ± 7, 64 ± 3, and 64 ± 2 and the SEF95 was 20.6 ± 4.6, 20.0 ± 2.0, 19.2 ± 2.2, 20.0 ± 2.1, and 21.0 ± 2.1 Hz at baseline and at 5, 10, 15, and 20 min, respectively, after the induction of anesthesia with 0.2 or 0.3 mg kg^{-1} i.v. midazolam without remifentanil ($n = 12$, mean age 59 ± 11 years). These data are comparable with those reported here with remifentanil, suggesting that the infusion of remifentanil unlikely affected the EEG data. Secondly, blood samples were collected for only 60 min after the induction of general anesthesia; this was a short period of time and may not have allowed precise calculation of the pharmacokinetic parameters, although they are comparable with those reported earlier [4–6]. We measured only midazolam, not its active metabolite, 1′-hydroxymidazolam. However, the plasma concentration of 1′-hydroxymidazolam is approximately 20% [21], and the affinity of 1′-hydroxymidazolam to the benzodiazepine receptor is approximately 60% of that of midazolam [22]. Of importance, approximately 90% of 1′-hydroxymidazolam is conjugated to form 1′-hydroxymidazolam glucuronide [21], which is only of clinical relevance in renal failure where accumulation occurs [22], suggesting that it has only a small effect on the EEG.

In conclusion, among our patients, the average BIS remained >60 for 60 min after the induction of general anesthesia with midazolam 0.2 or 0.3 mg kg^{-1} i.v. despite the rapid decrease in plasma midazolam concentration. This patterns is likely the result of the increased power of the electroencephalographic high-frequency band.

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