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Possibility of Influence of Midazolam Sedation on the Diagnosis of Brain Death: Concentrations of Active Metabolites after Cessation of Midazolam

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Midazolam and its active metabolites have a depressant effect on respiration and consciousness level, and therefore their effects should be considered in all patients for whom brain death testing is contemplated. The concentrations of midazolam and its active metabolites were measured in critically ill patients on a ventilator during and after continuous intravenous infusion of midazolam. Three days after cessation of midazolam infusion, the concentrations of midazolam and 1-hydroxymidazolam decreased to below the therapeutic range (100—1000 ng/ml) in all patients, although the concentrations of 1-hydroxymidazolam glucuronide remained extremely high in a patient who showed deteriorating renal function. The concentrations of 1-hydroxymidazolam glucuronide (19,497—29,761 ng/ml) were measured in this patient. When it is impossible to confirm factors consistent with irreversible brain death, such as the lack of cerebral blood flow, until 3 days after cessation of midazolam infusion, monitoring of the concentration of these substances should be carried out in all patients in whom suspicion exists prior to the evaluation of brain death. It is particularly imperative that monitoring of the 1-hydroxymidazolam glucuronide concentration be carried out in patients with poor renal function.

Key words——midazolam; pharmacokinetics; brain death testing; active metabolites; benzodiazepines

INTRODUCTION

The absence of evidence of drug intoxication or poisoning is one of the diagnostic criteria for the clinical diagnosis of brain death.[1] In other words, for a diagnosis of brain death, it is essential that the influences of drugs on brain function be excluded.

Since the 1980s, midazolam has been used frequently worldwide for induction of anesthesia and for long–term sedation of patients receiving mechanical ventilation, particularly in intensive care units (ICUs). Although there are many cases of brain death in ICUs, there are few reports concerning the influence of midazolam on the diagnosis of brain death. Evaluating the influence of midazolam on the diagnosis of brain death may be a common problem confronting every critical care department. All patients who may be brain dead are on ventilator management in ICUs. Mechanical ventilation decreases hepatic and renal blood flow, as well as glomerular filtration efficiency. Decreased hepatic and renal blood flow will decrease the clearance of drugs.[2] Thus the pharmacodynamic and pharmacokinetic properties of drugs are altered when patients are on a ventilator, and projections based on data derived from patients who are not on a ventilator or from normal volunteers are inappropriate.

In the present study, the concentrations of midazolam and its metabolites were measured in critically ill patients on a ventilator during and after continuous intravenous infusions of midazolam. Based on the pharmacokinetic parameters derived from these critically ill patients, the possible influence of midazolam, 1-hydroxymidazolam, and 1-hydroxymidazolam glucuronide on the diagnosis of brain death are discussed.

MATERIALS AND METHODS

Patients and Sedation This clinical study was carried out prospectively in medical ICU patients. All patients had been intubated and were being mechanically ventilated and sedated with continuous infusion

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of midazolam. The study protocol was approved by our institutional review board, and informed consent for participation was obtained from each patient or the patient's family before sedation. Their clinical features, duration of infusion, and infusion rate of midazolam are shown in Table 1. Sedation was started after the patients had been attached to a mechanical ventilator and midazolam was administered until mechanical ventilation was tolerated comfortably. The infusion rate was determined by the clinicians responsible for the patients' care and not influenced by the investigators. No other benzodiazepines were administered during the study period.

Blood Sampling and Drug Analysis Blood samples were obtained through an arterial cannula every day during the study period. Serum was separated and stored at −80°C until analysis. The concentrations of serum unconjugated midazolam and 1-hydroxymidazolam were determined with high-performance liquid chromatography (HPLC). The HPLC was assembled from the following components: LC-10AD constant-flow pump (Shimadzu), SIL-10Axl auto sample injector (Shimadzu), TSK-GEL Octyl-80TS column (150 mm×4.6 mm, Tosoh), and SPD-10A ultraviolet detector (242 nm, Shimadzu). The mobile phase was an acetonitrile-methanol 60 mM phosphate buffer (pH 6.5) (30 : 15 : 55 v/v). The flow rate was 1 ml/ min. Clotiazepam was used as the internal standard. The samples were extracted with ether and chromatographed on a reverse-phase column as described by Boukhabza et al.[3] with a slight modification. The 1-hydroxymidazolam glucuronide concentration was determined from aliquots after hydrolysis, which can be hydrolyzed to 1-hydroxymidazolam by β-glucuronidase for 18 h at 37°C. Midazolam was provided by Yamanouchi (Tokyo, Japan), and clotiazepam by Yoshitomi (Osaka, Japan). 1-Hydroxymidazolam was obtained from UFC Ltd. (Manchester, UK) and β-glucuronidase from Boehringer Mannheim. Other chemicals used were of analytical reagent grade. The quantitation limit was 8 ng/ml for both midazolam and 1-hydroxymidazolam.

Pharmacokinetic Analysis The concentrations in serum obtained after continuous intravenous infusion were used to calculate the elimination rate constant (ke) of midazolam based on a one-compartment open model with first-order kinetics. The elimination half-life ($t_{1/2}$) was calculated from ke using the equation: $t_{1/2}=0.693/\mathrm{ke}$. All data are presented as mean±SD.

RESULTS

The concentrations of midazolam and its active metabolites in nine patients (six men and three women) were studied. None of the patients were diagnosed as brain dead in this study. Continuous infusion of midazolam at 0.08—0.65 mg/kg body weight/h was maintained for long-term sedation during mechanical ventilation. The profiles of concentrations of the three substances in serum after cessation of infusion in nine patients are shown in Fig. 1. Three days after cessation of midazolam infusion, the concentrations of midazolam and 1-hydroxymidazolam

Table 1. Demographic Data

Patient	Sex	Age (y)	BW (kg)	Diagnosis	MDZ	
					Inf. rate (mg/kg/h)	Duration (days)
1	M	23	65	PF	0.15	8
2	M	57	72	SAH	0.30	3
3	M	69	65	SDH	0.08	2
4	F	28	46	BA	0.65	2
5	M	78	65	AAA, CRF	0.08	8
6	F	68	45	SDH	0.10	5
7	F	74	50	SAH	0.20	2
8	M	58	60	Burn	0.17	4
9	M	21	52	SDH	0.29	4
Mean±SD		52.9±22.7	57.8±9.7		0.2±0.2	4.2±2.4

BW: body weight, MDZ: midazolam,PF: pelvic fracture, SAH: subarachnoid hemorrhage, SDH: subdural hemorrhage, BA: bronchial asthma, AAA: abdominal aortic aneurysm, CRF: chronic renal failure.

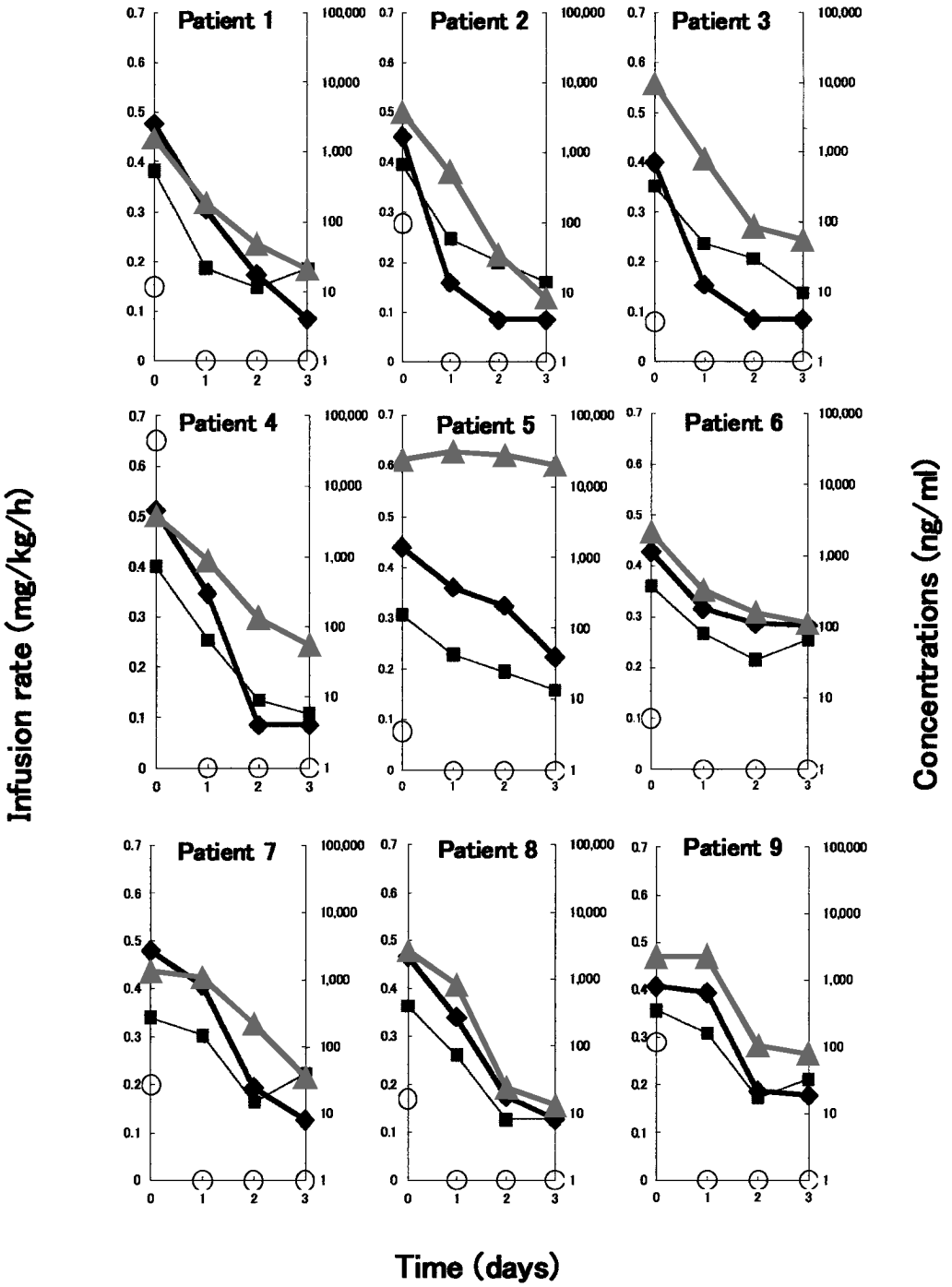

Fig. 1. Concentrations (ng/ml) of Midazolam (◆), 1-Hydroxymidazolam (■), 1-Hydroxymidazolam Glucuronide (▲) in Serum, and Infusion Rate (mg/kg Body Weight/h, ○) in Nine Patients under Intensive Care and Sedated with Midazolam Infusion

decreased to below the therapeutic range (100—1000 ng/ml[4]) in all subjects, although the concentration of 1-hydroxymidazolam glucuronide remained extremely high in patient 5 who showed deteriorating renal function (Table 2). Figure 2 shows the calculated terminal half-lives of midazolam, 1-hydroxymidazolam, and 1-hydroxymidazolam glucuronide in serum, which were in the range of 3.9—32.2 h, 7.3—33.2 h, and 5.5—205.1 h, respectively.

DISCUSSION

Midazolam is a water-soluble benzodiazepine with sedative-hypnotic, anticonvulsant, muscle relaxant, and anxiolytic effects.[5] Its elimination half-life is about 3 h, although a wide variation has been observed in healthy volunteers and patients.[6,7] Midazol-

Table 2. Biochemical Data and Creatinine Clearance In Critically Ill Patients

Patient	AST (IU/I)	ALT (IU/I)	SCR (mg/dl)	Albumin (g/dl)	Clcre (ml/min)
1	252	208	0.6	3.1	174.9
2	26	12	0.8	3.3	107.5
3	51	22	1.9	3	34.6
4	53	74	0.7	3.2	82.1
5	47	32	5.0	3.4	12.0
6	68	28	0.5	3.2	69.6
7	19	12	0.9	3	44.2
8	54	59	0.8	3.3	81.3
9	26	20	0.6	4.1	116.8
Mean±SD	66.2±71.5	51.9±62.2	1.3±1.4	3.3±0.3	80.3±49.0

AST: serum aspartate aminotransferase, ALT: serum alanine aminotransferase, SCR: serum creatinine, Clcre: creatinine clearance.

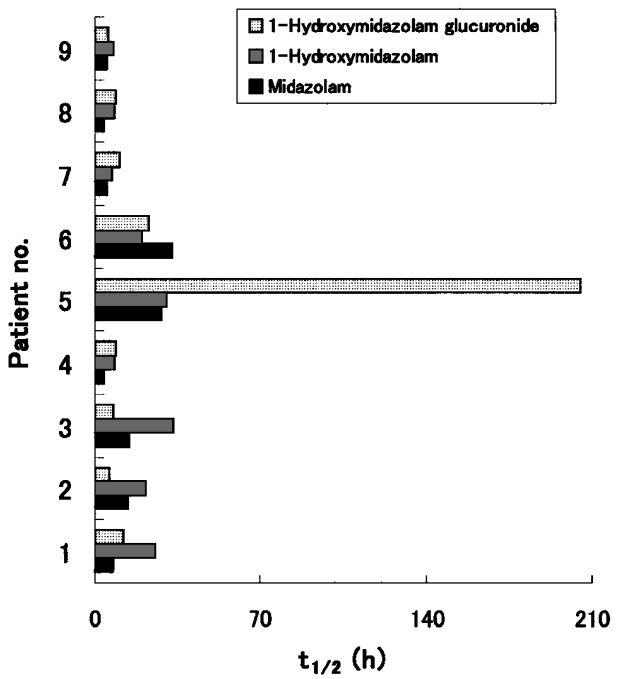

Fig. 2. Elimination Half-Lives ($t_{1/2}$) of Midazolam and Active Metabolites in Patients under Intensive Care

am is rapidly eliminated from the body by its transformation to three metabolites, 1-hydroxymidazolam (63% of the potency of midazolam), 4-hydroxymidazolam (inactive), and 1-hydroxymidazolam glucuronide (6% of the potency of midazolam).[8] Except for 4-hydroxymidazolam, they can induce prolonged coma and respiratory suppression. The first step in the metabolism of midazolam is hydroxylation by hepatic cytochrome P4503A4 (CYP3A4), followed by glucuronidation in the liver and then elimination from the kidneys.

The diagnosis of brain death must be beyond doubt, because it has crucial consequences for patients, relatives, and transplant programs. To make the diagnosis of brain death, three steps are necessary: first, determination of preconditions of comatose patients on a ventilator and positive diagnosis of the cause of coma; second, exclusion of reversible causes of apneic coma; and third, confirmation of brain stem areflexia and persistent apnea.[9] The following are mandatory criteria for the determination of brain death in Japan: deep coma, bilateral dilated pupils, brain stem areflexia, isoelectric electroencephalography, and apnea.[10]

When the clinical situation deteriorates to a level at which brain death is strongly suspected, the influence of drugs must be excluded before making the diagnosis of brain death.[11] Benzodiazepines suppress the respiratory center and affect the apnea test.[9] Similarly, midazolam and its active metabolites have a depressant effect on respiration and consciousness level, and therefore their effects should be considered in all patients for whom brain death testing is contemplated. The aim of this study was to investigate the possibility of the influence of midazolam, the most frequently used benzodiazepine for sedation in patients receiving mechanical ventilation, and its active metabolites on the diagnosis of brain death.

In all patients without renal failure, except for patient 5, it was possible to contemplate making the diagnosis of brain death 3 days after stopping midazolam infusion because the concentrations of midazolam had decreased to below the range (500—1500 ng/ml[12,13]) at which isoelectric electroen-

cephalography is induced, and those of its active metabolites had decreased to below the range where converted concentrations of midazolam render electroencephalography isoelectric. However, in patient 5, the diagnosis of brain death could not be made with certainty. The greatest change in the pharmacokinetics of midazolam occurred in patient 5 who had renal failure. Even though the concentrations of midazolam and 1-hydroxymidazolam decreased to below the therapeutic range, 1-hydroxymidazolam glucuronide, which penetrates the blood-brain barrier[14] and has substantial pharmacologic activity, was shown to accumulate in this patient. Prolonged sedation in critically ill patients after the withdrawal of midazolam despite a midazolam concentration well below the usual therapeutic threshold was reported.[6] In the report, high concentrations of 1-hydroxymidazolam glucuronide (5000—7000 ng/ml) were described. Higher concentrations of 1-hydroxymidazolam glucuronide (19,497—29,761 ng/ml) were measured during the 3 days after cessation of midazolam in patient 5. The possibility of accumulation of 1-hydroxymidazolam glucuronide in patients with renal failure who have prolonged sedation after cessation of midazolam can be considered. This finding is consistent with those in previous reports.[6,14,15]

In conclusion, until 3 days after stopping midazolam infusion, when it is impossible to confirm factors that are consistent with irreversible brain death, such as the lack of cerebral blood flow, monitoring of the concentration of these substances should be carried out in all patients in whom suspicion exists prior to the evaluation of brain death. It is particularly imperative that monitoring of the 1-hydroxymidazolam glucuronide concentration be carried out in patients with poor renal function.

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