—Regular Articles—

Impairment State of Cognitive Performance and the Affecting Factors in Outpatients Following Gastrointestinal Endoscopy after Single-dose Diazepam

Kenichi NAKAZONO,[a] Yoshiyuki WATANABE,[c] Shinichi NAKAYA,[c] Yoshie ASAMI,[d] Keisou MASUHARA,[d] Fumio ITOH,[c] and Hiroyasu OGATA*,[a,b]

Course of Clinical Pharmacy, Graduate School of Pharmaceutical Sciences,[a] and Department of Biopharmaceutics,[b] Meiji Pharmaceutical University, 2–522–1, Noshio, Kiyose, Tokyo 204–8588, Japan, Department of Gastroenterology and Hepatology, St. Marianna University School of Medicine,[c] and Department of Pharmacy, St. Marianna University School of Medicine Hospital,[d] 2–16–1, Sugao, Miyamae-ku, Kawasaki, Kanagawa 216–8511, Japan

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Diazepam is commonly used as premedicant for endoscopic procedures. Wide interindividual differences have been observed in the residual cognitive effects of the drug after gastrointestinal endoscopy. Our aim was to clarify the major factors, including pharmacokinetic factors, contributing to this wide variation in residual cognitive effect after gastrointestinal endoscopy in the study. Sixty-one outpatients undergoing gastrointestinal endoscopy participated in the study. Cognitive effects were evaluated in the diazepam group ($n=52$) by the digit symbol substitution test (DSST) twice before and 30 min after an intravenous administration of 5 mg diazepam; in the intervening time gastrointestinal endoscopy was performed. Plasma concentrations of diazepam were determined by HPLC. The control group ($n=9$) was tested by DSST in the same manner. The cognitive effects according to the change in DSST score was significantly decline in the diazepam group compared with the control group (by 0.2 versus -4.6; $P=0.014$). This prospective study confirmed that cognition was significantly impaired after gastrointestinal endoscopy by premedication to subjects with 5 mg diazepam. There were very wide variations in change in DSST score. However we could not identify the independent variables that best predicted DSST score difference in a multiple regression analysis for age, plasma albumin level, and plasma diazepam concentration 30 min after intravenous administration. We should pay attention to patients' individual states in cognitive performance following gastrointestinal endoscopy after single-dose diazepam.

Key words——diazepam; cognition; endoscopy; premedication; clinical study

INTRODUCTION

Gastrointestinal endoscopy is now performed for a multitude of clinical indications. This examination plays an important role in diagnosis by direct visualization, therapeutic procedures, and tissue biopsies. Therefore, we expect the procedure to be well tolerated and safe. Administration of sedative medication to patients before gastrointestinal endoscopy results in better patient acceptance and an increased probability of compliance if a repeat examination is necessary.[1] Diazepam, a benzodiazepine widely used as a sedative, muscle relaxant, anxiolytic, and anticonvulsant, is commonly used as a premedicant for endoscopic procedures and has been shown to be efficacious and safe.

Ideally, to optimize patient quality of life (QOL), any residual cognitive effects caused by diazepam administration should be minimized after the endoscopic procedure has been completed. However, wide interindividual differences in residual sedative effects have been observed after gastrointestinal endoscopy. The dose of diazepam should be adjusted to ensure sedation during the procedure and near-freedom from residual cognitive effects immediately after the procedure. Diazepam has been studied extensively as sedative agents for gastrointestinal endoscopy.[2—5] However, there have been no reports on the relationship between plasma benzodiazepine level and cognitive impairments (as measured by electroencephalogram or performance tasks) under gastrointestinal endoscopy.

To establish the optimal dose for each individual, we need to know the factors that determine the plasma concentration of diazepam (pharmacokinetic factors) and those that determine sensitivity to diazepam (pharmacodynamic factors).

Diazepam is metabolized mainly by hepatic

e-mail: hiroogat@my-pharm.ac.jp

cytochrome P450 enzymes, i.e., CYP3A4 and CYP2C19, to three major metabolites: *N*-desmethyldiazepam, temazepam, and oxazepam.[6,7] The pharmacogenetic entity of CYP2C19 shows a marked interethnic difference in incidence among poor metabolizers (PM); the PM frequency in the Japanese (18% to 23%) is greater than that in Caucasians (3% to 5%).[8] CYP2C19 has a wild-type allele and variant alleles at two sites, i.e. *CYP2C19*2* on exon 5 and *CYP2C19*3* on exon 4, in the Japanese, and a combination of both mutations leads to reduced activity of the enzyme.[9] Apart from differences in the generic entity of CYP2C19, previous studies have provided important data on the relative contributions of pharmacokinetic and pharmacodynamic factors in explaining altered clinical sensitivity to benzodiazepines. Such factors include advanced age, hepatic or renal disease, cigarette smoking, chronic benzodiazepine administration, and coadministration of ethanol or other centrally acting compounds.[10—12]

Therefore, our aim was to clarify the relevance of pharmacokinetic and pharmacodynamic factors to cognitive impairment after gastrointestinal endoscopy under diazepam.

PATIENTS AND METHODS

Selection of Subjects The study was carried out at the Endoscopy Unit at the St. Marianna University School of Medicine Hospital, Japan. Subjects aged 18 years or over and scheduled to undergo outpatient gastrointestinal endoscopy examination between November 2003 and March 2004 were considered eligible. The exclusion criteria were as follows: known allergy to diazepam; common use of benzodiazepines; anamnesis of epilepsy; need to undergo a surgical procedure; and pregnant or nursing. In addition, subjects with known cardiorespiratory or comorbid conditions that would preclude participation or with documented allergy to the anesthetic spray lidocaine were not included. Informed written institutional consent was obtained in all cases. The protocol for this study was approved by the Institutional Review Committee of the St. Marianna University School of Medicine Hospital.

All subjects accepted into the study underwent gastrointestinal endoscopy. They were divided into two groups, a diazepam group and control group, according to mainly patient's situation. The diazepam group was given 5 mg diazepam intravenously as a premedicant, and the control group received no diazepam. Both groups received the same basic endoscopic procedure.

Study Design After an overnight fast, subjects continued to fast until the procedure was performed. Subjects received a topical lidocaine spray before administration of diazepam. Five milligrams of diazepam was administered intravenously, after which subjects remained in left lateral recumbency and underwent gastrointestinal endoscopy. Blood samples were taken via an indwelling cannula in the subject's right forearm. One venous blood sample was obtained 30 min after diazepam administration, and two samples were collected randomly among the following times after administration: 5, 10, 15, 20, 40, and 50 min. DNA was isolated from leukocytes in the blood samples using a QIAamp DNA Blood Mini Kit (Qiagen, Tokyo, Japan), in accordance with the manufacturer's instructions. The separated plasma and isolated DNA were stored at −20℃ until analysis.

The digit symbol substitution test (DSST) was used to assess the cognitive level of each subject.[13—15] For each DSST, subjects were asked to accurately complete as many substitutions of symbols for numbers as possible in 120 seconds. Each test was scored as number of items correct. The DSST was done twice before and 30 min after diazepam administration (i.e. after the endoscopy had been completed). Control group subjects underwent the DSST on the same schedule.

Analytic Methods Diazepam, *N*-desmethyldiazepam, and alprazolam as an internal standard were obtained from Sigma-Aldrich (St Louis, MO). Acetonitrile (HPLC grade), methanol, and potassium dihydrogenphosphate were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). The plasma concentrations of diazepam were determined according to the HPLC method previously reported,[16] with a slight modification. The HPLC system consisted of an LC-9A HPLC pump and SPD-10A VP UV detector set at a wavelength of 250 nm (Shimadzu Co., Kyoto, Japan). We used an L-column ODS 5 μm (250×4.6 mm, Chemical Evaluation and Research Institute, Tokyo, Japan), which was maintained at 40 ℃. The mobile phase consisted of 0.05 M potassium phosphate buffer (pH 3.5), methanol, and acetonitrile (42 : 54 : 4, v/v/v). The flow rate was 1.0 ml/min. The lower detection limit of the assay, estimated on the basis of a signal-to-noise ratio of 3 : 1, was 10 ng/mL for diazepam and *N*-dimethyldiazepam. The

inter-day and intra-day variabilities for diazepam over concentrations of 50 to 200 ng/ml, based on five replicates, ranged from 2% to 4% and from 3% to 5%, respectively. These variabilities for *N*-desmethyldiazepam over the concentration of 20 to 100 ng/ml ranged from 2% to 8% and from 1% to 5%, respectively.

Genotyping The CYP2C19 wild-type allele (*CYP2C19*1*) and the two variant alleles, *CYP2C19*2* and *CYP2C19*3*, were identified by allele-specific polymerase chain reaction methods, using an SNP typing kit for Cytochrome P450 *2C19*2* and *2C19*3* (Toyobo Co., Ltd. Osaka, Japan).

Statistical Analysis Data were presented as mean ±SD. Characteristics of subjects in the diazepam and control groups were compared statistically by an unpaired Student's *t*-test or a Mann-Whitney test. DSST scores before and after diazepam administration were compared statistically by a paired Student's *t*-test or the Welch test. Statistical differences in mean plasma diazepam concentration among the three different CYP2C19 genotype groups were evaluated by analysis of variance (ANOVA). The relationship between DSST score difference and plasma diazepam concentration was tested by Spearman's correlation test. Multiple regression analysis was performed for subject age, logarithmic value of plasma diazepam concentration at 30 min after intravenous administration, and plasma albumin level, in order to identify the independent variables that best predicted the change in DSST score. $P<0.05$ was considered to be statistically significant. JMP software package version 5.0 J (SAS Institute Inc., Cary, NC) was used for statistical analysis.

RESULTS

Patient Enrollment A total of 70 subjects were enrolled in the study. Nine subjects withdrew: three refused to participate; two were not given the study protocol because of an oversight by medical staff; two could not perform DSST correctly; one could not administer diazepam accurately; and one was found to be taking diazepam for other medical purposes. Fifty-two subjects were entered into the final analysis as the diazepam group and 9 as the control group. All participants tolerated gastrointestinal endoscopy well and had no complications or adverse reactions to the diazepam.

Baseline Characteristics All of the subjects had no abnormal values in their clinical laboratory data. There were no significant differences in patient demographic and laboratory data between the diazepam and control groups (Table 1), although the control group tended to be slightly younger than the diazepam group.

Of the 52 subjects in the diazepam group, 18 (34.6%) were homozygous extensive metabolizers (Homo-EM), 22 (42.3%) were heterozygous extensive metabolizers (Het-EM), and 12 (23.1%) were poor metabolizers (PM) in terms of CYP2C19 polymorphism. Among these three subject groups (Homo-EM, Het-EM, and PM) there were no significant differences in demographic data or clinical characteristics (data not shown).

Digit Symbol Substitution Test The mean DSST scores before administration of diazepam were 46.3±18.0 points in the diazepam group and 57.1±25.4 points in the control group; this result was not significantly different between the two groups. At 30 min after diazepam administration the mean DSST score was 46.1±18.4 points. On the other hand, the mean DSST score in the control group after gastrointestinal endoscopy was 61.7±30.1 points. As shown by the mean scores in the control group, DSST scores seemed to increase when the test was repeated (Fig. 1).

The mean change in DSST score between the two tests (score before diazepam administration minus score after administration) was an increase of 0.2±0.7 points in the diazepam group. In contrast, on the contrary, there was a decrease of 4.6±1.7 points in the control group (Fig. 2). There was a significant

Table 1. Characteristics of Subjects Participated to the Study

	DZP group $n=52$	Control group $n=9$
Sex (male/female)	38/14	4/5
Age (years)	65.0±11.9	55.2±16.0
Body Weight (kg)	60.3±10.8	58.3±12.0
BMI (kg/m^2)	22.8±3.5	22.8±3.9
Smoker (%)	11 (21.2%)	2 (22.2%)
Alb (g/dl)	4.37±0.37	3.88±1.23
AST (IU/l)	24.5±8.8	14.6±2.9
ALT (IU/l)	23.4±14.1	16.0±8.4
Creatinine (mg/dl)	0.77±0.17	0.67±0.15
BUN (mg/dl)	14.8±3.97	12.8±3.87

DZP: Diazepam, Smoker was defined as more than 10 cigarettes per day, Each value is the mean±S.D.

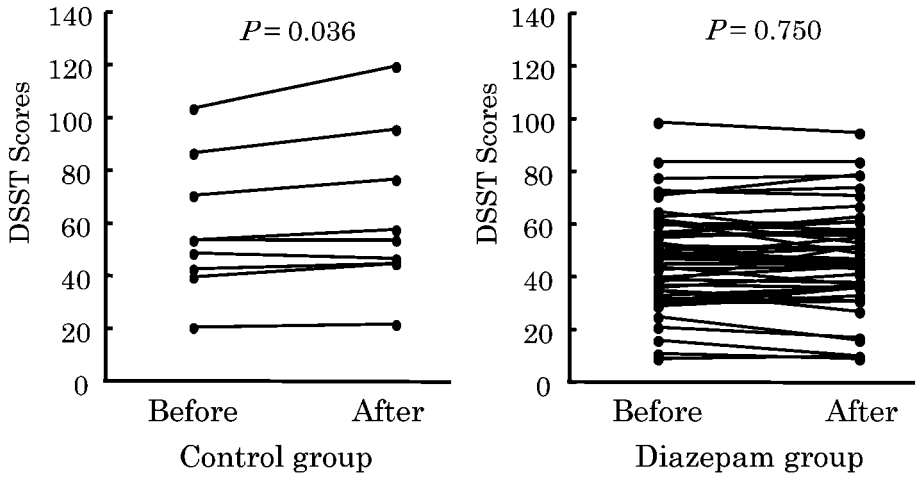

Fig. 1. Comparison of DSST Scores before and after Diazepam Administration
For statistical analysis we used a paired Student's *t*-test in the diazepam group and a Welch test in the control group.

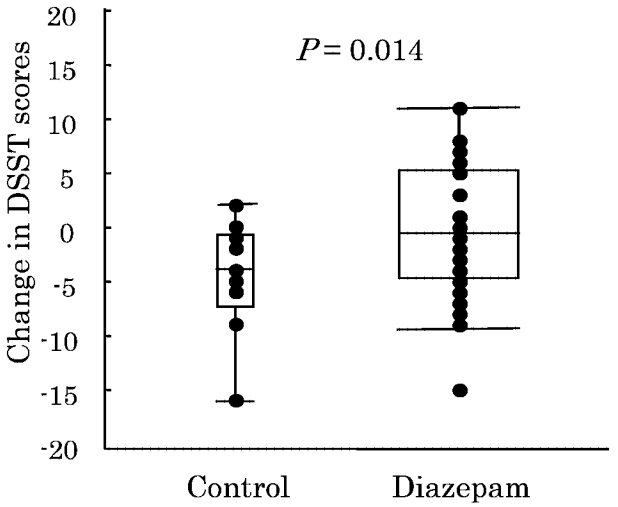

Fig. 2. Comparison of Changes in DSST Scores between the Diazepam and Control Groups
Data are expressed in box (three horizontal lines indicate 25th, 50th, and 75th percentiles of the data) and whisker (5th to 95th percentiles) plots. Statistic analysis was performed by the Mann-Whitney test.

difference between the mean changes in DSST score of the two groups when analyzed by the Mann-Whitney test (P=0.014). There was no significant difference in the mean changes in DSST score between smokers (more than 10 cigarettes per day) and nonsmokers (less than 10 cigarettes per day or none) (P =0.563; data not shown).

Plasma Diazepam Concentration After a single intravenous dose of 5 mg diazepam, drug concentrations in the plasma varied widely among subjects (Fig. 3). *N*-desmethyldiazepam could not be detected in any of the subjects over the sampling period. The mean plasma diazepam concentrations at 30 min after drug administration in Homo-EM, Het-EM, and PM were 268±70 ng/ml, 264±54 ng/ml, and 233±149 ng/ml, respectively (Fig. 4); these results were not significantly different (P=0.550) from each other.

Relationship between Changes in DSST Score, Plasma Concentration of Diazepam, and Other Demographic Data As factors that were potentially related to the variation in DSST score among subjects, we investigated subject age, plasma concentration of diazepam, and plasma albumin level. These factors showed no correlation with each other. The correlation between plasma diazepam concentration and change in DSST score was not significant (r= 0.071; P=0.621) (Fig. 5). On multiple regression analysis, the coefficient of determination did not show significant goodness of fit for the difference in DSST score (R^2=0.096; P=0.161), although logarithmic value of plasma diazepam concentration at 30 min (β =1.385; P=0.632) and age (β=0.082; P=0.374) showed proportional tendencies and plasma albumin level (β=−4.592; P=0.142) showed an inverse tendency (Table 2).

DISCUSSION

We assessed the subjects' cognitive levels immediately after gastrointestinal endoscopy had been performed. All subjects in the treatment group received the same dose of diazepam (5 mg) intravenously. Their cognitive levels, as assessed by the changes in their DSST scores from immediately before to 30 min after diazepam administration, showed very wide

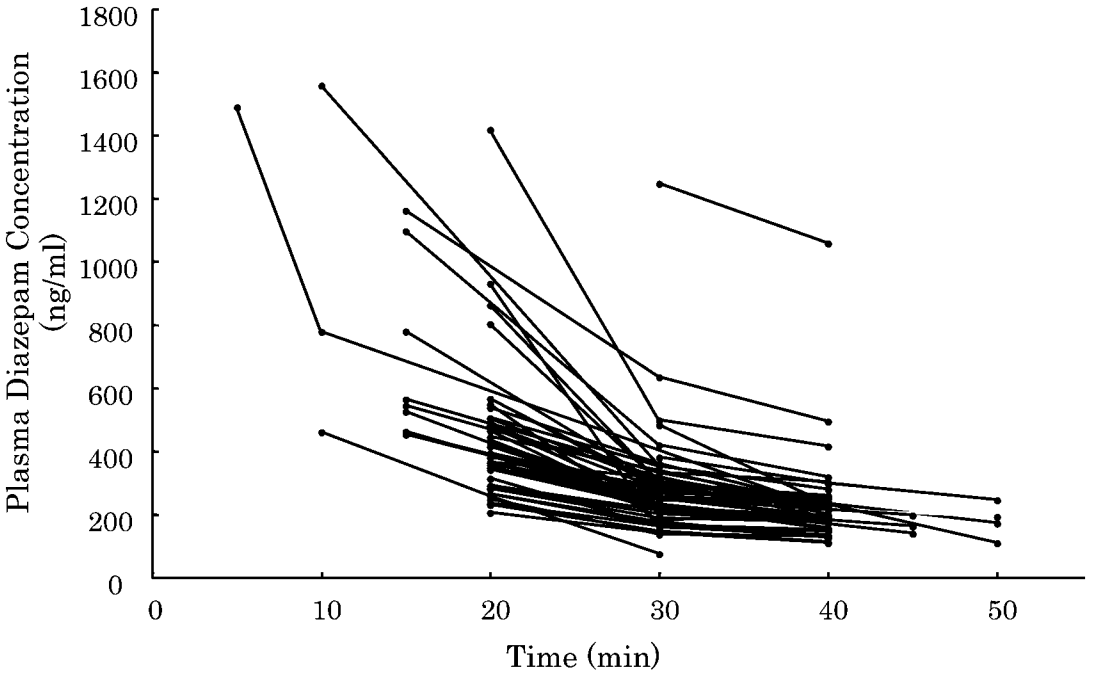

Fig. 3. Plasma Diazepam Concentration-Time Profiles after Intravenous Administration of 5 mg Diazepam

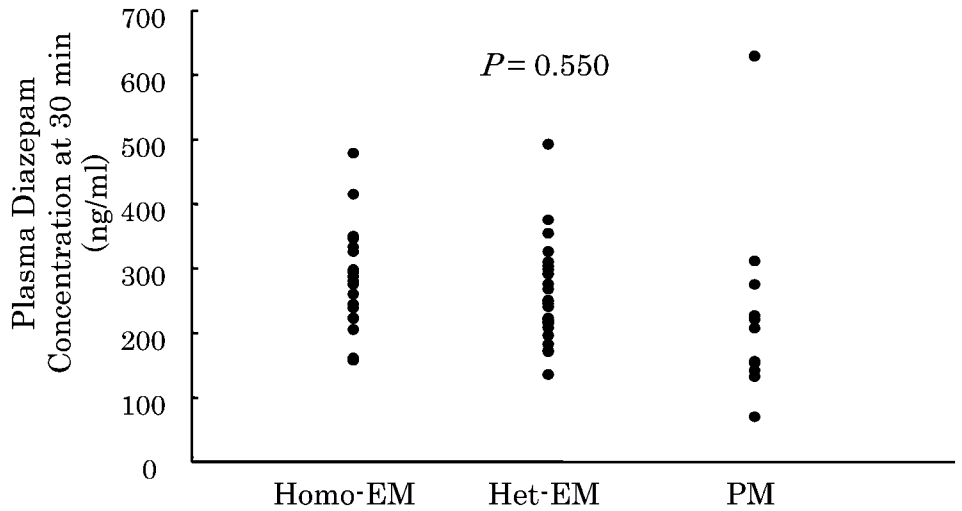

Fig. 4. Plasma Diazepam Concentrations in each of the Three CYP2C19 Polymorphism Groups 30 min after a Single Intravenous Dose of 5 mg Diazepam

Homo-EM: homozygous extensive metabolizer, Het-EM: heterozygous extensive metabolizer, PM: poor metabolizer. Mean plasma concentrations of diazepam were compared by ANOVA.

variations (Fig. 2). Subjects in the diazepam group showed a significantly higher level of residual impairment (Fig. 2) than those in the control group. The wide variation in differences in cognitive levels can be ascribed to two main factors: variation in the plasma concentration of diazepam in the biophase (pharmacokinetic variation) and variation in sensitivity to diazepam (pharmacodynamic variation) among subjects.

The plasma concentration and brain concentration of diazepam rapidly reach equilibrium after administration, and the onset of sedative action is rapid.[17—20] From these published data, the plasma concentration of diazepam can be regarded as equivalent to the biophase concentration, even when the drug is at the distribution phase of its pharmacokinetic profile. *N*-desmethyldiazepam, an active metabolite of diazepam, could not be detected during the study period.

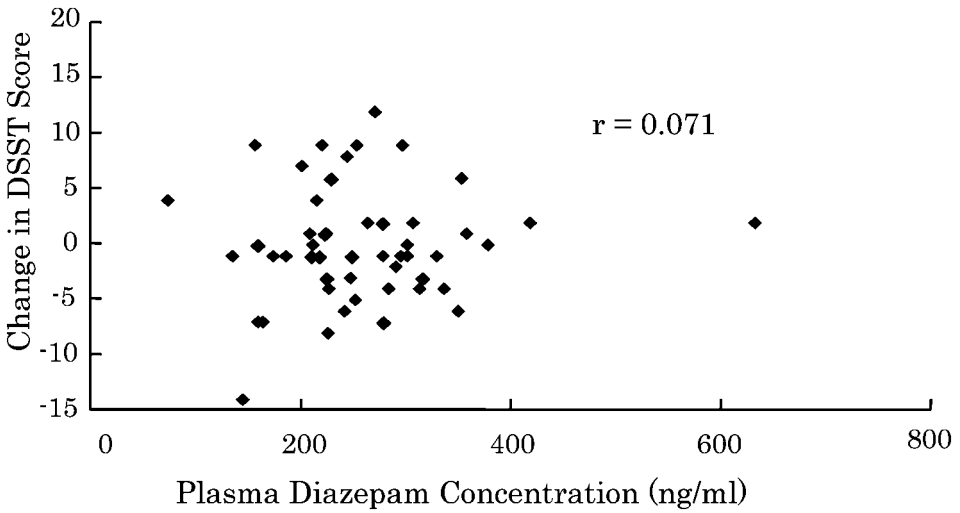

Fig. 5. Relationship between Change in DSST Score and Plasma Diazepam Concentration 30 min after a Single Intravenous Dose of 5 mg diazepam

Correlation coefficients (r) were compared by Spearman's correlation test.

Table 2. Relationship of the Change in DSST Score with Age, Plasma Concentration of Diazepam, and Plasma Albumin Levels Analyzed with Multiple Regression

	Regression coefficient	SEM	*p* value
Age	0.082	0.090	0.374
lnCp	1.385	2.850	0.632
Plasma albumin	−4.592	3.014	0.142

DSST: Digit Symbol Substitute Test, SEM: standard error of the mean, lnCp: logarithmic value of the plasma diazepam concentration at 30 min.

From these data, we can hypothesize that the variation in differences between pre- and post-diazepam cognitive levels among patients may be related to variations in the plasma concentration of diazepam.

In humans, diazepam is eliminated mainly via the hepatic metabolism, and this metabolism is mediated mainly by CYP2C19 and CYP3A4. CYP2C19 has a wild-type allele and variant alleles at two sites in Japanese people, i.e., *CYP2C19*2* on exon 5 and *CYP2C19*3* on exon 4, and a combination of both mutations leads to reduced activity of the enzyme.[9] The plasma elimination half-life value of diazepam in *CYP2C19*2/*2* was six times longer than in *CYP2C19*1/*1*,[21] which strongly suggests that diazepam metabolism may be mainly mediated by CYP2C19. Plasma concentrations of diazepam during the 50 min after administration showed a wide variation among subjects (Fig. 3). We compared plasma concentrations of diazepam 30 min after diazepam administration; the variation in concentration was very wide, ranging from 100 to 600 ng/ml, even though the diazepam had been administered intravenously. However, this wide variation could not be ascribed to the CYP2C19 genotypes of the subjects (Fig. 4). This result may in fact not be surprising, because the plasma diazepam concentration follows a two-compartment model, and the drug may still be in the distribution phase, not in the elimination phase, 30 min after intravenous injection. Another enzyme, CYP3A4, also take part diazepam metabolism in the liver.[6] Although numerous single nucleotide polymorphisms have been identified in CYP3A4,[22] the change of the enzymatic activity have not been related with these variants. In this report, we did not investigate the relationship between plasma concentration of diazepam and CYP3A4 variants.

The DSST score is a well-known psychometric marker, and it is commonly used to quantify the pharmacodynamic response associated with administration of benzodiazepines.[13,23] The relationship between plasma diazepam concentration and the DSST score difference from baseline is significantly correlated.[24] However, the relationship between plasma concentration of diazepam 30 min after administration and cognitive level after gastrointestinal endoscopy was not significant in this study (Fig. 5; $r=0.071$; $P=0.621$).

In principle, the pharmacological effect of the drug can be related to the drug's free concentration, not its

total concentration. Diazepam binds extensively to albumin in the plasma,[25] and the free fraction is thought to be inversely proportional to the plasma albumin concentration. Although the albumin concentration in the plasma did not range widely (from 3.3 to 4.8 g/dl) in our study, variation in the albumin concentration may complicate the relationship between total plasma concentration of diazepam and cognitive level.

The wide variation in cognitive levels may be induced mainly by variations in pharmacodynamic factors rather than pharmacokinetic factors. Previous reports have shown that the elderly exhibit increased sensitivity to the sedative effects of the benzodiazepines.[26,27] Elderly subjects require lower doses and lower plasma diazepam concentrations than younger patients to achieve the same degree of sedative effect.[26] Aging leads to an increase in pharmacological effects in humans and rats.[28—30]

To identify the independent variables that best predicted DSST score differences, we performed a multiple regression analysis for age, plasma albumin level, and plasma diazepam concentration 30 min after intravenous administration. We confirmed that these values changed independently of each other. In this analysis, we used the logarithmic value of the plasma concentration of diazepam, because pharmacological effect has often been related to the logarithmic value of drug concentration. This result in the multiple regression analysis was not significant as shown in Table 2.

We also have to consider the somewhat different conditions under which we observed the cognitive levels of our subjects. Most of the previous trials in humans have assessed the responses of subjects kept at rest during measurements. In contrast, our subjects had received a strong external stimulus—gastrointestinal endoscopy. The sedative effects of benzodiazepines seem to be disrupted under conditions of stimulation.[31,32] Therefore, the procedure of gastrointestinal endoscopy may have led to a discrepancy between our study and others in terms of the relationship between plasma concentration of diazepam and cognitive level.

In conclusion, this prospective study confirms that subjects given 5 mg diazepam before gastrointestinal endoscopy remained significantly cognitively impaired 30 min after diazepam administration. The cognitive levels showed very wide interindividual variations. However, we could not identify the independent variables that best predicted DSST score difference in a multiple regression analysis for age, plasma albumin level, and plasma diazepam concentration 30 min after intravenous administration. We should pay attention to patients' individual states in cognitive performance following gastrointestinal endoscopy after single-dose diazepam.

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