A Clinical Pharmacological Study to Evaluate the Sedative Effects of the Antihistaminic Drug, Diphenhydramine Hydrochloride (Drewell®) : Objective Evaluation of the Sedative Effects by Analysis of Saccadic Eye Movement

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The purpose of the present clinical study was to evaluate the sedative effects of Drewell® (50 mg of diphenhydramine hydrochloride). The study was conducted with a randomized, placebo-controlled, cross-over design in eight healthy male volunteers. Subjects took either a Drewell® or a matched placebo tablet on two occasions with more than a 6-day wash-out period. The sedative effects on central nervous system (CNS) were assessed using both objective evaluation (saccadic eye movement analysis) and subjective evaluation (Visual Analogue Scale : VAS). Statistical analyses for pharmacodynamic parameters were performed by repeated measures-ANOVA. Saccadic peak velocity (SPV), one of the sensitive parameters for sedative effects, showed a significant decline in the Drewell group compared with the placebo group. In the VAS evaluation, a sedative effect was also detected in the Drewell group. Despite some inter-individual differences of SPV values, in both the Drewell and the placebo sessions SPV baseline values within the same subject were quite similar. This suggests that subjects might have their own SPV values and those values might be reproducible. In addition, it was also confirmed that objective and quantitative evaluations of the sedative effects of Drewell® were possible using saccadic eye movement analysis.

Key words : diphenhydramine hydrochloride, objective evaluation, sedative effects, VAS, saccadic peak velocity

Introduction

Sleep is a basic function for human life. Insomnia is defined as a sleep disorder and its morbidity rate is relatively high. In 1997 a large-scale epidemiological study about sleep was conducted in Japan. According to the report, 21.4% of subjects enrolled in the research had a sleep disorder symptom¹⁾. Barbiturates and later non-barbiturates have been prescribed for insomnia treatment. Recently, however, the benzodiazepines and the non-benzodiazepines have been developed as sleeping pills and are frequently used due to low dependency and safety profiles^{2,3)}.

There are no parameters that directly show the

effect of a drug on the central nervous system (CNS). In the CNS research field, there is no “biomarker” such as blood pressure in hypertension or blood sugar level in diabetes research. Therefore, a self-assessment questionnaire, defined as a subjective evaluation, has generally been used in CNS research. The psychomotor performance test has often been used to evaluate CNS function as an objective and/or a quantitative evaluation. However, the peripheral nervous system and skeletal muscles are contaminated with “noise” when subjects are carrying out the test. Eye movement analysis picks up little such noise due to its simple regulation^{4,5)}. Therefore, eye movement analysis is useful to evaluate drug effects on the CNS directly

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with higher sensitivity⁴⁻⁸⁾.

Diphenhydramine hydrochloride is an antihistaminic drug which has been widely used to treat allergy reactions. However, the first generation antihistaminic drugs, such as diphenhydramine hydrochloride, pass through the blood brain barrier resulting in sedative side effects. In April 2003, Drewell® (SS Pharmacy Cooperation) appeared on the market as an over-the-counter (OTC) sleep-inducing drug in Japan. Drewell was originally based on the sedative side effect of diphenhydramine and was expected to induce and to maintain sleep. Clinical trials with Drewell were not conducted to investigate the sedative effects using objective and quantitative evaluation⁹⁾. The purpose of the present clinical study was to evaluate sedative effects of Drewell. This study was composed of a combination of two evaluation methods: saccadic eye movement analysis, which is used to measure the sedative effects objectively, quantitatively and sensitively; and the Visual Analogue Scale (VAS)¹⁰⁾, which is used to evaluate the subjective sedative effects. In addition, plasma drug concentrations were also measured. For pharmacokinetics-pharmacodynamics (PK/PD) analysis, the concentration-effect relationship in individual subjects was evaluated as well.

Methods

1. Design and plan of study

The protocol of this study was reviewed and approved by the Institutional Review Board (IRB) of the medical corporation Yakusen-kai Kannon-dai Clinic (approved date: Dec/01/2005) which examined the scientific and ethical adequacy. This study was conducted from February to March in 2005 complying with the World Medical Association Declaration of Helsinki and Ethical Principles for Medical Research Involving Human Subjects and Ethical Guidelines for Clinical Studies (July 30, 2003 Amended December 28, 2004, Ministry of Health, Labour and Welfare).

2. Subjects

Each subject gave informed consent to participate in this study based on a written informed consent form approved by the IRB revealing this study's object, methods, anticipated effects, freewill participation, and free-to-withdraw agreement.

Eight healthy male volunteers, whose physical examinations and clinical laboratory data indicated healthy/normal medical history, participated in this study. The subjects were prohibited to take any oral medications 2 weeks before the study and they were prohibited to drink alcohol during the study period. Subjects were instructed to enter the clinic the day before drug administration and they were prohibited to smoke until discharged from the clinic.

3. Design and schedule

A randomized, placebo-controlled crossover study was carried out with eight volunteers. Subjects were randomly divided into two groups; one group was administered Drewell at the first session (the Drewell preceding group: 4 subjects), and the other was administered matched placebo at the first session (the placebo preceding group: 4 subjects). The matched placebo tablet, provided by SS Pharmacy Cooperation, was prepared by replacement of diphenhydramine hydrochloride with lactose. Subjects were instructed to take two Drewell (diphenhydramine hydrochloride 25 mg) tablets or two matched placebo tablets in the early morning with 100 mL water; there was a six-day wash-out period between the two dosages. After administering the drugs, subjects were instructed to maintain a sitting position except when measuring vital signs, etc. and eating lunch. Eye movement, for an objective evaluation of pharmacodynamics, was performed before intake and 30, 60, 90, 120, 180, 240, 360 and 480 minutes after drug administration, 9 times. Results of measurement were automatically recorded on a hard disc, and an analysis was carried out after all study sessions. Subjects entered in VAS for a subjective evaluation of pharmacodynamics 5 minutes before eye movement measurement, 9 times. Blood withdrawals to measure plasma drug concentrations were carried out before intake and 60, 120, 180, 240, 360 and 480 minutes after drug administration, 7 times. To confirm the safety of this study, blood pressure and pulse rate were measured and a doctor examined subjects for adverse events before intake and at 120 and 480 minutes after administration. In consideration of the effects of needle injection for blood withdrawal on sedative evaluation, first we measured blood pressure and pulse rate, then VAS and

eye movement; blood withdrawal was carried out last. Eye movement was set up at "0" minute after drug administration.

4. Pharmacokinetic analysis

Blood sampling (6 mL) was carried out from the cubital vein with a disposable needle syringe following the schedule time. Blood was left for 30 minutes in the sample tube containing heparin. The samples were centrifuged at 3,000 rpm for 10 minutes at 4°C. Plasma was transferred to another sample tube and immediately frozen at -80°C. The specimens were transferred to the 2nd Department of Pharmacology, Showa University, School of Medicine, and plasma diphenhydramine concentrations were measured according to the method of a previous report from Simons KJ et al¹¹⁾ with a minor modification. Diphenhydramine was separated with CAPCELL PAK C18 SG120 (1.6 mm × 250 mm × 5 μm Shiseido Co., Ltd.) and detected using a fluorescence detector (Ex 233 nm, Em 286 nm) by high performance liquid chromatography (HPLC). The area under the plasma concentration versus time curve up to 8 hours after drug administration (AUC_{0-8}), maximum plasma concentration (C_{max}), time to maximum concentration (t_{max}) and elimination half life ($t_{1/2}$) of diphenhydramine were calculated.

5. Pharmacodynamic analysis

1) Eye movement analysis (objective evaluation)

Disposable electrodes (MEDICOTEST Co.) were used for electrooculographic registration. Electrodes were attached to both the outer canthi and the nasal root for reference ground. The skin was cleaned thoroughly at the contact points before electrodes were applied using skin-pure (Nihon Kohden Cooperation, Tokyo, Japan). Skin resistance was kept below 5 kΩ. Head movements were restrained using a fixed head support. The target consisted of an array of light emitting diodes on a bar fixed at 50 cm in front of the head support. Stimuli consisted of 20 step-like displacements of 30 degrees (+/- 15 degrees) at random intervals ranging from 3 to 5 seconds. Ten cycles of a series of random stepwise jumps were measured in saccadic eye movement. The following parameters were obtained for each saccade: saccadic reaction

time (latency), saccadic peak velocity (SPV), and inaccuracy (IAC). The average values of each parameter were measured and were set as parameters of eye movement. Latency is the value of reaction time, when the subject's eyes started to pursue a target. SPV is the value of the peak velocity of eye pursuing the target. IAC was defined as the absolute value of the difference between the stimulus angle and the corresponding saccadic, expressed as a percentage of the stimulus angle. To measure eye movement, we used the Nistagmo-stimulus system (SLE-5100, Nihon Kohden Cooperation, Tokyo, Japan), Nistagmo-guidance panel (PN-640G), and Nistagmo-amplifier. The amplified signal of eye movement data were converted by an analog-to-digital conversion system to digital and automatically input into microcomputer. CED-1401 (Cambridge Electronic Design Ltd., UK) was used as a waveform analyzer, and an analysis was carried out with software (CED). The measured data and the results of each point were automatically put into a hard disk. Measurement of one point of eye movement required about 3 minutes.

2) Subjective symptom analysis (subjective evaluation)

Visual Analogue Scale (VAS) was used to evaluate subjective symptoms after drug administration. VAS is based on the Bond & Lader scale¹⁰⁾ which was translated into Japanese. In the VAS questionnaire, there are 16 items of question pairs. Between the question pair, there is a 10 cm-long ungraded horizontal line. Subjects were instructed to mark the horizontal line with a check (/) as their own estimate of sedation. Subjects were asked to mark the check on the middle of the horizontal line if they felt normal. An ungraded 10 cm line was read "alert" at the left end and "drowsy" at the right end, for example. Other pairs in the VAS questionnaire sheet are shown in Table 1. The distance from the left edge (or right edge when the adjective describing a more sedate state was on the left) of the 10 cm line to the subject's mark was used for analysis. In this study we measured 9 items concerning alertness. The average values from nine items were calculated as the sedative score on each point. Nine question pairs and the direction of measurement are also shown in Table 1. New questionnaires were distributed to subjects in every time point, and subjects did not know their own

Table 1 Question pairs of visual analogue scale and direction of measurement

Alert (目が覚めている)	→	Drowsy (眠い)
Calm (落ち着いている)	—	Excited (興奮している)
Strong (精力的である)	→	Feeble (意志が弱くなっている)
Confused (混乱している)	←	Clear-headed (頭が冴えている)
Well-coordinated (身体がよく動く)	→	Clumsy (身体がぎこちない)
Lethargic (無気力である)	←	Energetic (活気に満ちている)
Contented (機嫌が良い)	—	Discontented (不機嫌である)
Troubled (困っている)	—	Tranquil (平静である)
Mentally slow (理解が遅い)	←	Quick-witted (理解が早い)
Tense (緊張している)	—	Relaxed (リラックスしている)
Attentive (集中している)	→	Dreamy (夢心地である)
Incompetent (うまくできない)	←	Proficient (うまく出来る)
Happy (幸せである)	—	Sad (悲しい)
Antagonistic (反抗的である)	—	Amicable (協力的である)
Interested (興味深い)	→	Bored (退屈である)
Withdrawn (孤立的になっている)	—	Gregarious (外交的になっている)

English is from the original words in the Bond & Lader scale ; Japanese translation is shown in parenthesis. Nine question pairs concerned with alertness are in boldface. The direction of measurement is shown as → or ←. The averages from nine alertness questions were calculated as the sedative score.

previous estimates.

6. Pharmacokinetics-Pharmacodynamics (PK/PD) relationship

For the analysis of relationships with pharmacokinetics and pharmacodynamics, statistics analysis was not performed. Individual concentration-effect relationships were discussed with the figure that was plotted with the pharmacodynamics on the vertical axis and the plasma diphenhydramine concentration on the horizontal axis.

7. Statistic analysis

The difference of subjects' backgrounds between the Drewell preceding group and the placebo preceding group was analyzed by the Student's t-test. Because of the crossover design, the time difference of Drewell administration or "time effects" may have affected the results. To confirm whether time effects had occurred or not, the pharmacokinetics parameters of the two groups (the Drewell preceding group and the placebo preceding group) were analyzed by the Student's t-test.

In the evaluation of pharmacodynamics, all results after the administration of Drewell were Drewell group data, and those after the matched placebo were the placebo group data. A statistical analysis of parametric data of pharmacodynamics

was performed by repeated measures-ANOVA : RM-ANOVA with time and effects as factors between the Drewell and placebo groups. When significant treatment effects or treatment x time interactions were found at the $p < 0.05$ level, the differences between Drewell and placebo at each time point were analyzed with a paired t-test. All statistic analyses were performed using the software package SPSS version 12 (SPSS Inc., Chicago, Illinois, USA).

Results

All subjects completed the study without any adverse events. The average age of eight Japanese healthy male volunteers was 24.1 ± 4.8 (20-34) years old (average $\pm$ SD, range). The average height was 172.7 ± 6.9 (162.3-180.8) cm, the average weight was 70.3 ± 6.0 (64.9-79.3) kg, and the average BMI was 23.3 ± 2.3 (20.1-27.5). There was no significant difference between the Drewell preceding group and the placebo preceding group in age, height, weight and BMI.

1. Pharmacokinetics of diphenhydramine hydrochloride

Individual pharmacokinetics parameters were calculated from the plasma diphenhydramine concentrations (Table 2). Subject 1 and subject 3

Table 2 Individual pharmacokinetics parameters after Drewell® administration

Subject No.	C _{max}	t _{max}	AUC ₀₋₈	t _{1/2}
1	145.7	3	784.2	4.7
2	59.3	4	298.2	4.1
3	116.4	2	553.2	5.0
4	66.3	2	351.0	9.0
5	71.1	2	321.9	3.1
6	69.1	2	313.7	4.2
7	72.0	2	310.1	5.7
8	58.1	3	319.6	6.8
Average of all subjects	82.2±31.5	2.5±0.8	406.5±173.9	5.3±1.8
Drewell preceding group	97.8±40.5	2.3±0.5	494.8±221.9	4.9±1.5
Placebo preceding group	66.7±5.5	2.5±1.0	318.2±22.8	5.7±2.3

Values are average±S.D. The value in the Drewell® preceding group was calculated from data of subjects 1, 3, 5 and 8. And the value in the placebo preceding group was calculated from the data of subjects 2, 4, 6 and 7. No significant differences between the Drewell preceding and placebo preceding group was observed ($p>0.05$ by Student *t*-test).

revealed high values of AUC₀₋₈ and C_{max} compared to the other subjects. There was no significant difference between the Drewell preceding group and the placebo preceding group in AUC₀₋₈, C_{max}, t_{max}, and t_{1/2}. Therefore, no time effects on diphenhydramine pharmacokinetics were detected in the present study.

2. Pharmacodynamics of diphenhydramine

1) Eye movement analysis

The changes of SPV values of saccadic eye movement after administration are shown in Figure 1. SPV in the Drewell group showed a significant decline compared with the placebo group ($p=0.022$ by RM-ANOVA). SPV values before drug administration were 520.09 ± 47.86 deg/sec in the Drewell group, 542.72 ± 50.93 deg/sec in placebo group. There was no statistically significant difference between the two groups ($p>0.05$ by Student's *t*-test). SPV values in the Drewell group from 30 minutes to 180 minutes after drug administration declined significantly ($p<0.05$ by paired *t*-test). The largest SPV change from the baseline in the Drewell group was observed at 120 minutes after drug administration 429.75 ± 96.43 deg/sec. There was no significant difference in latency ($p=0.237$ by RM-ANOVA) or in IAC ($p=0.094$ by RM-ANOVA).

Table 3 shows the individual values of SPV. "Pave" was the average of all SPV values during the placebo session. Pave and the SPV baseline

Fig. 1 Saccadic peak velocity (SPV) after administration of Drewell® (solid circles) and matched placebo (open square)

All data are shown as average±S.D. (n=8)

* : $p<0.05$ by paired *t*-test followed by repeated measured ANOVA ($p=0.022$)

value of the same subject were similar. The intra-individual difference in SPV was very small. However, there were some inter-individual differences in both Pave and SPV baseline values. The value of the largest SPV change from the baseline in the Drewell group is described as ΔE_{max}. The ΔE_{max} of subject 1 was -50.36 deg/sec and that of subject 4 was -245.60 deg/sec. Large inter-individual differences in the ΔE_{max} values were also observed. Typical cases of SPV and latency are shown in figure 2. The time course of the largest change (subject 4) and the smallest change (subject 1) in SPV are shown in the upper section of figure 2. In subject 1, the changes of SPV were nearly the same

Table 3 Individual effects on saccadic peak velocity after Drewell® administration

Subject No.	Baseline (deg/sec)	Pave (deg/sec)	E _{max} (deg/sec)	ΔE _{max} (deg/sec)	E _{tmax} (hr)	ΔE% from baseline (%)	ΔE% from Pave (%)
1	460.83	457.64	410.47	-50.36	2	-10.93	-11.00
2	521.42	537.38	442.69	-78.74	1.5	-15.10	-14.65
3	556.80	530.92	456.28	-100.52	2	-18.05	-18.93
4	485.49	469.14	239.89	-245.60	2	-50.59	-52.35
5	611.67	562.33	561.76	-49.91	1	-8.159	-8.87
6	481.70	563.89	344.71	-136.99	1	-28.44	-24.29
7	528.90	568.87	417.27	-111.63	1.5	-21.11	-19.62
8	513.92	509.74	422.41	-91.51	4	-17.81	-17.95
Average (S. D.)	520.09 (47.86)	524.99 (42.94)	411.93 (92.29)	-108.16 (62.87)	1.88 (0.95)	-21.27 (13.36)	-20.96 (13.61)

Baseline, SPV value at preadministration ; Pave, average of all SPV values during placebo session ; E_{max}, maximal measured effect on peak saccadic velocity ; E_{tmax}, time to of E_{max}.

The following parameters were calculated with the following formulas :

ΔE_{max}, Baseline-E_{max} ; ΔE% from baseline, expressed as ΔE_{max} percentage of baseline (ΔE_{max}/Baseline)*100 ; ΔE% from Pave, expressed as ΔE_{max} percentage of Pave (ΔE_{max}/Pave)*100

*Note : E_{max} is not used in the classic sense of a maximum possible effect.

Fig. 2 The typically opposite responses of SPV (upper figures) and latency (lower figures) after administration of Drewell® (solid circles) and matched placebo (open square)

whether administered Drewell or placebo. Typical changes of latency, which were not significant in this study, are shown on the lower section of Figure 2. Subject 5 had no changes of latency after Drewell administration. Compared with subject 5, a remarkable delay of latency at 2 and 3 hours after Drewell administration was observed in subject 3.

2) Visual Analogue Scale (VAS)

The sedative scores increased significantly in the Drewell group ($p=0.026$ by RM-ANOVA) signifying that the sedative effects of Drewell were also

detected by subjective evaluation. The paired t-test was carried out on each point ; the sedative scores of the Drewell group increased significantly from 30 minutes to 180 minutes after dosage.

3. Examination of pharmacokinetics-pharmacodynamics (PK/PD) relationship

Because of the remarkable changes of SPV values in the study, the plasma diphenhydramine concentrations versus SPV values were plotted for the pharmacokinetics-pharmacodynamics relationship.

In Figure 3, the concentration versus effects curves of the two subjects who had totally opposite reactions are plotted. Subject 4 had the largest SPV change, and subject 1 showed almost no change of SPV even though the plasma diphenhydramine concentration was the highest of 8 volunteers in this study (reference Table 2). In subject 4, SPV decreased with increasing plasma diphenhydramine concentration, and SPV recovered with decreasing plasma diphenhydramine concentration. On the other hand, the concentration versus effect line was almost flat in subject 1. There was no SPV change related to blood concentration levels.

Discussion

In April 2003, Drewell appeared on the market as an OTC sleep-inducing drug in Japan. Drewell uses the sedative side effect of diphenhydramine and is expected to maintain sleep as well. To date, clinical studies have been carried out with diphenhydramine hydrochloride for insomniac patients. The previous studies used a self-assessment questionnaire^{9,12)} as subjective evaluation for sedative effects and reported that the qualities of sleep as sleep latency or depth of sleep were improved. In CNS research field, there is no validated “biomarker” such as blood pressure in hypertension or blood sugar level in diabetes research. Subjective evaluation has a tendency to be influenced by the physical and mental condition of the study subject. Therefore, careful discussion is necessary to determine whether the measurements are quantitative and reproducible. SPV is a very sensitive measurement of CNS sedation and has the advantage of being beyond voluntary control. Each subject has his own peak velocity value; therefore, the peak velocity can be easily reproduced. In addition, the peak velocity is a measurement, which is able to evaluate CNS sedation objectively and quantitatively^{7,8)}. The study was performed with two evaluating methods in combination. One was the saccadic eye movement analysis as an objective assessment, and the other was VAS¹⁰⁾, which is able to evaluate the subjective sedative effects.

The pharmacokinetic parameters of diphenhydramine in this study were similar to those reported previously¹⁵⁾. However, AUC values of subject 1 and subject 3 were 784.2 ng·h/mL and 553.2 ng·h/mL, respectively (Table 2), and C_{max} values of

Fig. 3 Plasma diphenhydramine concentration effect curves for saccadic peak velocity in two typically opposite response subjects after Drewell® administration

subject 1 and subject 3 were 145.7 ng/mL and 116.4 ng/mL, respectively. These values were approximately 1.5 – 2.0 times higher compared to the other 6 subjects. The metabolism of diphenhydramine occurs principally in the liver after oral administration. Diphenhydramine receives the first-pass effect in the liver, and the bioavailability of the drug has been reported to be approximately 42 – 62%^{13–16)}. Some reports suggest that diphenhydramine might be metabolized by successive N-demethylations in man¹⁷⁾, and other reports suggest that diphenhydramine could inhibit CYP2D6^{18,19)}. However, detailed examination, using CYP isoforms for example, which involve diphenhydramine metabolism or a phenotype study in human subjects, has not been reported previously. In this study, the pharmacokinetics parameters of 2 subjects suggest the possible existence of CYP2D6 polymorphism although the details are still unclear.

Sedative side effects of the first generation antihistaminic drugs were caused by passage through the blood brain barrier and blockage of the histaminic nervous system that works to activate wakefulness and cognitive function. The strength of the sedative effects was related to the amount of passing drugs^{21–24)}; using positron emission tomography (PET), the passing amount of histamine H_1 -receptor antagonist was quantified by the receptor occupancy in the brain. According to reports of the first generation antihistaminic drugs, chlorpheniramine, ketotifen, and oxatomide blocked more than 50% of the H_1 -receptor in the brain. Diphenhydramine is one of the first generation of

antihistaminic drugs. Therefore, it is speculated that a similar extent of diphenhydramine might be passed through the blood brain barrier or as much as that after Drewell administration.

Eye movement analysis, for the pharmacodynamics evaluation of Drewell, showed that SPV in the Drewell group decreased significantly compared with the placebo group from 30 minutes to 180 minutes after administration. From this study SPV was confirmed as a very sensitive evaluation tool for CNS sedation. Furthermore, in this study there were no significant differences in changes of latency or IAC. However, typical reactions in latency were observed. As shown in the lower section of figure 3, there were no latency changes in subject 5 but remarkable changes in subject 3 were observed. These results show that the effect of diphenhydramine on saccadic eye movement parameters resulted in large inter-individual differences. It can be speculated that these large inter-individual differences were caused by the extent of action to the CNS area which regulates eye movement. However, the areas of CNS or detailed functions regulating saccadic eye movement are still uncertain. There is the possibility that inter-individual differences observed in this study were connected with the inter-individual differences of the passing rate and/or the affinity to histamine receptor in CNS. A complex study, which investigates the histamine H_1 -receptor occupancy and/or the binding area assessment by using PET, should clarify the inter-individual differences in detail.

VAS was also used to evaluate subjective sedative effects after Drewell administration. For further details, nine items in the Bond & Lader scale consisted of questions for mental sedation and physical sedation^{10,20}. The total sedative scores could be divided into mental sedation scores and physical sedation scores. As an additional comparative analysis, physical sedation and mental sedation between the Drewell and the placebo groups were analyzed separately. Similarly, for the total sedation score, both the mental sedation and the physical sedation scores were significantly increased in the Drewell group (mental sedation $p=0.014$, physical sedation $p=0.049$ by RM-ANOVA). The mental sedation score increased much more than the physical sedation score. Because of the subjective

characteristics of the VAS assessment, the answers could be influenced by the physical and the mental conditions of subjects, the circumstances at the study site and at the assessment time, the instructions for answering the VAS sheet, and so on. In a crossover study like this, careful consideration of the above issues should be made when discussing the VAS results. However, in the additional comparative analyses, there was a tendency for the mental sedation score to increase somewhat more than the score of physical sedation. There could be a possibility that muscular relaxation action affected the results. Barbiturates and benzodiazepines have muscular relaxation actions but not diphenhydramine hydrochlorides. Further investigation to compare the effect on physical sedation and mental sedation of diphenhydramine and drugs that have muscular relaxation profiles is needed.

A comparison between the Drewell and placebo groups showed that the baseline values of all saccadic eye movement parameters were similar within the same subject. In particular, the baseline values of SPV were intra-individually quite similar. Although there were some inter-individual differences in the baseline of SPV (range : 453 deg/sec-611 deg/sec), the average and a 95% confidence interval of ratio of the baseline value of Drewell by placebo (Drewell/placebo) was 0.96 (95% CI : 0.89, 1.03). There was no difference within the same subject in the baseline SPV value. Therefore, SPV was confirmed to be reproducible in each subject whenever measured because of his/her own individual SPV value. Similarly, the ratio of the baseline of the Drewell session by Pave (the average of all SPV values during the placebo session) was calculated for each subject. The average ratio was 0.99 (95% CI : 0.93, 1.05). In a pharmacodynamics study for CNS, it is preferable to use a crossover design as in the present study. SPV is reproducible in individuals and the placebo value is stable. SPV could be useful in a study in which it is difficult to adopt a crossover design due to the study characteristics.

There are some reports which evaluate sedative effects using the same eye movement analysis system. The mean $\Delta E\%$ from Pave (Table 3) after administration of diphenhydramine hydrochloride 50 mg was -21% in this study. Compared to previ-

ous studies, the degree was 78% of temazepam 20 mg (mean $\Delta E\%$ from Pave : -27%)²⁵⁾, and 68% of nitrazepam 5 mg (mean $\Delta E\%$ from Pave : -31.1%)⁴⁾. The subject who had the strongest sedative reaction showed -52.4% of $\Delta E\%$ from Pave. This extent of sedative effect was 1.9 times of temazepam 20 mg, and 1.7 times of nitrazepam 5 mg. This suggests that the quantitative profile of SPV can be widely applicable to the comparison of sedative effects among several drugs.

Conclusion

It was confirmed that the objective and quantitative evaluation of the sedative effects of Drewell was possible using saccadic eye movement analysis. SPV in each subject was reproducible and useful to evaluate the sedative effect. These characteristics of SPV were useful to compare the results of the present study with previous reports using the same analysis system.

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References

- 1) Uchiyama M, Kim K. Classification epidemiology and impact of sleep disorders. *Pharma Medica* 2000 ; **18**(11) : 13-8 (in Japanese).
- 2) Uchiyama M (Ed). Diagnosis and treatment of sleep disorders society. *The Correspondence Treatment Guidelines of Sleep Disorders*. Jihou Co., Ltd, 2002 : 99-101 (in Japanese).
- 3) Hukutome K, Kubo C. Pharmacotherapy of sleep disorders. *The Japanese J of Clinical and Experimental Medicine* 2005 ; **82**(5) : 771-4 (in Japanese).
- 4) Yasuda K, Uchida E, Uchida N, Oguma T, Shimada K, Koda T, Watanabe M, Kurata N, Yasuhara H. Evaluation of pharmacokinetic-pharmacodynamic relationship of nitrazepam using computerized eye movement analysis and visual analogue scales in healthy Japanese volunteers. *Jpn J Clin Pharmacol Ther* 1997 ; **28**(3) : 703-13 (in Japanese).
- 5) Koda T, Uchida E, Uchida N, Oguma T, Watanabe M, Yamada Y, Yasuda K, Yasuhara H. Gender differences in pharmacokinetics and pharmacodynamics of nitrazepam in healthy young Japanese volunteers. *Jpn J Clin Pharmacol Ther* 1999 ; **30**(2) : 443-55 (in Japanese).
- 6) van Gerven JM, Uchida E, Uchida N, et al. Pharmacodynamics and pharmacokinetics of a single oral dose of nitrazepam in healthy volunteers : an interethnic comparative study between Japanese and European volunteers. *J Clin Pharmacol* 1998 ; **38**(12) : 1129-36.
- 7) van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. *J Psychopharmacol* 1999 ; **13**(1) : 10-7.
- 8) Griffiths AN, Marshall RW, Richens A. Saccadic eye movement analysis as a measure of drug effects on human psychomotor performance. *Br J Clin Pharmacol* 1984 ; **18** : 73 S-82 S.
- 9) Kudo Y, Sakai T, Kawakita Y, et al. A clinical evaluation of hypnotic efficacy of antihistaminic, diphenhydramine hydrochloride, in Japan. *J of Clinical Therapeutics & Medicines* 1989 ; **5**(5) : 195-222 (in Japanese).
- 10) Bond A, Lader M. The use of analogue scales in rating subjective feelings. *Br J Med Psychol* 1974 ; **47** : 211-8.
- 11) Simons KJ, Watson WT, Martin TJ, Chen XY, Simons FE. Diphenhydramine : pharmacokinetics and pharmacodynamics in elderly adults, young adults, and children. *J Clin Pharmacol* 1990 ; **30**(7) : 665-71.
- 12) Rickels K, Morris RJ, Newman H, Rosenfeld H, Schiller H, Weinstock R. Diphenhydramine in insomniac family practice patients : a double-blind study. *J Clin Pharmacol* 1983 ; **23** : 235-42.
- 13) Higuchi N, Matumoto H. Pharmacokinetics of antihistaminic agents. *The Journal of Practical Pharmacy* 1987 ; **38**(6) : 837-45 (in Japanese).
- 14) Carruthers SG, Shoeman DW, Hignite CE, Azarnoff DL. Correlation between plasma diphenhydramine level and sedative and antihistamine effects. *Clin Pharmacol Ther* 1978 ; **23**(4) : 375-82.
- 15) Spector R, Choudhury AK, Chiang CK, Goldberg MJ, Ghoneim MM. Diphenhydramine in orientals and caucasians. *Clin Pharmacol Ther* 1980 ; **28**(2) : 229-34.
- 16) Berlinger WG, Goldberg MJ, Spector R, Chiang CK, Ghoneim M. Diphenhydramine : kinetics and psychomotor effects in elderly women. *Clin Pharmacol Ther* 1982 ; **32**(3) : 387-91.
- 17) Chang T, Okerholm RA, Glazko AJ. Identification of diphenhydramine (Benadryl) metabolites in human subjects. *Res Commun Chem Pathol Pharmacol* 1974 ; **9**(3) : 391-404.
- 18) Hamelin BA, Bouayad A, Drolet B, Gravel A, Turgeon J. In vitro characterization of cytochrome P450 2D6 inhibition by classic histamine H1 receptor antagonists. *Am Soc Pharmacol Exper Ther* 1998 ; **26**(6) : 536-9.
- 19) Sharma A, Pibarot P, Pilote S, Dumesnil JG, Arsenault M, Belanger PM, Meibohm B, Hamelin BA. Modulation of metoprolol pharmacokinetics and hemodynamics by diphenhydramine coadministration during exercise testing in healthy premenopausal women. *Am Soc Pharmacol Exper Ther* 2005 ; **313**(3) : 1172-81.
- 20) Norris H. The action of sedatives on brain stem oculomotor systems in man. *Neuropharmacology* 1971 ; **10**(21) : 181-91.
- 21) Yanai K, Tashiro M, Okamura N. Fexofenadine, the second-generation antihistamines, not passing through blood brain barrier. *Molecular Medicine* 2004 ; **41**(8) : 1008-15 (in Japanese).
- 22) Tashiro M, Sakurada Y, Iwabuchi K, et al. Central

- effects of fexofenadine and cetirizine : measurement of psychomotor performance, subjective sleepiness, and brain histamine H1-receptor occupancy using 11C-doxepin positron emission tomography. *J Clin Pharmacol* 2004 ; **44**(8) : 890-900.
- 23) Tashiro M, Sakurada Y, Yanai K. Sedative effects of fexofenadine and brain histamine H1-receptor occupancy : clinical pharmacological study of fexofenadine. *Jpn J Clin Pharmacol Ther* 2002 ; **33**(1) : 163 S-4 S (in Japanese).
- 24) Tagawa M, Yanai K, Watanabe T. Elucidation of mechanism in central sedative effects occurrence by antihistaminines : evaluation of blood-brain barrier transport of ebastine using positron emission tomography. *Progress in Medicine* 2001 ; **21**(12) : 2882-8 (in Japanese).
- 25) van Steveninck AL, Verver S, Schoemaker HC, Pieters MSM, Kroon R, Breimer DD, Cohen AF. Effects of temazepam on saccadic eye movements : concentration-effect relationships in individual volunteers. *Clin Pharmacol Ther* 1992 ; **52**(4) : 402-8.