Factors Influencing Serum Concentration of Zonisamide in Epileptic Patients

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The relationship between daily dose and serum concentration of zonisamide (ZNS) and the effects of patient age on the serum level/dose (*L/D*) ratio for ZNS were studied in epileptic patients (mean age ± S.D. = 10.6 ± 6.2 years) who chronically received ZNS. The influence of phenytoin (PHT), phenobarbital (PB), carbamazepine (CBZ) and valproic acid (VPA) on the serum protein binding of ZNS *in vitro* and the correlation between total and unbound serum levels of ZNS in partients were also examined. Significant correlations were obtained between daily dose per body weight or per body surface area and serum level of ZNS. The correlation coefficient of the latter was higher than that of the former. There was no effect of age on the *L/D* ratio on the basis of body surface area, whereas that on the basis of body weight increased significantly with age. No significant increase in the free fraction of ZNS was observed in the presence of PHT, PB and CBZ except VPA *in vitro*. There were significant correlations between total and unbound serum levels of ZNS in the two patient groups coadministered with and without VPA. Although the free fraction of ZNS in the former was significantly higher than that of the latter, the increase was small. These results suggest that dosage regimens on the basis of body surface area would be more accurate than those on a body weight basis and that there is little effect of other antiepileptics on the serum protein binding of ZNS.

Keywords zonisamide; refractory seizure; serum level/dose ratio; body surface area; age; protein binding

Antiepileptic drugs have a narrow margin of safety between effective and toxic levels. Furthermore, long-term medication and/or multi-drug therapy cannot be avoided because of the characteristics of convulsive disorders. Therefore, several confounding factors (*e.g.*, age of patients, concurrent drugs) influencing serum drug concentrations must be taken into account when making dosage adjustments.

Zonisamide (1,2-benzisoxazole-3-methanesulfonamide, ZNS), a newly developed antiepileptic drug, has been reported to have an anticonvulsant effect in experimental animals,[1,2] and to show clinical efficacy for various types of epilepsy, especially refractory seizures.[3,4] However, factors affecting the serum levels of ZNS have not yet been fully elucidated,[5] and the pharmacokinetic and pharmacodynamic behavior of ZNS including its effective serum levels still remain unclear.

The present study was carried out to ascertain the relationship between daily dose and serum concentration of ZNS and the effects of age on the ratio of serum level to dose (*L/D*) for ZNS in epileptic patients who chronically received ZNS. In addition, the influence of phenytoin (PHT), phenobarbital (PB), carbamazepine (CBZ) and valproic acid (VPA) on the serum protein binding of ZNS *in vitro* and the correlation between total and unbound serum levels of ZNS in patients were examined.

Materials and Methods

Patient Population One-hundred and seven outpatients (mean age ± S.D. = 10.6 ± 6.2 years; range = 0.6—31.1 years) at Hiroshima University Hospital participated in this study after giving informed consent. All were treated with ZNS (Excegran®, conventional tablets or powder, Dainippon Pharmaceutical Co., Tokyo, Japan) at a uniform dosage in two or three divided doses for refractory seizures (mean daily dose ± S.D. = 5.7 ± 3.4 mg/kg, range 1.2—23.1 mg/kg; 158.2 ± 96.7 mg/m^2, range 29.6—534.9 mg/m^2). The total body surface area was estimated using the equation reported by DuBois and DuBois[6]: surface area (m^2) = weight(kg)$^{0.425}$ · height(cm)$^{0.725}$ · 0.007184. Since the duration of treatment was in exess of two weeks in all patients, it was considered that steady-state serum levels had been achieved. The present study was proposed as add-on medication in the treatment of refractory seizures, so that one to three other antiepileptics (PHT, PB, CBZ or VPA) were coadministered to all patients. Patients suspected of noncompliance were excluded from the study.

Blood samples were collected between 09:00 and 12:00 h during clinic visits (2—5 h after the last oral dose). The fluctuations of steady-state serum levels of ZNS at different sampling times were considered to be negligible because of its long elimination half-life.[5,7] Serum samples were obtained by centrifugation. The ultrafiltrates for the determination of free ZNS levels were prepared by an Amicon Centrifree TM micropartition system (Amicon Co., Danvers, MA, U.S.A.) in accordance with the manufacturer's instructions. All samples were stored at −20 °C until analyzed.

***In Vitro* Study of Serum Protein Binding for ZNS** For determination of the extent of the protein binding of ZNS at various concentrations *in vitro*, ZNS was added to each serum obtained from six healthy male volunteers to make a concentration of 10, 25, 50, or 100 μg/ml.

The effects of other antiepileptic drugs on the protein binding of ZNS in the serum were determined by experiments performed at a near upper end of the therapeutic range for each drug: 25 μg/ml for ZNS, 20 μg/ml for PHT (Dainippon Pharmaceutical Co.), 10 μg/ml for CBZ (Ciba-Geigy Ltd., Hyogo, Japan), 25 μg/ml for PB (Sankyo Pharmaceutical Co., Tokyo, Japan), or 100 μg/ml for VPA (Dainippon Pharmaceutical Co.).

The mixture was voltexed for 30 s and then incubated for 1 h at 37 °C. A part of each serum sample and the rest were used for the determination of total and free levels of ZNS, respectively.

Analytical Method for ZNS in the Serum Serum levels of ZNS were determined by high-pressure liquid chromatography (HPLC) using a partial modification of the methods of Noguchi *et al.*[8]: briefly, 0.2 ml of serum (or ultrafiltrate) sample was mixed with 0.3 ml of 0.1 M phosphate buffer (pH 7.4). After adding 20 μl of a 200-μg/ml solution of *N,N*-dimethyl-ZNS (Dainippon Pharmaceutical Co.) as an internal standard and 5 ml of a chloroform–ethanol mixture (10 : 1) to each sample, the resulting mixture was shaken for 15 min and centrifuged for 15 min at 3000 rpm. The organic layer (4 ml) was transferred to another tube and then evaporated to dryness. The residue was dissolved with 50 μl of ethanol, and a 20-μl aliquot of this solution was injected into the HPLC system. The HPLC system consisted of a solvent delivery pump (Shimadzu LC-6A, Kyoto, Japan), a variable wave-length ultraviolet (UV) detector (Shimadzu spectrophotometer SPD-6A, 235 nm), an integrator (Shimadzu chromatopack C-R3A), and a reversed-phase column (Develosil ODS-7, 4.6 × 250 mm, Chemco Co., Tokyo, Japan). The mobile phase consisted of a 70 : 11 : 10 mixture of 1% acetic acid, isopropyl alcohol and acetonitrile, and was pumped at a rate of 1.0 ml/min. Column temperature was kept at 35 °C. The minimum

detectable concentration of ZNS was 0.5 μg/ml.

Data Analysis Relationships between variables were analyzed by least-squares linear regression analysis. The statistical significance of the difference between groups was determined by the unpaired t test or paired t test. A p value of <0.05 was considered to be statistically significant.

Results and Discussion

Relationship between Daily Dose and Serum Level of ZNS Significant positive correlation was obtained between ZNS dose per body weight and steady-state serum concentration of ZNS (Fig. 1A, $r=0.793$, $p<0.001$) in spite of multi-drug therapy. Previous investigators have reported a good correlation between daily dose per body weight and serum level of ZNS,[3,4] being in good agreement with our results. Wagner *et al.*,[9] however, reported a nonlinear relationship of ZNS when studied intraindividually. We do not have enough data in this experiment to elucidate the intraindividual relationship, and further detailed study should be made to clarify this.

In the present study, most patients were under eighteen years of age, so that there were large inter- and intra-individual differences in their growth and development. Since most drugs distribute throughout the extracellular water (ECW) space in order to reach their receptors, the size of the ECW compartment is of importance for the ultimate concentration of these drugs. It is thus considered that dosage regimens on the basis of ECW space would be more accurate than those on a body weight basis. Furthermore, it is well known that the ECW space correlates closely with body surface area.[10] Therefore, the relationship between daily dose per body surface area and serum level of ZNS was also investigated, and showed a better correlation ($r=0.872$, $p<0.001$) than that obtained with ZNS dose per body weight (Fig. 1B). These results suggest that body surface area could be a better indicator for the determination of ZNS daily dose in children.

Effects of Age on Serum Level/Dose (L/D) Ratio of ZNS A number of studies have examined the L/D ratios with the purpose of using such data to make adjustments to dosage regimens so that therapy can be tailored to individual patients.[11,12] In addition, the L/D ratios of a drug have been reported to increase with age.[13-16] Therefore, the effects of age on the L/D ratios of ZNS were studied (Table I). Ratios on the basis of body weight in elder groups (aged 12.0—17.9 years and above 18.0 years) were higher than those in younger groups (aged 0.6—5.9 years and 6.0—11.9 years). These findings coincided with those reported by previous investigators.[13-16] By contrast, no significant difference in the L/D ratios on the basis of body surface area was observed among the groups examined, indicating no effect of age on these ratios where the dosage is expressed as mg/m^2/d. No correlation in the L/D ratio was found between body surface area and age, whereas a significant relationship ($r=0.445$, $p<0.001$) was observed between age and the L/D ratio on the basis of body weight. From these results, it is suggested that body surface area would be a more reliable indicator when making dose adjustments as stated earlier. Careful drug monitoring should, however, be made for each epileptic patient because of large interindividual variations as shown in Fig. 1 and Table I.

***In Vitro* Serum Protein Binding of ZNS** Unbound drug concentrations in the blood are closely associated with the clinical efficacy and toxicity of the drug.[17,18] In addition, the free fraction of VPA increases due to its saturable protein binding.[19,20] Therefore, the extent of the protein binding of ZNS was determined at various concentrations of ZNS *in vitro* (Table II). The free fractions of ZNS at concentrations of 10—100 μg/ml ranged from 60.2 to 61.7%, indicating a nonsaturable binding phenomenon. Cornford and Landon[21] reported that ZNS is highly bound to human serum albumin. Total serum levels of ZNS examined were equal to 47—470 μM, which was less than the albumin concentration in the serum (530—830 μM). It was consequently considered that few changes in the free fraction of ZNS could be attributed to the presence of nonsaturated binding sites on human albumin.

Multi-drug therapy is the usual treatment for refractory seizures, so the effects of PHT, PB, CBZ and VPA on the serum protein binding of ZNS were studied *in vitro* (Table

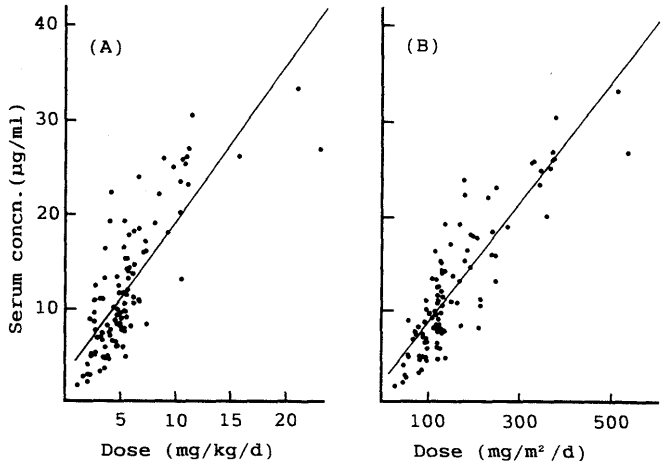

Fig. 1. Correlations between ZNS Daily Dose per Body Weight (A) or per Body Surface Area (B) and ZNS Serum Level

(A) $Y=1.635\cdot X+2.851$, $r=0.793$, $p<0.001$, $N=107$. (B) $Y=0.063\cdot X+2.195$, $r=0.872$, $p<0.001$, $N=107$.

TABLE I. Ratios of Serum Level to Daily Dose per Body Weight and per Body Surface Area for ZNS[a]

Age (years)	Number of patients	L/D_w[b]	L/D_s[c]
0.6— 5.9	27	1.89±0.53	0.080±0.023
6.0—11.9	40	1.86±0.64	0.077±0.024
12.0—17.9	28	2.48±0.79[d]	0.075±0.023
18.0<	12	3.36±1.05[e]	0.092±0.025

a) Each value represents the mean±S.D. *b*) L=steady-state serum level (in μg/ml). D_w=dose based on body weight (in mg/kg/d). *c*) D_s=dose based on body surface area (in mg/m^2/d). *d*) $p<0.01$, *vs.* aged 0.6—5.9 years group and $p<0.001$, *vs.* aged 6.0—11.9 years group. *e*) $p<0.001$, *vs.* aged 0.6—5.9 years and 6.0—11.9 years groups, respectively.

TABLE II. Serum Protein Binding of ZNS at Various Concentrations of ZNS

ZNS (μg/ml)	Free fraction of ZNS[a] (%)
10	60.2±2.7
25	60.6±2.5
50	61.6±3.1
100	61.7±1.8

a) Each value represents the mean±S.D. of six subjects.

TABLE III. Serum Protein Binding of ZNS in the Presence of Other Antiepileptics

ZNS (μg/ml)	Other antiepileptics (μg/ml)	Free fraction of ZNS[a] (%)
25	—	60.6±2.5
25	PHT 20	61.2±2.3
25	PB 25	62.9±2.6
25	CBZ 10	63.6±2.5
25	VPA 100	63.6±2.3[b]

a) Each value represents the mean±S.D. of six subjects. *b*) $p<0.05$, *vs.* corresponding control.

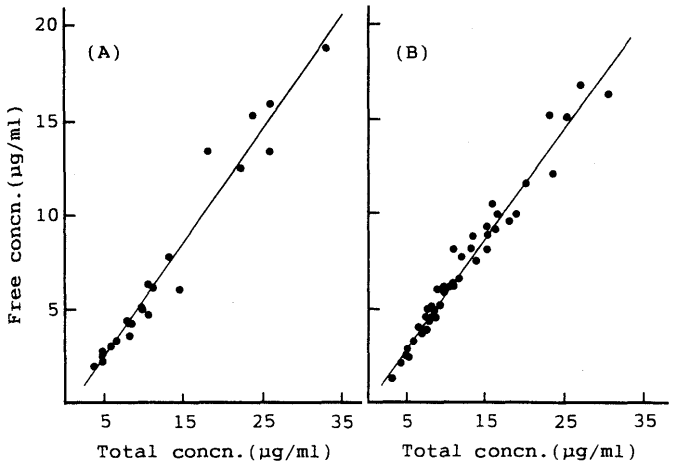

Fig. 2. Correlations between Total and Unbound Serum Levels of ZNS without (A) and with VPA (B)

(A) $Y=0.598\cdot X-0.506$, $r=0.980$, $p<0.001$, $N=23$. (B) $Y=0.576\cdot X+0.118$, $r=0.983$, $p<0.001$, $N=45$.

III). There was no significant difference in the unbound fraction of ZNS in the presence of PHT, PB and CBZ, but significant increase ($p<0.05$) was observed in the free fraction of ZNS when VPA was present, suggesting that more tightly bound VPA might be expected to cause significant displacement of ZNS. The increase, however, was small (approximately 3%). There thus appears to be little effect of other antiepileptics on the serum protein binding of ZNS.

Relationship between Total and Unbound Serum Concentrations of ZNS in Patients The correlations between total and unbound serum levels of ZNS in 68 patients whose free levels could be determined were investigated. Since a significant increase was observed in the free fraction of ZNS when VPA was present *in vitro*, these patients were allocated to two groups: coadministered with and without VPA. Significant linear relationships ($p<0.001$, respectively) were observed between the two in both groups, indicating that the protein binding of ZNS is not saturable over the range of concentrations observed (Fig. 2). In addition, the overall mean±S.D. of 58.3±5.5% in the free fraction of ZNS with VPA was significantly higher ($p<0.05$) than that without VPA (54.3±6.9%) as well as the results obtained by *in vitro* study (Table III). The increase was small, however, the influence of VPA on the clinical efficacy of ZNS should be further evaluated.

Conclusion

This study, using ZNS dose-level data from epileptic patients received co-medication and the protein binding data of ZNS examined *in vitro*, showed the following: (1) the correlation between daily dose per body surface area and serum level of ZNS was more significant than that found with daily dose per body weight. There was also no effect of age on the L/D ratios on the basis of body surface area, suggesting that dosage regimens determined with this factor would be more accurate than those on a body weight basis; (2) there was no alteration in the serum protein binding of ZNS within the concentration range examined *in vitro*; (3) there was no marked effect of other antiepileptics except VPA on the serum protein binding of ZNS *in vitro*; and (4) apparently linear relationships were observed between total and unbound serum levels of ZNS in patients concurrently administered with and without VPA.

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