Pharmacokinetics of Zonisamide; Saturable Distribution into Human and Rat Erythrocytes and into Rat Brain

Kohshi NISHIGUCHI,* Noriaki OHNISHI,* Seigo IWAKAWA,* Jiro YAGI,** Shin-ichi NAKAYAMA,** Satoshi TAKADA,** Hajime NAKAMURA,** Teruyoshi YOKOYAMA,*** and Katsuhiko OKUMURA*.*

Departments of Hospital Pharmacy* and Pediatrics, ** School of Medicine, Kobe University, Chuo-ku, Kobe 650, Japan and Department of Hospital Pharmacy, Kyoto Pharmaceutical University, *** 5 Misasaginakauchi-cho, Yamashina-ku, Kyoto 607, Japan

(Received March 13, 1992)

The distribution of zonisamide, a new antiepileptic drug, in erythrocytes and in brain was studied to clarify the factors influencing its distribution in epileptic patients. In both humans and rats, zonisamide was concentrated significantly in erythrocytes in a saturable manner. When the effective concentration of zonisamide in serum was compared with that in blood in nine refractory epileptic patients taking zonisamide chronically, the variation in effective serum concentration was significantly larger than that in blood concentration. In rats, the distribution in the brain also showed saturability. These results suggest that differences in saturable binding to various tissues may contribute to the wide variation that occurs in the effective serum concentration of zonisamide in epileptic patients and that monitoring of the blood concentration of zonisamide may provide useful information for treatment with this drug.

Keywords — zonisamide (ZNA); pharmacokinetics; saturable distribution; erythrocyte; brain; refractory seizure; epilepsy; human; rat

Introduction

Zonisamide (1,2-benzisoxazole-3-methanesulfonamide, ZNA), a new antiepileptic drug used in Japan, is an effective anticonvulsant in various types of epilepsy, particularly in refractory seizures.1-3) Matsumoto et al.4) demonstrated the saturable binding of ZNA to erythrocytes and suggested that binding to carbonic anhydrase contributed to this saturable binding.

Three clinical studies of ZNA have reported linear pharmacokinetics in the therapeutic dose range.5-7) However, Wagner et al.8) and Wilensky et al.9) indicated that ZNA had nonlinear pharmacokinetics in adult epileptic patients. The effective serum concentration of ZNA in humans has been reported to be 10—30 μg/ml. However, the relationship between the pharmacokinetics and pharmacodynamics of ZNA still remains unclear. In a previous study, we reported that the effective serum concentration of ZNA was rather variable in pediatric patients.10) In one patient who responded well to ZNA, the effective serum concentration was less than 5μg/ml.

We have already reported the changes in pharmacokinetics of cyclosporin in pediatric patients by food11) or by renal transplantation,12) and also investigated the pharmacokinetics of pirarubicin in pediatric patients.13) These two drugs were also known to be concentrated in blood cells and showed wide variations in these pharmacokinetic parameters. In this study we examined the distribution of ZNA in rats to clarify the factors influencing its distribution in epileptic patients.

Materials and Methods

Materials — ZNA, N,N-dimethyl ZNA, and sodium ZNA were provided by Dainippon Pharmaceutical Co. Ltd. (Osaka, Japan). All other chemicals used were obtained from commercial sources and were of reagent grade.

Animals — Male Wistar rats, weighing 180—240 g, were used throughout the study. The rats were housed under controlled environmental conditions and fed a normal diet (MF food,
Oriental Yeast Co., Tokyo, Japan).

**ZNA Distribution in Human Erythrocytes** — Heparinized blood from three healthy subjects, aged 25—38 years, was used for this study; none of the subjects had received any medication during the one-month period preceding this study. The hematocrit (Ht) of each sample was determined immediately. Three ml of blood was preincubated for 15 min at 37 °C and each sample was spiked with sodium ZNA dissolved in saline. Each sample was incubated for 30 min (this incubation period had been found to be sufficient for equilibration) and centrifuged at room temperature to separate the plasma aliquot. The plasma samples were then stored in the freezer (−20 °C) until analysis was carried out. ZNA concentration in erythrocytes (CE) was calculated from the plasma concentration (CP), the blood concentration (CB), and Ht using the following equation:

\[ CE = \frac{CB - (1 - Ht) \times CP}{Ht} \]

Nine patients with refractory seizures in Kobe University Hospital were selected for this study. They all gave their informed consents. Since all patients had been treated with ZNA (Excegran®, tablets or powder, Dainippon Pharmaceutical Co. Ltd., Osaka, Japan) for at least two weeks, it was considered that steady-state blood levels had been achieved at the sampling time-point. Blood samples were collected from two to four hours after ZNA administration to determine serum and blood concentration. All samples were stored at −20 °C until assayed.

**ZNA Distribution in Rats** — For four consecutive days sodium ZNA dissolved in saline (5, 20, and 80 mg/kg as ZNA) was administered orally to four rats in the morning. Blood samples were obtained from the abdominal artery, while the animals were under pentobarbital anesthesia (50 mg/kg, i.p.), at 10 and 24 h after the last doses. The entire brain of each rat was also collected. Ht of each blood sample was determined immediately, and the plasma was separated by centrifugation. Both blood and plasma samples were stored at −20 °C until assayed. The brain was homogenized with 0.1 M phosphate buffer (pH 6.0) using a glass homogenizer. ZNA content in the homogenate was analyzed by the following method.

**Assay Method for ZNA** — All the blood samples were assayed by high-performance liquid chromatography (HPLC), using a previously reported method. Briefly, to 0.2 ml of serum (or blood) sample, 0.3 ml of distilled water and 1.0 ml of 0.1 M phosphate buffer (pH 6.0) were added. After adding 50 µl of I.S. solution (0.1 mg/ml of dimethyl ZNA dissolved in chloroform:ethanol = 10:1) and 6.0 ml of chloroform—ethanol (10:1) mixture to each sample, the resulting mixture was shaken for 10 min and centrifuged at 3500 rpm for 10 min. The supernatant extract (5.0 ml) was transferred to a clean tube, and the solvent was evaporated to dryness under N2 gas stream. The residue was dissolved in 0.1 ml of ethanol and centrifuged at 10000 rpm for 1 min. Twenty µl supernatant was then injected into the HPLC column (Develosil ODS-7, 4.6 × 250 mm, Chemco Co., Osaka, Japan).

The brain samples were also assayed by HPLC. Briefly, to 0.5 ml of the brain homogenate, 50 µl of I.S. solution and 1.0 ml of acetonitrile were added. After being allowed to stand for 30 min at room temperature, the sample was centrifuged at 14000 rpm for 1 min. The supernatant was evaporated under N2 gas stream to a volume of 0.2 ml, and this was centrifuged at 14000 rpm. A 20-µl aliquot was then injected into the HPLC column.

The mobile phase consisted of 1% acetic acid—acetonitrile—isopropylalcohol (70:11:10, v/v/v). The flow rate was 1.0 ml/min, and the detection wavelength was 285 nm. The column temperature was set at 35 °C. The coefficients of variation (C.V.) of the blood and brain assays were less than 6% over the range of ZNA concentrations studied and the lower limits of the determinations were 0.1 µg/ml for the blood samples and 0.05 µg/g wet tissue for the brain samples.

**Analysis of Distribution Parameters and Statistical Analysis** — The parameters for ZNA distribution to erythrocytes (or brain) were calculated from the following equation, in accordance with Boddy et al.: \(^{14}\)

\[ CE \text{ or } CB = B_{\text{max}} \times C_P / (K_d + C_P) + k \times C_P. \]
where $C_E$: erythrocyte concentration of ZNA, $C_B$: brain concentration of ZNA, $B_{\text{max}}$: maximum binding capacity, $C_P$: plasma concentration of ZNA, $K_d$: dissociation binding constant, and $k$: proportionality constant relating to the plasma concentration of ZNA. Boddy et al.,\textsuperscript{14} in studying the binding of sulfonamides to carbonic anhydrase, estimated binding parameters using unbound and total plasma concentration. However, we estimated the parameters for ZNA using only total plasma concentration, as described by Wagner et al.,\textsuperscript{8} since it was reported that ZNA had a constant value for plasma protein binding (about 45\%) within a wide range of plasma concentration.

The results, shown as the mean $\pm$ S.D., were statistically evaluated with Student's $t$-test, taking $p < 0.05$ as the level of significance.

Results

Initially we studied ZNA distribution in human erythrocytes. Figure 1 shows that ZNA was markedly concentrated in erythrocytes, with saturable and nonsaturable components, as previously described by Matsumoto et al.\textsuperscript{15}

The variations in the effective serum and blood concentrations of ZNA were compared in nine refractory epileptic patients who received ZNA chronically and were well controlled (Fig. 2). Their ranges of ZNA concentration in serum and blood were 8.7—31.2 $\mu g$/ml and 23.2—38.4 $\mu g$/ml, respectively, and the variations in the serum (C.V. 36.0\%) were more than about 2 times larger than those in the blood (C.V. 17.0\%). The blood concentration was always higher than the serum level in these patients. These results indicate that erythrocytes may play an important role as a depot for ZNA distribution in the blood.

Next, we studied the distribution of ZNA in the rat brain. In a preliminary study, we found no difference between the plasma and serum concentrations of ZNA either in humans or rats. Therefore, we used the plasma concentration of ZNA instead of the serum concentration in the following experiments.

Figure 3 shows the ZNA concentration-time profiles in plasma, erythrocytes, and brain at 10 and 24 h after the last dose of ZNA (5, 20, or 80 mg/kg, p.o., once a day for 4 d) in rats. The plasma and erythrocytes concentration-time profiles were almost the same as those for single doses (data not shown). Although the brain concentration-time profiles were almost the same as that of the plasma, the ZNA levels in the brain were higher than in the plasma at these sampling times. Figure 4 shows the relationship between the ratio of ZNA concentration in erythrocytes (or brain) to plasma and the plasma concentration. These ratios decreased with increasing plasma concentration.

The distribution parameters for both the erythrocytes and the brain are shown in Table I. The $k$ and $K_d$ values for erythrocytes (1.23 and 0.36 $\mu g$/ml, respectively) were comparable
Fig. 3. Time Profiles of ZNA in Plasma, Erythrocytes, and Brain at 10 and 24 h after Last Oral Administration of 5, 20, and 80 mg/kg/d of ZNA for 4 d in Rats
a) plasma, b) erythrocytes, c) brain.
○, 5 mg/kg/d; △, 20 mg/kg/d; □, 80 mg/kg/d. Each value represents the mean ± S.D. of 4 rats.

to those for the brain (1.27 and 0.21 μg/ml, respectively), but the estimated $B_{\text{max}}$ values for the erythrocytes (21.5 μg/ml) were about 30-fold higher than those for the brain (0.73 μg/g wet tissue).

Figure 5 shows the calculated concentration of the saturable and nonsaturable components in rat erythrocytes and brain at 10 and 24 h after the last dose of ZNA. The concentrations of saturable and nonsaturable components were calculated from the parameters shown in Table I. At 24 h, the nonsaturable components in both brain and erythrocytes were significantly decreased, to about 30% of those at 10 h, for

Fig. 4. Erythrocyte/Plasma and Brain/Plasma Ratios at Various Plasma Concentrations after Last Oral Administration of 5, 20, and 80 mg/kg/d of ZNA for 4 d in Rats
a) erythrocytes, b) brain.
Saturable Disposition of Zonisamide

Table 1. Partition Parameters of ZNA in Erythrocytes and Brain in Rats

<table>
<thead>
<tr>
<th>Sample</th>
<th>( k^a )</th>
<th>( B_{\text{max}} ) ( ^b ) (( \mu g/\text{ml or g wet tissue} ))</th>
<th>( K_d ) ( ^c ) (( \mu g/\text{ml} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>1.23</td>
<td>21.5</td>
<td>0.36</td>
</tr>
<tr>
<td>Brain</td>
<td>1.27</td>
<td>0.73</td>
<td>0.21</td>
</tr>
</tbody>
</table>

\( a \) Proportionality constant relating to the plasma concentration. \( b \) Maximal binding capacity. \( c \) Dissociation binding constant.

each dose. However, the saturable components at 24 h were more than about 75% of those at 10 h for each dose. These results indicate that the saturable components in such tissues as erythrocytes and brain can remain at a high level, even if the serum ZNA level is low.

![Graphs showing comparison of saturable and nonsaturable components in erythrocytes and brain at 10 and 24 h after last oral administration of 5, 20, and 80 mg/kg/d of ZNA for 4 d in rats.](Image)

Fig. 5. Comparison of Saturable and Nonsaturable Components in Erythrocytes and Brain at 10 and 24 h after Last Oral Administration of 5, 20, and 80 mg/kg/d of ZNA for 4 d in Rats

a) nonsaturable components in erythrocytes, b) nonsaturable components in brain, c) saturable components in erythrocytes, d) saturable components in brain.

Each value represents the mean ± S.D. of 4 rats. The left and right columns of each dose show 10 and 24 h after last dosing, respectively. a) Significantly different from value at 10 h (\( p < 0.001 \)).
Discussion

The purpose of this study was to characterize the regulatory factors that control the distribution of ZNA in epileptic patients. We used rats as experimental animals to analyze the distribution of ZNA in the brain, as well as in the erythrocytes.

Matsumoto et al.\textsuperscript{4, 15} reported that ZNA was concentrated in human erythrocytes and they demonstrated that this uptake profile was specific to sulfonamide drugs. They consider that there are two uptake processes for ZNA, saturable binding to carbonic anhydrase and nonsaturable distribution. Thus, the saturable component involved in the distribution of ZNA to erythrocytes, observed in this study, may also reflect the binding of ZNA to carbonic anhydrase.

Conford and Landon\textsuperscript{16} studied the brain capillary transit of ZNA by an intracarotid injection technique and they suggested that ZNA that is present in both plasma and erythrocytes can equilibrate across the blood-brain barrier in the course of a single transcapillary transit. Approximately half of the ZNA gaining access to the brain in a single transcapillary passage was reported to be erythrocyte-borne. Further, Hamberger et al.\textsuperscript{17} demonstrated that probigade, a similar anticonvulsant drug, also showed similar transport through the blood-brain barrier. The variation of effective concentration of that drug was also less in blood than in serum. A low serum level of ZNA may not necessarily mean low brain levels, since as we found in rats, ZNA in erythrocytes may also be transported into the brain.

We also investigated the distribution of ZNA in the brain and in erythrocytes after a steady-state was reached in the rat. A saturable distribution of ZNA in the brain was observed. The maximum binding capacity of brain was less than 4% that of erythrocytes. It was reported that human carbonic anhydrase consists of at least two isozymes.\textsuperscript{18} Maren\textsuperscript{19} then reported that the contents of carbonic anhydrases in rat brain was about 3% that of erythrocytes. Thus, the main saturable component of ZNA in the brain may be the bindings to carbonic anhydrases. The wide distribution of carbonic anhydrases in various tissues other than brain and erythrocytes\textsuperscript{20} may contribute to the saturable distribution of ZNA in humans, as well as in rats. However, the bindings of ZNA to each isozyme of carbonic anhydrase in various tissues remains to be studied.

The anticonvulsant activity of acetazolamide, a sulfonamide similar to ZNA, is related to the inhibition of brain carbonic anhydrase.\textsuperscript{21, 22} Compared with acetazolamide, the inhibitory potency of ZNA was very weak in an in vitro system.\textsuperscript{23} However, the brain concentration of ZNA was about 50 times higher than that of acetazolamide, and the inhibitory activity of ZNA on carbonic anhydrase in rat brain was reported to be 30% that of acetazolamide.\textsuperscript{24} Therefore, this ZNA inhibitory effect on carbonic anhydrase may be related to its anticonvulsant activity.

The reduction of the saturable component of ZNA in brain did not parallel the plasma concentration profile, indicating that the antiepileptic action of ZNA is not directly related to its plasma (or serum) concentration. We consider that the differences in ZNA binding to saturable components such as carbonic anhydrase in various tissues in epileptic patients are one of the factors that control the wide variation in ZNA distribution and the effective serum concentration in these patients. The present findings provide information which will be important for the treatment of epilepsy with ZNA. Monitoring of ZNA in blood and serum may also be valuable in rationalizing ZNA therapy.

Acknowledgement We thank Dainippon Pharmaceutical Co. Ltd. (Osaka, Japan) for providing ZNA, N,N-dimethyl ZNA, and sodium ZNA.

References


