The Effects of Body Weight and Combination Drug Therapy on the Serum Concentrations of Zonisamide in 94 Epileptic dogs: An Epidemiological Analysis

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ABSTRACT

To clarify the effects of background factors such as body weight, combination drug therapy with phenobarbital (PB) and potassium bromide (KBr), age, gender, neuter status, and blood collection time on the serum concentrations of zonisamide (ZNS) in 94 epileptic dogs, serum ZNS concentrations of 141 samples from 94 epileptic dogs were measured by high-performance liquid chromatography with UV detection. Serum ZNS concentrations were divided by the dosage of ZNS, to obtain normalized ZNS concentrations. Multiple linear regression analysis revealed that body weight and combination therapies with KBr, PB, and KBr + PB affected the normalized ZNS concentrations, and normalized ZNS concentrations

were positively correlated with body weight. The normalized ZNS concentrations in dogs with PB combination therapy were lower when compared with those concentrations in dogs receiving ZNS alone, but were higher in dogs receiving KBr combination therapy. These results suggest that serum ZNS concentrations are influenced by body weight and combination drug therapies using KBr, PB, and KBr + PB. This information of ZNS characteristics may be useful in determining the initial and modified doses of ZNS in the therapy of epileptic dogs.

INTRODUCTION

Zonisamide (3-sulfamoylmethyl-1,2-benzisoxazole, ZNS) has been recently and widely used as a new-generation anticonvulsant drug in the treatment of canine epilepsy. Unlike the pharmacological actions of phenobarbital (PB) and potassium bromide (KBr), which open gamma-aminobutyric acid subtype A receptor (GABAA)-associated chloride channels[1], it has been suggested that ZNS reduces the number, frequency, and duration of seizures by altering the fast inactivation threshold of voltage-dependent sodium channels[2] and by inhibiting low-threshold T-type calcium channels in neurons[3]. Zonisamide has been shown to have a beneficial effect, reducing seizures in dogs with refractory epilepsy (defined as a lack of sufficient response to PB and/or KBr treatment despite therapeutic concentrations of these drugs[4,5]).

It is necessary to maintain ZNS concentrations within a therapeutic range in order to prevent seizures in dogs. In humans and rats, body weight, age, and combination drug therapies, including PB, valproic acid, carbamazepine, and phenytoin, reportedly affect ZNS concentration[6,7]. In dogs, it has been reported that ZNS concentrations decreased after repeated administrations of PB[8], and the half-life of ZNS in Beagles (medium size breed) and Greyhounds (large size breed) were also different at 13.8 h[8] and 17.2 h[9], respectively. Thus, it is possible that combination drug therapies and body weight differences affect the plasma concentration and half-life of ZNS. However, such factors have not been evaluated so far in epileptic dogs. The present study quantitatively examined whether body weight and PB and/or KBr combination drug therapies altered ZNS concentrations by using an epidemiological method.

MATERIALS AND METHODS

We collected serum samples between June 2006 and October 2008 at Azabu University, the Watanabe Animal Hospital, the Kariya Animal Hospital, and the Kobayashi Animal Clinic. We screened 224 samples from 104 epileptic dogs suffering from more than 1 seizure per month and were diagnosed with epilepsy in any of the aforementioned hospitals. To assess potential factors affecting the serum ZNS concentrations, the epileptic dogs used in this test had to fulfill the following inclusion criteria: (a) treatment with ZNS (Excegran®; Dainippon Sumitomo Pharma Co., Osaka, Japan) alone or in combination with PB, KBr, and PB+KBr; (b) 1 to 13 years of age; (c) availability for collecting more than 1 sample of serum ZNS concentration at steady state (more than 2 weeks after the last change in the dose of ZNS without modification of other drugs therapies); (d) availability of reliable information on the clinical status and details of all medications, including blood collection time, body weight, breed, gender, and neuter status. If the dogs received ZNS at a consistent dose, and the serum concentration of ZNS was measured more than once, only the latest concentration was included. If the dose changed or if therapy was changed from ZNS alone to ZNS in combination with PB or KBr, concentrations of ZNS before and after these changes were included in the present study. The study protocol was approved by the ethics committee of the Azabu University animal hospital, and all dogs' owners provided written informed consent.

Serum concentrations of ZNS were determined according to a previously described method[10] with a modification that involved the use of high-performance liquid chromatography with ultra-violet detection (λ =

***Table 1**. Association between normalized zonisamide serum concentrations and body weight, combination drugs, age, neuter status, gender, and blood collection time in a multiple linear regression*

Variable	Coefficient	95% CI		P value
		Lower	Upper	
Body weight	0.056	0.027	0.085	<0.001*
Combination drugs	–0.311	–0.598	–0.023	0.035*
Age	–0.113	–0.228	0.001	0.053
Neuter status	0.671	–0.028	1.371	0.060
Gender	0.694	–0.041	1.429	0.064
Blood collection time	–0.160	–0.472	0.153	0.314
Eliminated	5.221	1.456	8.984	0.007*

Abbreviation: CI, confidence interval.
Combination drugs: (ZNS alone, 0; ZNS+KBr, 1; ZNS+PB, 2; ZNS+KBr+PB, 3), Gender: (female, 0; male, 1), neuter status: (without spay or castration, 0; with spay or castration, 1).

215 nm, SPD-10A; Shimadzu Co., Kyoto, Japan). Separation was achieved with a 4.6 × 150 mm column (ODS-pak F-411 C18; particle size, 5 µm; Showa Denko K.K., Tokyo, Japan), and the temperature was maintained at 37°C. The flow rate of the acetonitrile-H3PO4 (8 mM) mobile phase (30:70, v/v) was 1.8 mL/min.

Serum samples (300 µL) were mixed with 30 µL of 5-methyl-5-phenylhydantoin (10 µg/mL; Wako Pure Chemical Industries, Osaka, Japan), which was used as an internal standard; 60 µL of hydrochloric acid (4.0 M); and 1.8 mL of dichloromethane. The mixture was shaken for 3 min and centrifuged at 980 × g for 15 min. The organic layer was evaporated to dryness and then reconstituted with 300 µL of the mobile phase, with injection of 65 µL. The standard curve was linear with a range of 0.05–240 µg/mL, and the lower limit of quantification was 0.05 µg/mL. The mean recovery was above 99.0%, calculated by adding ZNS at concentrations of 0.1, 10, and 100 µg/mL to the plasma. The inter- and intra-day precisions and accuracies were less than 10%.

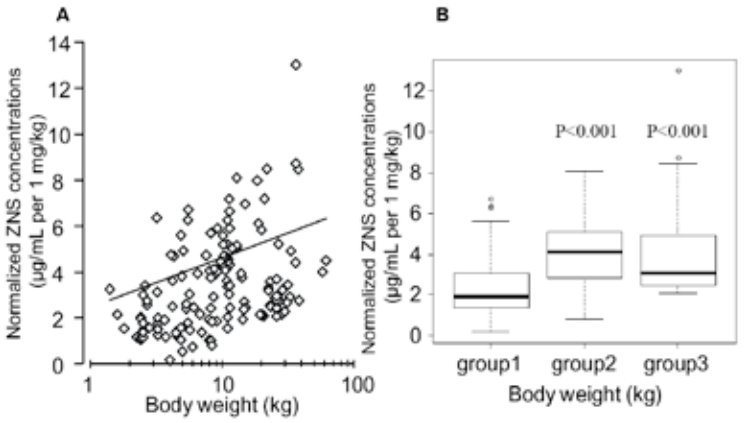
All epileptic dogs were evaluated at different dose levels, and the relationship between serum ZNS concentrations and dosage was linear over the previously assessed dose range of 5–30 mg/kg in dogs[10]. Thus, ZNS concentrations were corrected to the normalized ZNS concentrations as reported previously using the following equation[11,12]: normalized ZNS concentration (µg/mL per 1 mg/kg of dosage) = serum ZNS concentration (µg/mL)/ZNS dosage (mg/kg)

Statistical analyses were performed using the R software package, version 2.10.1 (http://www.r-project.org/), and the results are presented as mean ± SD. To investigate the relative contribution of each covariate to the variability in the normalized ZNS concentrations, a multiple linear regression model was developed using the following variables: body weight (kg), drug combinations (ZNS alone, 0; ZNS+KBr, 1; ZNS+PB, 2; ZNS+KBr+PB, 3), age (years), neuter status (without spay or castration, 0; with spay or castration, 1), gender (female, 0; male, 1), and blood collection time (h). A univariate linear regression analysis was conducted to assess the independent effect of body weight on the normalized ZNS concentrations. A group analysis was performed using the Kruskal-Wallis one-way analysis of variance (ANOVA). A P value below 0.05 was considered to be statistically significant.

RESULTS

We obtained 141 samples from 94 dogs (48 males, 46 females) that fulfilled the criteria

Fig. 1.A. *The association between body weight and normalized ZNS concentrations. (Normalized ZNS concentrations = 2.68 + 0.06 (body weight in kg), r2 = 0.10, P < 0.01).* ***B.*** *Box plots showing the normalized ZNS concentrations for group 1 (0–9 kg), group 2 (9–20 kg), and group 3 (20–62 kg) in dogs. Boxes represent the 25th–75th percentiles, and horizontal lines within the boxes represent median values. P values are for those groups versus group 1 (0–9 kg).*



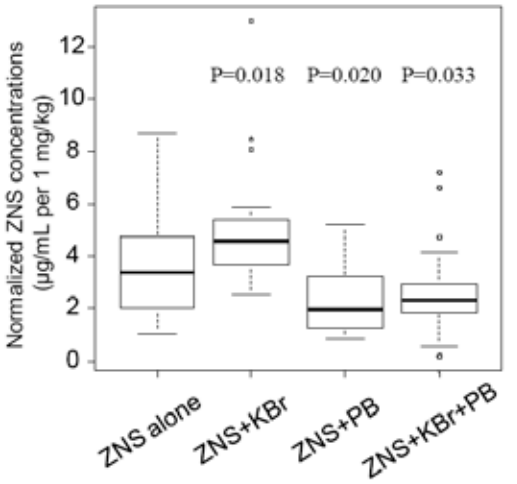
described in the Materials and Methods. Forty-eight samples were obtained from castrated or spayed dogs. Doses of ZNS were 8.3 ± 4.4 mg/kg, twice daily (BID) in dogs with ZNS alone (n = 63). In the combination therapies of KBr, PB, and KBr+PB, doses were 10.4 ± 2.9 (n = 24), 7.5 ± 3.2 (n = 34), and 13.8 ± 5.9 (n = 20) mg/kg BID, respectively. Doses of KBr in the combination therapies of KBr and KBr+PB were 25.5 ± 8.7 and 17.9 ± 5.9 mg/kg BID, respectively. Doses of PB in combination therapies of PB and KBr+PB were 3.8 ± 2.3 and 4.3 ± 2.3 mg/kg BID, respectively. Body weight was 13.0 ± 11.1 kg, and age was 5.7 ± 3.1 years. Blood samples were drawn from the epileptic dogs 11.9 ± 1.0 h after the last dosage.

The results of the multiple linear regression analysis used to explore the relative contribution of a number of variables to the variability in normalized ZNS concentrations are summarized in Table 1. Normalized ZNS concentration was influenced by body weight and combination drug therapies of PB, KBr, and PB+KBr. ZNS concentration, however, was not influenced by age, gender, and/or blood collection time. Further analyses revealed that normalized ZNS concentrations were positively correlated with body weight (Fig. 1.A). When samples were stratified into 3 groups of body weight (group 1: 0–9 kg; group 2: 9–20 kg; group 3: 20–62 kg), the normalized ZNS concentrations of group 1 were significantly lower than those of groups 2 and 3(Fig. 1.B). The normalized ZNS concentration in combination with KBr was significantly higher than that of ZNS alone, while the concentration of ZNS in combination with PB and KBr+PB was significantly lower than those of ZNS alone (Fig. 2.)

DISCUSSION

In the present study, a multiple linear regression analysis revealed that age, neuter status, gender, and blood collection time had no effect on the normalized ZNS concentrations in dogs with epilepsy. Normalized ZNS concentrations, on the other hand, positively correlated with body weight. This finding is in agreement with previous research in humans6 demonstrating that ZNS concentrations in patients older than 10 years were higher than those in patients who were 10 years and younger. It is possible that differences in dog breeds rather than merely body weight may also influence normalized ZNS concentrations. Dogs normally reach maturity by 1 year of age, and the age range of the present sample was between 1 and 13 years. Thus, the differences of body weight here might also reflect the contribution of breed differences, as previous studies have shown that when the activity of cytochrome

Figure 2. *Box plots showing the normalized ZNS concentrations for ZNS alone, ZNS+KBr, ZNS+PB, and ZNS+KBr+PB. Boxes represent the 25th–75th percentiles, and horizontal lines within the boxes represent median values. P values are for each drug type versus ZNS alone.*



P450 (CYP), a drug-metabolizing enzyme, was evaluated as evidence for propofol hydroxylation, the CYP levels in the liver microsomes of Beagles were 3-fold higher than that in the Greyhound[13].

The present study reveals that normalized ZNS concentrations were low when ZNS was used in combination with PB than when ZNS was used alone. This result is consistent with a previous report that showed that serum ZNS concentrations decreased after repeated administrations of PB[8]. Conversely, protein content and drug metabolic activity for CYP2B, CYP3A, GST-D, and UDP-GT enzymes increased after PB treatment in male Beagles[14]. Although no specific enzymes associated with drug interactions between ZNS and PB have been identified in dogs, it is possible that ZNS is metabolized by any of these enzymes, thereby explaining why the serum concentration of ZNS might decrease.

Combination therapy with KBr led to higher normalized ZNS concentrations. To our knowledge, this is the first report of a drug interaction between ZNS and KBr. As KBr is a simple compound and is not metabolized, it is believed that it does not interact with other drugs[15]. The present study demonstrates that the increasing effect of KBr on serum ZNS concentrations was abolished by a combination drug therapy using KBr+PB. Therefore, the lowering effect of PB on the ZNS concentration might be stronger than the increasing effect of KBr. Further studies are needed to elucidate the mechanism of drug interaction between ZNS and KBr, as well as between ZNS, KBr, and PB.

In conclusion, the present study reveals, by epidemiological analyses, that serum ZNS concentrations were not influenced by age, neuter status, or gender. ZNS concentrations were influenced by body weight and possibly by breed type, as well as by a combination of PB, KBr, and PB+KBr. This information characterizing ZNS may be useful in determining initial and modified doses of ZNS in the therapy of epileptic dogs.

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