Zonisamide: Pharmacology and Clinical Efficacy in Epilepsy

Yoshinobu Masuda, Masayuki Ishizaki, and Masanao Shimizu

Dainippon Pharmaceutical Co., Ltd., Research Laboratories, Osaka, Japan

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INTRODUCTION

The discovery of zonisamide (ZNS) did not result from a search for new antiepileptic drug (AED). A continuous random screening for anticonvulsant activity had been in progress for many years at the research laboratories of Dainippon Pharmaceutical Co., Ltd. ZNS was discovered by serendipity in 1974 during routine testing of 1,2-benzisoxazole derivatives which were synthesized for the management of psychiatric diseases (49,101). Among the derivatives tested, some compounds including ZNS, were found to have a potent anticonvulsant activity in experimental animals. ZNS has a benzisoxazole structure that distinctly differs from any existing AED. The chemical structure of ZNS is shown in Fig. 1.

After comprehensive preclinical studies, clinical development of ZNS in Japan started in September 1979. ZNS was approved for the control of partial seizures and generalized seizures in March and launched in June, 1989, in Japan. ZNS was introduced to the Korean market in 1992. In the United States, a New Drug Application (NDA) was submitted in March 1997. The Food and Drug Administration (FDA) issued an approvable letter to the company in March, 1998, for use as adjunctive therapy in the treatment of partial seizures in adults with epilepsy (80). This review will focus mainly on the pharmacodynamic properties of ZNS and its clinical efficacy in epilepsy.

GENESIS AND CHEMISTRY

1,2-benzisoxazole bears a close resemblance to indole, and the 1,2-benzisoxazole nucleus can substitute for the indole nucleus as far as auxin (plant cell growth substance)-like activity is concerned (18). The derivatives of 1,2-benzisoxazole should be of biologic interest because many indole derivatives such as serotonin are biologically important. Starting from 1,2-benzisoxazole-3-acetic acid, which is easily prepared by the Posner re-
action (71,72) between 4-hydroxycoumarin and hydroxylamine, several 3-substituted-1,2-benzisoxazole derivatives were synthesized (98–100). Among the derivatives tested, 1,2-benzisoxazole-3-methanesulfonamide (ZNS, AD-810, CI-912) exhibited a potent anticonvulsant effect as measured by protection against maximal electroshock seizure (MES) in mice (49,101).

Studies of the structure-activity relationship showed that introduction of a halogen atom to the 5 position of the benzisoxazole nucleus of ZNS increased the anticonvulsant potency in the following order: Br > Cl > F > H. However, neurotoxicity measured by the rotarod test increased in the same order to an even greater extent. Studies of N-substitution of the side chain of ZNS revealed that the introduction of a simple monoalkyl did not abolish the activity, but when the substituent was an amino, a dimethylamino, a benzyl, or longer chain, the activity was generally lost. The disubstituted compounds were inactive with the exception of the dimethylamino analog and the anticonvulsant potency of compounds with N-methyl for alkylated derivatives was as follows: –SO₂NH₃ (ZNS) > –SO₂NHCH₃ > –SO₂NHC₂H₅ = –SO₂N(CH₃)₂ > –SO₂NH₂ (101). Since this order is in general agreement with the relative extent of in vitro dealkylation of monoalkylated sulfonamide derivatives (88), the potency of these compounds is thought to result from biotransformation. ZNS was finally selected as a promising candidate for further development of a new AED.

ZNS has a melting point of 164 to 168°C, and is a nonhygroscopic white to pale yellow crystal or a crystalline powder with a slightly bitter taste. It is freely soluble in acetone, sparingly soluble in methanol, slightly soluble in ethanol, and very slightly soluble in ether and chloroform. The solubility in water is dependent on the pH, since the pKa value of ZNS is 9.66. ZNS in aqueous acidic, neutral, or alkaline solutions and in the solid state was proven to be a highly stable compound under severe conditions of heat and light exposure (82). However, ZNS is highly reactive to hydroxyl radicals and easily decomposed by them; ZNS was reported to be decomposed in Fenton reagent solution up to 90% in 30 min. ZNS scavenges hydroxyl and nitric oxide radicals in a dose-dependent manner (62).

**PHARMACOLOGY AND MECHANISM OF ACTION**

**Anticonvulsant Properties**

The antiepileptic potential of ZNS has been evaluated in laboratory animals by studying seizure control and electroencephalographic epileptiform activity. The anticonvulsant effect as measured by protection against maximal electroshock seizure (MES) in mice (49,101).

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vulsant profile of ZNS is presented here, together with a summary of other pharmacological properties.

Since the MES test was introduced by Toman et al. (97), many AEDs have been found by this method. Activity in the MES test correlates reasonably well with effectiveness in patients with epilepsy. Indeed, drugs effective in controlling human tonic-clonic and temporal lobe epilepsies are effective in blocking MES in rodents, while those effective in absence seizures are less active in this animal model (56). ZNS was as potent as comparative drugs in blocking MES. This fact prompted us to study the profile of this new compound as a potential AED in various animal models of epilepsy.

First, the anticonvulsant effects of ZNS against MES were examined using species other than mice, i.e., rats, rabbits, and dogs. In these species, ZNS exhibited anticonvulsant activity which was as potent as that of phenobarbital (PB) or carbamazepine (CBZ) and more potent than phenytoin (PHT). The time course of ZNS’s anticonvulsant activity has been assessed and compared with that of other AEDs when administered orally to rats up to 24 h before maximal electroshock (49). In this model, the anticonvulsant effect of ZNS peaked 2 h after administration and lasted for at least 10 h after administration. This profile was essentially similar to that of PB but different from CBZ or PHT.

In mice, ZNS was effective against maximal seizures induced by electroshock or pentylentetrazol, but not against minimal seizures induced by pentylentetrazol, as shown in Table 1. The effects of ZNS in the electro- and chemoshock seizure tests were similar to those observed with PHT and CBZ (29,49,50), suggesting that ZNS, like PHT and CBZ, exerts an anticonvulsant effect by inhibiting the spread or propagation of seizures. This observation was supported by electroencephalographic studies (30,31) which showed that ZNS restricted the spread of cortical seizures evoked by electrical stimulation and prevented cortical focal spikes elicited by cortical freezing from developing into generalized seizures in cats. ZNS suppressed focal seizure activity in the cat cortex due to electrical stimulation and produced a dose-related significant increase in afterdischarge threshold (103).

ZNS was effective against interictal spiking activity within cortical foci. ZNS suppressed or abolished spikes occurring interictally between recurrent episodes of generalized seizures and reduced the frequency and amplitude of focal spikes in the cat cortex by freezing (30) or by application of conjugated estrogens (31). These effects, not observed with PHT, PB, or trimethadione, would be to suppress activity within the focus, especially in the cortex. On the other hand, ZNS demonstrated little effect on seizures induced by subcortical stimulation. ZNS was almost without effect against thalamic afterdischarges induced by electrical stimulation of the nucleus centralis lateralis in cats and reticularis thalami in rats, while some standard AEDs were effective against these afterdischarge (30).

ZNS suppressed seizures and electroencephalographic afterdischarges in various kindling models, thought to be models of epilepsy. In neocortex- and hippocampus-kindled rats, ZNS markedly shortened the duration of afterdischarges and elevated the seizure threshold (35). In amygadaloid-kindled rats, however, ZNS was ineffective (35) in this study, but ZNS was effective in amygadaloid-kindled rats in another study (20). This discrepancy may be due to the difference of stimulation conditions, i.e., the former is estimated by an alteration of the convulsive threshold and the latter by the seizure triggering threshold as a stimulation condition. When the effects of ZNS were compared to those of PHT on electroencephalographic afterdischarges in hippocampus-kindled rats, ZNS pre-
vented focal seizure activity at doses that did not affect motor behavior (96). In amygdalo-
id-kindled cats, consecutive oral administration of ZNS for several days reduced or abol-
ished both electroencephalographic afterdischarges and seizures (34). ZNS also
suppressed photically evoked myoclonus in lateral geniculate-kindled cats (104).

In the limbic seizure model in rats, which was induced by a unilateral microinjection of
kainic acid into the amygdala, stable seizure development was observed from focal
amygdala epilepticus to limbic seizure status epilepticus (91,93,94,95). Systemic adminis-
tration of ZNS during the generalized seizure suppressed not only seizure propagation to
hippocampus, but also blocked cortical seizure propagation observed by the electroence-
phalographic or glucose metabolic activity. In the same experiment, ZNS did not suppress
both activities in the primary epileptogenic focus of amygdala, suggesting that main anti-
convulsant action of ZNS is a suppression of seizure propagation from the epileptogenic
focus (93).

Linear relationships between drug concentrations in plasma and brain were observed in
mice and rats after oral doses of ZNS, PHT, CBZ, and PB, as revealed by high correlation
coefficients (48). In addition, the brain-to-plasma ratios of these drugs in either species
were approximately one, which was consistent with the ratio reported in humans
(28,86,102). A minimal effective plasma concentration at which animals were protected
against MES was estimated for each drug. It was similar to the effective concentration in
humans. ZNS was effective against MES irrespective of species at plasma concentrations

\[ \text{TABLE 1. Anticonvulsant activity of zonisamide and other AEDs against maximal or minimal seizures induced by electroshock or pentylenetetrazole in mice} \]
over 10 μg/ml with a wider therapeutic range of plasma concentrations than that of other AEDs (48).

Development of tolerance to the anticonvulsant action of ZNS was examined by repeated administration to rats and compared with PHT, CBZ, and PB. Short-term pharmacokinetic or pharmacodynamic tolerance to the anticonvulsant activity of ZNS did not occur (47). In contrast, repeated administration of PHT, CBZ, and PB resulted in the development of tolerance. The time course of plasma ZNS levels was unaltered after repeated administration of either ZNS or PB in rats, differing from other AEDs (47,49). This also means that ZNS does not induce its own metabolism.

ZNS reduced spontaneous alternation and active avoidance behavior in mice at doses of 100 and 300 mg/kg which were two to three times higher than those of CBZ (27). ZNS was also less potent in mice than CBZ, PHT, or PB in eliciting motor disturbance, inducing hypnosis, and potentiating hexobarbital narcosis, all which are indicators of the potency for neurological adverse effects (49). Sublethal doses produced marked sedation within 1 h which lasted for 2 to 3 d. Sedation developed progressively with increasing doses until loss of righting reflex and death due to respiratory failure occurred (49).

**Neuroprotective Properties**

Several reports concerning the neuroprotective effect of ZNS indicated mechanism other than its anticonvulsant action. ZNS reduced hypoxic-ischemic brain damage in neonatal rats by a mechanism independent of its anticonvulsant properties (22). ZNS reduced cortical and striatal infarct volumes induced by hypoxic-ischemic insult. It also reduced neuronal cell death in CA1, CA3, CA4, the dentate gyrus, and the subiculum regions of hippocampus, but without effect on electroencephalographic seizures during hypoxia-ischemia in rats.

In a brief period of global cerebral ischemia, damage occurred in selectively vulnerable regions of the brain (21). In the gerbil, a 5-min ischemic insult produced substantial damage to the CA1 sector of the hippocampus (6,36). During a brief ischemic insult, there was a rise in extracellular glutamate levels in the hippocampus (5,68) which caused pyramidal cell damage and impairment of cognitive memory task (65). ZNS reduced ischemic damage to the CA1 sector of the hippocampus in a gerbil model of transient global forebrain ischemia (17,68). It almost completely prevented the elevation of glutamate release in the hippocampus during, and after ischemia. ZNS also reduced ischemia-induced memory impairment in the water maze task in gerbils (68).

In the model of transient focal cerebral ischemia with reperfusion, ZNS reduced infarct volume and neurological deficits in rats (59). ZNS reduced the infarct area in both cortical and subcortical regions including the caudatum, putamen, hippocampus, and corpus callosum in a dose-dependent manner at anticonvulsive doses that did not affect body temperature (59) or physiological parameters such as blood gases, pH, blood pressure, and regional cerebral blood flow (27). ZNS also reduced the neurological deficit of the contralateral fore and hind paws in a dose-dependent manner. In contrast to ZNS, CBZ, and sodium valproate (VPA) did not reduce infarction nor neurological deficits even at doses which were two to five times higher than their anticonvulsant doses (59).
1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes irreversible destruction of the dopaminergic nigrostriatal pathway. MPTP is used extensively in various mammalian species to produce a model of Parkinson’s disease (23,24,33,40,45). MPTP selectively causes a marked reduction of dopamine and elevation of dopamine turnover rate in the striatum of mice. ZNS exerted a potent preventive effect on MPTP-induced dopaminergic neurodegeneration in a dose-dependent manner (53). ZNS almost completely prevented the reduction of dopamine and its metabolites, homovanillic acid (HVA) and dihydroxyphenyl acetic acid (DOPAC), and the elevation of dopamine turnover rate in the striatum of mice as shown in Fig. 2. Preventive effects of various other AEDs (CBZ, PHT, PB, VPA, ethosuximide, lamotrigine, diazepam, acetazolamide, topiramate, tiagabine, and gabapentin) against MPTP-induced dopaminergic neurodegeneration have been examined for the purpose of comparison with ZNS under the same conditions. Table 2 shows the results. Among those tested, only PHT and lamotrigine exerted weak preventive effects at doses that were 4 to 11 times higher than their median anticonvulsivive doses. These results suggest that ZNS has a potential neuroprotective effect against MPTP-induced dopaminergic neurodegeneration which is different from other AEDs.

Fig. 2. Protective effect of zonisamide against the dopaminergic neurodegeneration induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice. Male C57 black mice were intraperitoneally injected with 30 mg/kg of MPTP daily for 8 d. Zonisamide was administered orally at 10, 30, and 100 mg/kg 30 min before every MPTP injection. Twenty-four hours after the last MPTP injection, mice were treated with a microwave applicator and the striata were dissected. Concentrations of dopamine (DA), homovanillic acid (HVA) and dihydroxyphenyl acetic acid (DOPAC) in the striatum were determined by HPLC-ECD. Statistical analysis was performed by Dunnett’s multiple comparison test. *, **: Significantly different from vehicle control (P < 0.05, P < 0.01).
Mechanisms of Action

Because ZNS possesses a sulfamoyl group in the side chain of the molecule, the anti-convulsant action may result from inhibiting carbonic anhydrase activity in the brain, similar to acetazolamide. This possibility has been ruled out by several lines of evidence. Although ZNS has been shown to inhibit brain and blood carbonic anhydrase in vitro and ex vivo, the potency in vitro was 100 to 200 times less than that of acetazolamide (49,51). Doses 100 to 1000 times higher than acetazolamide were required to produce a comparable inhibition of the enzyme ex vivo (49). The 7-methylated analog of ZNS, which has the same potency as ZNS in inhibiting carbonic anhydrase in vitro did not exert any anti-convulsant effects (52). In addition, ZNS and acetazolamide produced different effects on the functions of central nervous system. ZNS did not cause a decrease in brain pH or an increase in cerebral blood flow of rats in contrast to the marked changes in these parameters with acetazolamide (27). Moreover, ZNS reduced the turnover of monoamine transmitters, especially norepinephrine and dopamine, in the mouse and rat brain, while acetazolamide enhanced them (27,67). These results demonstrate that ZNS, unlike acetazolamide, does not exert anticonvulsant effects through inhibition of carbonic anhydrase.

A binding study of ZNS suggests that ZNS may have specific binding sites in the crude membrane fraction of rat brain. These sites may have some relationship to benzodiazepine receptors (58). However, in the electrophysiological study of ZNS even at high concentrations, ZNS did not alter the post-synaptic response to GABA or glutamate in cultured...
mouse spinal cord neurons, suggesting that the anticonvulsant effects of ZNS are not due to a modulation of GABA or glutamate receptors (96).

Studies of ZNS cellular mechanisms of action have demonstrated that ZNS blocks voltage-sensitive Na\(^+\) and Ca\(^{2+}\) channels. Without blocking the initial firing, ZNS specifically blocked the sustained repetitive firing of action potentials induced by the depolarizing current pulse in cultured spinal cord neurons from mouse embryo similar to PHT (76). Since ZNS blocked only the later action potentials of the evoked current pulses, it is likely that repetitive firing is abrogated by an effect on Na\(^+\) current-inactivation kinetics. In support of these findings, ZNS caused a hyperpolarizing shift in the steady-state fast inactivation curve and retarded recovery from fast and slow Na\(^+\) inactivation in voltage-clamped Myxicola giant axons (78).

ZNS has been shown to selectively reduce the transient inward current (T-type Ca\(^{2+}\) current) without affecting long lasting inward currents (L-type Ca\(^{2+}\) current) in cultured neurons of the rat cerebral cortex in a dose-dependent manner at concentrations within the therapeutic range (89). The methylated analog of ZNS, which is ineffective against MES in mice, was tested at a high concentration of 500 µM, and reduced neither T-type nor L-type Ca\(^{2+}\) current. These findings suggest that the effects of ZNS against MES might occur through the reduction of T-type Ca\(^{2+}\) current. Because drugs effective against MES are thought to prevent seizure discharge spread, T-type Ca\(^{2+}\) channels could underlie a cellular mechanism of spreading activity in epileptic seizures.

Both voltage-dependent Na\(^+\) and Ca\(^{2+}\) channels have a pivotal role in membrane excitability (3). Blockade of Na\(^+\) channels suppresses Na\(^+\)-dependent action potentials, and suppression of T-type Ca\(^{2+}\) channels inhibits sharp depolarization of the membrane potential underlying Na\(^+\)-dependent action potentials. The effects of ZNS on these ion channels suggests that ZNS disrupts neuronal synchronized firing and epileptic activity, thereby limiting spread or propagation of seizures (89).

Although the cellular ion mechanisms of ZNS inhibitory action on seizure spread or propagation has been clarified, the mechanism underlying the suppression of epileptogenic focus activity caused by applications of tungstic acid gel, conjugated estrogens, or tissue freezing is still unclear. Several reports of neuroprotective effects describe possible mechanism(s) for them. ZNS inhibited the elevation of thiobarbituric acid reactive substances (TBRS), an index of lipid peroxidation in the rat cortex with iron-induced epileptic foci (38) which is a model for post-traumatic epilepsy (107). It also inhibited TBRS formation in rat cortex homogenates induced by ascorbic acid and ferrous chloride (38). Oxygen free radicals have been implicated as cause factor in neuronal cell damage following both traumatic head injury and cerebral ischemia.

ZNS reduced the cerebral damage by hypoxic-ischemic insult and also transient global and focal ischemia with reperfusion in rats and gerbils, in contrast to CBZ and VPA, which had no effect against focal ischemia with reperfusion (17,22,59,68). The principle free radicals that originate during cerebral ischemia are superoxide anions, which generate hydroxyl radicals through the iron-catalyzed Haber–Weiss reaction. Hydroxyl radicals are capable of damaging protein, carbohydrate, and DNA and may be the most harmful radicals for neuromembranes (61,62). Moreover, NO released from NO synthase activated by rising intracellular Ca\(^{2+}\) concentration during cerebral ischemia can react with superoxide anions to form peroxynitrite anions, which then generate hydroxyl radicals (4). Free radical generation depends on reperfusion (110). ZNS scavenged DPPH (1,1-diphenyl-2-pycrylhydrazyl) and carbon-centered radicals in a dose-dependent manner (38). ZNS also
scavenged hydroxyl and nitric oxide radicals detected by using electron spin resonance (62). However, there are no reports about a radical scavenging effect of other AEDs. These results suggest that the mechanism of action of ZNS may involve protection of brain tissue from free radical damage.

Systemic administration of MPTP to primates induces Parkinson’s disease-like symptoms associated with a selective destruction of dopamine-producing neurons in the substantia nigra pars compacta and a reduction in striatal dopamine content (10,15,32,43). Narcotic abuse patients who took meperidine contaminated with MPTP experienced the above symptoms (43,45). The neurotoxicity of MPTP appears to be due to the formation of the 1-methyl-4-phenyl-pyridinium ion (MPP+) (11,13,42,77). MPP+ is then selectively taken up by the dopamine transporter into dopamine neurons (14). The cytotoxic effects of MPP+ are due to its accumulation in mitochondria (74) and inhibition of complex I of the respiratory chain (63), thereby impairing ATP production (12). These biochemical changes may ultimately lead to increased free radical production (2). In addition, these neurotoxins are able to induce oxygen free radical generation and increase lipid peroxidation in vitro (70,75) and also increase striatal lipid peroxidation in vivo (1,73).

ZNS prevented dopaminergic neurodegeneration induced by MPTP in a dose-dependent manner. The preventive effect of ZNS against MPTP-induced striatal dopamine depletion was different from those of other AEDs which exerted virtually no preventive effect (53). ZNS has radical scavenging effects (38,62). Consequently, the difference between ZNS and other AEDs in preventing MPTP-induced dopaminergic neurodegeneration as well as cerebral damage induced by ischemia or chemicals, may depend on the radical-scavenging action. As mentioned above, despite being stable to heat and light exposure, ZNS is highly reactive to free radicals such as the hydroxyl radical, and, easily decomposed by them. The neuroprotective effect of ZNS could lead to new clinical applications, including neurodegenerative disorders such as cerebral infarction after stroke and Parkinson’s disease, in addition to epilepsy.

CLINICAL EFFICACY IN EPILEPSY

Pharmacokinetics

A full program of pharmacokinetic studies have been conducted, including single- and multiple- dose studies in both normal subjects and epileptic patients and radiolabelled studies. Interactions of ZNS with food or other AEDs plus the effect of gender, age, and renal impairment have been studied.

ZNS has a prompt $T_{\text{max}}$ of 2.8 to 3.9 h. Oral absorption is complete and unaffected by food. In a $^{14}$C study, essentially only ZNS was detected in the plasma. Because ZNS metabolites are inactive, plasma ZNS levels should reflect the pharmacodynamic activity: 62% of the total dose was recovered in the urine as ZNS (35% of recovery), N-acetyl ZNS (15%), or 2-sulfamoylacetyl phenol (SMAP, 50%); 3% was recovered in the feces. ZNS reduction to SMAP is mediated by the cytochrome P450 isozyme 3A4. Plasma levels were proportional to dose over the dose range of 200 to 1000 mg/d. ZNS kinetics were unaffected by renal insufficiency, age, or gender. The serum concentrations were similar on once- or twice-daily dosing (7).
Yagi et al. (109) studied the relationship between ZNS dose and serum concentration in 22 patients with epilepsy. A linear relationship was observed at ≤13 mg/kg/d. Twice-daily administration of 200 mg every 12 h produced a 14% serum level fluctuation across the 12-h dose interval at steady state. After once-daily administration of 400 mg, a 27% serum level fluctuation was observed over 24 h (37). A peak-to-trough difference in plasma concentrations of ZNS (200 mg/d) in healthy volunteers was small (105). This small mean fluctuation across the dose interval also is a potentially significant advantage in clinical practice.

Drug Interactions

Although metabolized by the 3A4 isoform of the hepatic cytochrome P450 enzyme system, ZNS does not inhibit the P450 system (54). The addition of ZNS has no effect on steady-state levels of PHT, CBZ, or VPA in clinical trials, consistent with the absence of cytochrome P450 inhibition. ZNS may, therefore, be added to existing regimens of currently used AEDs without adjustment of their doses. When liver enzyme-inducing AEDs are added to ZNS, the relatively long elimination half-life of ZNS is reduced from 52 to 66 h when given alone to ~27 h with PHT, 38 h with CBZ, 38 h with PB, and 46 h with VPA (8). Shinoda et al. (87) observed that in epileptic patients the concomitant administration of PB, PHT, or CBZ with ZNS significantly decreased the ratio of the steady-state plasma concentration to the administered dose (C/D ratio) of ZNS, whereas clonazepam and VPA when administered concomitantly with ZNS did not change the C/D ratio, again consistent with the above observations. McJilton et al. (55) reported two patients treated with ZNS whose serum levels became elevated when lamotrigine was introduced.

Clinical Efficacy

Yagi and Seino (108) have assessed the clinical efficacy of 1008 patients with various types of epilepsy (605 adults and 403 children) who were treated with ZNS over a 7-y period in phase II and III studies at 56 centers across Japan. Despite variations in clinical efficacy by seizure type, the results indicate that ZNS was effective for both partial and generalized seizures. Analysis by type of epilepsy confirmed that ZNS was effective not only for partial epilepsy with a 50% response rate of 51 to 57%, but also for symptomatic generalized epilepsies, including West Syndrome and Lennox–Gastaut Syndrome. The response rate in generalized epilepsies ranged from 22% in West Syndrome to 66% in idiopathic generalized epilepsy.

In one open-label trial of 35 patients in Korea, the overall response rate was 50% in 14 patients with epileptic encephalopathy, 33% in childhood-onset symptomatic partial epilepsy (6 cases), 32% in adults onset partial epilepsy (12 cases). Two patients with progressive myoclonic epilepsy of the Unverricht–Lundborg type responded well to ZNS. The Korean experience was comparable to the Japanese results and confirms that ZNS is a broad-spectrum AED (46).

The antiepileptic efficacy of ZNS has been demonstrated in two multicenter, comparative studies with CBZ and VPA in Japan, and in three multicenter, placebo-controlled
studies in the U.S. and Europe in which ZNS was administered as add-on therapy to patients with refractory partial seizures and generalized seizures (Table 3).

Two randomized controlled trials were conducted in Japan to compare the efficacy of ZNS with that of CBZ and VPA. In the CBZ comparison (83), 123 adults patients with complex partial seizures refractory to one to three conventional AEDs were enrolled and treated with ZNS or CBZ for 16 w. The average monthly frequency of simple and complex partial seizures decreased from 14.9 to 3.4 in the ZNS group and from 13.3 to 4.4 in the CBZ group. A similar reduction in mean monthly frequency (from 2 to 0.6 in the ZNS group, from 2 to 0.7 in the CBZ group) was observed for secondarily generalized tonic-clonic seizures. The percent of patients showing a ≥ 50% reduction in seizure frequency were 82% in the ZNS group and 71% in the CBZ group at week 16.

The efficacy of ZNS was compared with VPA for 8 w in 32 children (≤ 15 y old) who had four or more convulsive or non-convulsive generalized seizures per month, refractory to up to three other AEDs (66). The median percent reduction in seizure frequency of generalized tonic-clonic seizures was 67% for VPA and 81% for ZNS from baseline, and the percent of patients showing a 50% or more reduction was 53 and 77% in the VPA and ZNS groups, respectively. The overall improvement rate was 43.8% for VPA and 50.0% for ZNS.

In Korea, the efficacy of ZNS is being investigated in a multicenter, double-blind comparison with CBZ in newly diagnosed epilepsy with idiopathic generalized tonic-clonic or partial seizures with or without generalization. After 4 w of planned fixed-dose escalation (ZNS 300 mg/d, CBZ 600 mg/d), doses were adjusted according to efficacy, adverse events, and plasma concentrations. The trial continued until the patients withdrew for adverse events or inadequate efficacy, or were seizure free for 24 w. Efficacy was assessed by time to first seizure, time to 24-w remission, and the proportion of patients maintaining a 24-w remission. Approximately 90% (17 of 19 patients) of the ZNS group and 95% (20 of 21 patients) of the CBZ group maintained a 24-w remission. The most common side effects leading to withdrawal were gastrointestinal symptoms in the ZNS group (11.5%) and rash in the CBZ group (18.5%). Anorexia and gastrointestinal symptoms were more common in ZNS than in CBZ (42.3 vs. 7.4%), while rash was observed more frequently in CBZ recipients (22.2 vs. 11.5%) (26).

In Europe, Schmidt et al. (79) conducted a multicenter, placebo-controlled, double-blind trial of ZNS as add-on treatment for 139 patients with refractory partial epilepsy. During the 12-w treatment phase, a ≥ 50% reduction (response rate) of all generalized and partial seizures was found in 29.9% of the ZNS group and 9.4% of the placebo group.

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**TABLE 3. Multicenter efficacy study of zonisamide**

<table>
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<tr>
<th>Country</th>
<th>Duration (w)</th>
<th>Design</th>
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<th>No. of patients</th>
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<td>double blind, add-on</td>
<td>CBZ</td>
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<td>83</td>
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</tbody>
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In one placebo-controlled American study, 152 adult patients with complex partial seizures (≥ 4 seizures/m) refractory to other AEDs were enrolled for ZNS add-on therapy for 12 w (106). The median percent change in seizure frequency (28 to 30%) and the response rate (29 to 30%) in the ZNS group were similar to the results of European trial. In this study, the final median percent change in seizure frequency was a 30.1% decrease in the ZNS group and a 0.3% increase in the placebo group (p < 0.01). The response rate was 28.6% in the ZNS group and 13.2% in the placebo group (p = 0.03). The overall improvement was higher in the ZNS group (62.3%) than in the placebo group (11.0%; p < 0.01).

In the U.S., one double-blind, parallel-group study that evaluated patients with partial seizures was conducted to investigate the clinical efficacy, dose response, and the relationship between efficacy and serum levels (9). Two hundred and three patients entered a 4-w single-blind placebo baseline. Those who continued to have at least four seizures/m were randomly assigned to receive ZNS or placebo. The ZNS group was further subdivided to receive either 100 or 200 mg/d for 5 w. This period allowed a low-dose comparison to placebo. Then ZNS was increased to 400 mg/d by the end of week 7 for a comparison with placebo to confirm efficacy at the therapeutic dose. At week 12, placebo recipients were crossed over to ZNS 400 mg/d while maintaining the blind. The dose of other concurrent AEDs was held constant throughout the study. At the end of week 5, the median reduction in seizure frequency for the ZNS group receiving 200 mg/d was 20.4% versus 4.0% for placebo (p ≤ 0.05). From week 8 through week 12 (5 w), the ZNS groups combined achieved a 40.5% reduction vs. 9.0% for placebo (p < 0.05), establishing ZNS efficacy. When placebo patients were crossed over to ZNS 400 mg/d at week 12, the median seizure reduction improved from 9.0% at week 12 to 40.7% at week 20 (p < 0.05). For those receiving ZNS 200 mg/d, the mean ZNS serum level was 6.94 μg/ml. When ZNS was increased to 400 mg/d, the median reduction in seizure frequency about doubled and the mean serum level increased to 19.0 μg/ml, thereby demonstrating a pharmacodynamic relationship between ZNS serum levels and seizure control. These results confirm the efficacy of ZNS 400 mg/d in patients with unresponsive partial seizures.

ZNS monotherapy was investigated in an open-label trial of 72 previously untreated children with cryptogenic localization-related epilepsies (60). A daily dose of 2 mg/kg of ZNS was introduced at first, then was doubled at weekly intervals until the initial maintenance daily dose of 8 mg/kg was reached. ZNS was given once a day in the morning. ZNS produced complete control of seizures in 57 out of 72 patients (79.2%). Once-daily administration of ZNS monotherapy was effective for the control of partial seizures in children. In another ZNS open monotherapy study (2 to 12 mg/kg/d), in which 68 pediatric patients with generalized (20 cases) or partial epilepsy (48 cases; 4 idiopathic, 44 cryptogenic/symptomatic) were enrolled, the remarkable improvement rate was 90% in patients with generalized epilepsy and 83.3% in patients with partial epilepsy (84). The short-term efficacy of ZNS monotherapy was also studied in 11 newly diagnosed patients with infantile spasms (IS) (90). ZNS at doses of 3 to 10 mg/kg was administered as the second-choice drug to patients with IS (3 cryptogenic, 8 symptomatic) who failed to respond to high-dose vitamin B6. Four infants with symptomatic IS had cessation of spasms and disappearance of the hypsarrhythmia. In these responders, spasms ceased at a ZNS dose 4 to 5 mg/kg/d, which produced plasma concentrations from 5.2 to 16.3 μg/ml (mean = 9.8 μg/ml). Two patients relapsed 4 to 6 w after cessation of seizures. Heo (26) com-
pared the efficacy of ZNS and CBZ monotherapy in patients with newly diagnosed epilepsy. Their interim report demonstrates that there was no statistical difference between two groups with regard to the remission rate of seizures.

ZNS efficacy was evaluated in five long-term studies to ensure that efficacy was maintained with no evidence of tachyphylaxis or pharmacologic tolerance. Two were extensions of placebo-controlled studies, two were extensions of baseline-controlled studies, and one was an extension of an uncontrolled study. The primary efficacy parameter was median reduction in frequency of all partial seizures compared to entry. Median reduction in seizure frequency ranged from 37.6 to 50.0% in four of five long-term studies (total n = 453). Median reduction in seizure frequency in the largest study (n = 137) with mean dose (mg/d) and mean plasma level (µg/ml) at each interval are shown in Table 4. ZNS efficacy was maintained in long-term studies with no evidence of tachyphylaxis or pharmacologic tolerance. This efficacy was achieved by a ZNS dose between 400 and 500 mg/d with a mean plasma level of about 20 µg/ml (106).

ZNS may be a useful AED in progressive myoclonic epilepsy (PME). Henry et al. (25) reported two patients with PME of the Unverricht-Lundborg type with intractable seizures in spite of standard AEDs regimens. After ZNS therapy was initiated, both had a marked decrease in seizure frequency and significant improvement of function. Serum ZNS concentrations were 27 and 43 µg/ml at doses of 8.8 and 10.5 mg/kg/d, respectively. A recent report on the use of ZNS as add-on therapy for seven patients (ages 19 to 42) with Unverricht–Lundborg’s disease (ULD) and one Lafora body disease is encouraging (41). ZNS was given at doses of 100 to 600 mg/d for 2 to 3 y. Concomitant AEDs were usually VPA and a benzodiazepine. ZNS dramatically reduced the number of myoclonias and generalized seizures. In three patients, the initial dramatic effect on myoclonias wore off after 2 to 4 y of treatment but patients still experienced control of generalized tonic-clonic seizures. The dramatic reduction of stimulus by light, touch, and startle by ZNS was sustained in all patients with ULD. In the patients with ULD treated concomitantly with acetylcysteine, a potentiating effect on the reduction of seizure activity and improvement of the general neurological condition was also observed (41).

**Tolerability**

Among 1008 patients treated with ZNS in phase II and III studies in Japan, 517 patients (51.3%) experienced adverse events and 185 patients (18%) discontinued ZNS therapy be-

### Table 4. Zonisamide efficacy in long-term studies

<table>
<thead>
<tr>
<th>Duration (m)</th>
<th>n</th>
<th>Median reduction (%)</th>
<th>Mean dose (mg/d)</th>
<th>Mean plasma level (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–7</td>
<td>120</td>
<td>42.1</td>
<td>491</td>
<td>21.0</td>
</tr>
<tr>
<td>8–10</td>
<td>95</td>
<td>45.2</td>
<td>503</td>
<td>21.5</td>
</tr>
<tr>
<td>11–13</td>
<td>74</td>
<td>50.7</td>
<td>502</td>
<td>21.3</td>
</tr>
<tr>
<td>14–16</td>
<td>63</td>
<td>43.2</td>
<td>445</td>
<td>20.9</td>
</tr>
<tr>
<td>&gt; 16</td>
<td>40</td>
<td>45.9</td>
<td>414</td>
<td>19.5</td>
</tr>
</tbody>
</table>
cause of adverse events (81). Drowsiness was the most commonly reported adverse event (24.3%), followed by ataxia (12.7%), anorexia (11%), gastrointestinal discomfort (6.2%), decreased spontaneity (5.5%), mental slowing (5.3%), weight loss (3.4%), and skin rash/itching (2.4%). Among 55 patients with ZNS monotherapy, adverse events were less frequent: drowsiness (9%), anorexia (7%), gastrointestinal discomfort (7%), decreased spontaneity (6%), headache (6%), weight loss (6%), and skin rash/itching (6%).

The incidence of adverse events in the study with ZNS and CBZ in Japan was 52% in the ZNS group and 57% in the CBZ group (not statistically different). The incidence of somnolence, mental slowing, decreased spontaneity, and skin rash/itching were almost the same in the two groups, whereas anorexia was more frequent in the ZNS group (12 vs. 0%, \( p = 0.02 \)), and ataxia was observed more frequently in the CBZ group (9 vs. 35%, \( p < 0.01 \)). The withdrawal rate for adverse events was 12% in the ZNS group and 16% in the CBZ group. The overall safety ratings of ZNS and CBZ evaluated according to adverse events and laboratory test deviations were 83% and 66% (cumulative of “safe” and “almost safe [practically no problems]”), respectively. The incidence of adverse events in the placebo-controlled studies was 92% in ZNS group vs. 58% in placebo group and 69% in ZNS vs. 64% in placebo in two U.S. studies, and 59% in ZNS vs. 28% in placebo in one European study. The withdrawal rate because of adverse events in one U.S. study was 14% in the ZNS group and 1% in the placebo group. In the European study, the rate was 3% in the ZNS group and none in the placebo group (79,82).

In Japan, two patients (0.2%) developed renal calculi among 1008 patients in phase II and III studies; both had family histories of renal calculi and the stones passed spontaneously. Furthermore, in the postmarketing surveillance program (PMS) conducted over 6 y since launch in 1989, only three cases with renal calculi (0.06%) were reported out of 5418 cases surveyed. Yagi et al. (82) measured 24-h urine volume and calcium, magnesium, citrate, and phosphate excretion in ten inpatients before and 1-m after the initiation of ZNS treatment (80 to 300 mg/d). Excretion of citrate decreased significantly after the administration of ZNS; however, other measures (calcium, magnesium, phosphate and urine volume) did not change significantly. In two initial U.S. studies and one initial European study, 13 (11 in the U.S. and 2 in Europe) of 505 patients (2.6%) developed symptomatic nephrolithiasis. Ten of the 13 patients above had histories of renal calculi and urinary tract surgery (81,82). In three subsequent U.S. studies, a renal ultrasound examination was performed prior to entry and periodically on each patient. This appears to be the first systematic search for renal calculi by means of objective imaging in epileptic patients receiving an antiepileptic drug; 514 patients (429 on ZNS and 85 on placebo) participated. Of the 85 patients initially randomized to placebo, 72 were crossed over to ZNS so 501 received ZNS. Of 501 patients, 17 (3.4%) developed calculi by ultrasound, but three dropped out of the study while receiving ZNS so the frequency of calculi possibly attributable to ZNS was 14 of 501 (2.8%). Four (0.8%) were symptomatic. Two of 85 (2.4%) developed calculi while receiving placebo (69).

In the PMS in Japan, anhydrosis accompanied by increased body temperature was reported in pediatric patients during the summertime. The dyshydrosis subsided with a decrease of ZNS doses or withdrawal (16,92).

Kondo et al. (39) described 26 offspring exposed to ZNS (4 monotherapy, 22 polypharmacy) in 22 mothers. Malformations were detected in two offspring (7.7%) exposed to ZNS polypharmacy. Anencephaly was detected in one case at 16 w of gestation (artificial abortion), and an atrial septal defect was detected in another case at 37 w of gestation (Ce-
The authors concluded that teratogenic effects of ZNS were not clearly defined from these results since malformations were detected in two polypharmacy cases but not in four monotherapy cases. The use of ZNS is recommended for women who are pregnant or suspected to be pregnant only if the therapeutic benefits outweigh the potential risks to the fetus.

**Dosage and Administration**

ZNS is available in tablets (100 mg) and powder form (200 mg/g) in Japan. The approved dosage and administration schedule of ZNS is as follows. The initial total daily dose is 100 to 200 mg, p.o.. It is usually divided into three equal dosages. The total daily dose is gradually increased every 1 to 2 w, up to 200 to 400 mg. The maximal total daily dose is 600 mg. The initial total daily in children is 2 to 4 mg/kg, p.o.. It is also usually divided into three equal dosages and gradually increased, every 1 to 2 w, up to 4 to 8 mg/kg. The maximal daily dose in children is 12 mg/kg. Clinical experience in the U.S. and Europe shows that the majority of adult patients will derive maximum benefits from a dose of 400 to 600 mg/d. In order to reduce adverse events, the dose of ZNS should be titrated slowly over 2 to 4 w.

**SUMMARY**

We have presented the chemistry and pharmacological properties of ZNS and its clinical efficacy in epilepsy. The profile of ZNS can be summarized as follows:

ZNS is effective in various animal models of epilepsy. ZNS was effective against maximal seizures induced by electroshock and pentylenetetrazole, but had no effect against minimal seizures induced by latter analeptic. This anticonvulsant profile is similar to those of PHT and CBZ, suggesting that ZNS exerts an anticonvulsant effect by inhibiting the spread or propagation of seizures. ZNS restricts the electroencephalographic spread of cortical seizures evoked by electrical stimulation.

ZNS suppresses seizures and electroencephalographic afterdischarges in various kindling models. ZNS shortened the duration of afterdischarge and elevated the seizure threshold in neocortex-, hippocampus-, and amygdaloid-kindled rats. ZNS also reduced or abolished both electroencephalographic afterdischarges and seizures in amygdaloid-kindled cats.

Studies of ZNS on cellular mechanisms of antiepileptic action have demonstrated that ZNS blocks voltage-sensitive Na⁺ and Ca²⁺ channels. ZNS specifically blocked the sustained repetitive firing of action potentials in cultured spinal cord neurons of the mouse embryo. ZNS caused a hyperpolarizing shift in the steady-state fast inactivation curve and retarded recovery from fast and slow Na⁺ inactivation in voltage-clamped *Myxicola* giant axons. ZNS selectively reduced the transient Ca²⁺ current (T-type Ca²⁺ current) with no effect on long-lasting Ca²⁺ currents (L-type Ca²⁺ current) in cultured neurons. The effect of ZNS on both voltage-dependent Na⁺ and Ca²⁺ channels disrupts neuronal synchronized firing and epileptic activity, thereby limiting the spread and propagation of seizures.
ZNS has a neuroprotective effect which is thought to be due to other mechanism than its antiepileptic action. ZNS prevented cerebral damage after hypoxic-ischemic insult or a transient global forebrain ischemia. ZNS reduced the infarct volume and neurological deficits in focal ischemia with reperfusion, although CBZ and VPA did not show any preventive effect. ZNS exerted a potent preventive effect against MPTP-induced dopaminergic neurodegeneration in contrast to other AEDs. ZNS has radical scavenging activity on hydroxyl and nitric oxide radicals as well as DPPH and carbon centered radicals. The difference between ZNS and other AEDs in preventing cerebral damage may depend on this radical scavenging action.

In humans, absorption of ZNS is prompt and complete. ZNS kinetics are not affected by food, age, gender, or renal impairment. Within the therapeutic dose range, plasma concentrations of ZNS are proportional to dose. ZNS has a relatively long elimination half-life, 50 to 69 h, allowing once- or twice-daily dosing. ZNS shows a small peak-to-trough differences in plasma concentrations after repeated administration. The elimination half-life of ZNS is reduced and clearance increased when liver enzyme-inducing AEDs are added to ZNS. ZNS has no appreciable effect on the steady state plasma concentrations of PHT, CBZ, or VPA.

ZNS is effective for the management of partial-onset seizures, with or without secondarily generalized seizures, and generalized seizures as adjunctive therapy and as monotherapy. Multicenter, double-blind, controlled trials were conducted in Japan, Europe, and the U.S. in which ZNS was administered as adjunctive therapy to patients with refractory seizures. The efficacy of ZNS was comparable to that of CBZ or VPA and significantly superior to placebo. Clinical results from these comparative and open studies show that ZNS is indicated for patients with localization-related epilepsies, and also for patients with generalized epilepsies.

The most commonly observed adverse events are drowsiness, ataxia, anorexia, gastrointestinal symptoms, decreased spontaneity, mental slowing, and weight loss. The incidence of adverse events can be significantly reduced by gradual introduction of ZNS with a slow titration to the therapeutic dose. In Japan, the frequency of renal calculi in the registration trials and PMS program was low, 0.2 and 0.06%, respectively. In the U.S. trials in which renal ultrasound examinations were performed before entry and periodically while receiving ZNS, 14 of 501 patients (2.8%) developed calculi. On the other hand, 2 of 85 patients (2.4%) developed calculi while receiving placebo.

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