Cerebral Protective Effect of Zonisamide

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ABSTRACT. We have been studying the cerebral protective effect of various anticonvulsants and have elucidated the relationship between various types of anticonvulsants and their protective effects. The present study was carried out to evaluate the cerebral protective effect of Zonisamide (ZNS : 1,2-benzisoxazole-3-methanesulfonamide) known to be clinically effective against refractory epilepsy. Experiments on the following effects of ZNS were conducted in mice ; 1) the effect of ZNS on the survival time after hypoxia, 2) its effect on the duration of the gasping movement induced by decapitation, and 3) its effect on the cerebral trauma of Manaka's model. The effect of ZNS on delayed neuronal death after a transient cerebral ischemia was studied in the gerbils by means of histological examination of the neuronal density in hippocampal CA1 sections. In the ZNS-treated group ; 1) protective effects against hypoxia, ischemia, and trauma were confirmed, and 2) delayed neuronal death in hippocampal CA1 sections was suppressed significantly. In summary, ZNS clearly demonstrated its cerebral protective effect in animal models. Thus, anticonvulsants like ZNS and PHT, with an action to block the propagation of discharges are considered to possess a protective action, suggesting their future applicability for protection against various forms of cerebral damage in the acute phase.

Key words : cerebral protection — anticonvulsant — zonisamide

Zonisamide (ZNS) is an anticonvulsant characterized by a completely new chemical structure (Fig. 1). ZNS does not have the ureide structure seen in many anticonvulsants. It can also treat a wide spectrum of various types of seizures related to partial epilepsy and generalized epilepsy. The profile of the anticonvulsant action of ZNS resembles that of phenytoin (PHT),[1,2] and its range of effective blood concentration is wide.[3] In this article, we discuss the fact that this agent has not only an anticonvulsant action but also a cerebral protective action similar to phenobarbital (PB) and PHT. Our study is based on various models of experimental hypoxia, cerebral ischemia, and traumatic cerebral lesions.

ANIMALS AND METHODS

1) Mice

ZNS was orally administered to male mice (weighing 22-25 g). After the

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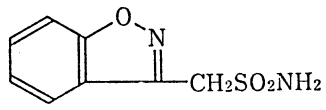

1, 2-benzisoxazole-3-methanesulfonamide

Characteristics

- Broad antiepileptic spectrum (usefulness for refractory epilepsy)
- Anticonvulsive action similar to PHT, CBZ
- Unique chemical structure without ureide moiety
- Long half life (60hrs)
- Wide therapeutic concentration

Fig. 1. Chemical structure and characteristics of ZNS

peak time, (1) normobaric hypoxia was introduced by placing the mice in a 2.5 L plastic chamber through which a gas mixture of nitrogen and oxygen (96 : 4) was allowed to flow at a rate of 4 L/min. The length of time from the onset of hypoxic seizure to the time of respiratory cessation (survival time) was measured. Subsequently, the drug concentration in the brain was examined and correlated with the survival time. (2) Complete cerebral ischemia was performed by decapitation, after which the gasping movement intervals and the persistent time were measured and correlated with the cerebral drug concentration. (3) The cerebral traumatic consciousness damage model of Manaka[4] was used here. The mouse's head was fixed by holding both of its ears, and a 20 g cylindrical bakelite rod was dropped through a clear, cylindrical, plastic tube from 40 cm above onto the top of the mouse's head. The degree of cerebral lesion was evaluated by measuring the righting reflex time (RR time) from the occurrence of the coma caused by the impact. Afterwards the period between the RR time and the time of the appearance of spontaneous movement (SM time) was also measured.

2) Gerbils

One hour after orally administering ZNS to male gerbils (weighing 65-85 g), the cervical region was incised at the midline under ether anesthesia. The bilateral common carotid arteries were exposed and occluded with Zen clips. Five minutes later, the clips were removed to resume the blood flow. After a similar anesthetic surgical operation, we prepared a group of gerbils who underwent no clipping of the arteries as a "sham" operation group. Four days later, the brain was perfused through the left ventricle of the heart with a fixative solution containing 2.5% glutaldehyde and 2% paraformaldehyde in a 0.1 M phosphate buffer (pH 7.4). A tissue specimen containing the dorsal

hippocampus was dewatered and, through penetration, was embedded in a paraffin block. Sections of 4 μm thickness were prepared from this paraffin block and dyed with hematoxylin and eosin. The number of pyramidal cells remaining in the hippocampal CA1 field in those sections was measured using an image processor (model: MCID, Imaging Research Inc.) under 200 magnification to calculate the cell density per mm^2 of the bilateral hippocampal pyramidal cell layer. Student's t-test was uesd to analyze the data.

RESULTS

1) ZNS significantly prolonged the length of time between the onset of hypoxic seizure and the survival time in hypoxic mice dose-dependently (Table 1). A positive correlation between the survival time and the

TABLE 1. Effects of ZNS on the latency of hypoxic convulsion and the survival time in normobaric hypoxic mice

	Control (n=8)	Treated (dose: mg/kg.p.o.)			
		10(n=8)	20(n=8)	50(n=8)	100(n=8)
Latency time of convulsion (sec ± SE)	154±12	173±20	164±15	242±19**	318±46**
Survival time (sec ± SE)	242±11	261±16	249±13	323±15**	449±35**

** Significantly different from the value of vehicle control ($p<0.01$)

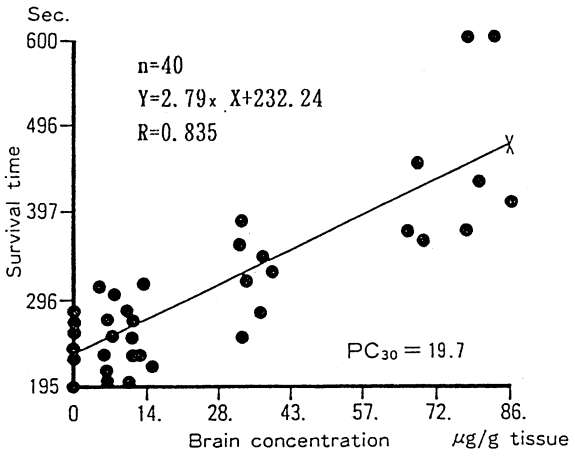

Fig. 2. Effect of ZNS on survival time in hypoxic mice

A positive correlation between the survival time and the cerebral ZNS concentration in individual mice was observed. Based on these correlations, the drug concentration (30% protective concentration; PC_{30}) necessary to prolong the survival time by 30% was calculated as 19.7 μg/g tissue.

cerebral ZNS concentration in individual mice was observed. The reciprocal relationship in each group showed a higher correlation coefficient. Based on these correlations, the drug concentration (30% protective concentration ; PC_{30}) necessary to prolong the survival time by 30% was calculated as 19.7 μg/g tissue (Fig. 2).

2) The persistent time and intervals of gasping movement after decapitation were prolonged significantly with ZNS (Table 2). There was a positive correlation between the gasping persistent time and the cerebral concentration in individual mice. The drug concentration necessary to prolong the gasping persistent time by 15% was 15.6 μg/g tissue (Fig. 3).

TABLE 2. Effects of ZNS on gasping caused by decapitation in mice

	Control (n=8)	Treated (dose : mg/kg.p.o.)			
		10(n=8)	20(n=8)	50(n=8)	100(n=8)
Persistent time (sec ± SE)	22.0±0.8	22.1±0.7	24.8±1.3	27.4±1.1**	32.8±1.2**
Gasping interval (sec ± SE)	1.7±0.1	1.9±0.2	2.0±0.1	2.3±0.1**	2.7±0.1**

** Significantly different from the value of vehicle control ($p<0.01$)

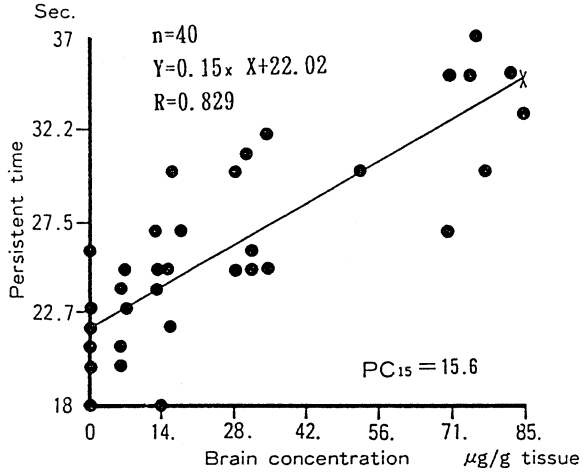

Fig. 3. Effect of ZNS on the persistent time of gasping caused by decapitation in mice

There was a positive correlation between the gasping persistent time and the cerebral concentration in individual mice. The drug concentration necessary to prolong the gasping persistent time by 15% was 15.6 μg/g tissue.

3) The RR time and SM time in the control group of the mouse head trauma model were 20.3 sec and 54.3 sec respectively. These were shortened to 9.7 sec and 12.4 sec, respectively, with ZNS (Table 3).

4) In the gerbil ischemia model, ZNS inhibited, in a dose-dependent manner, the decrease in the neurocyte density of the hippocampal CA1 field caused by transient ischemia (Table 4, Fig. 4).

DISCUSSION

Among anticonvulsants, PB and PHT have already been clinically used as cerebral protective agents. However, their cerebral protective action has not been correlated with standard antiepileptic activity, and many unclear issues remain. We therefore conducted experiments to determine whether an anticonvulsant can be evaluated generally as a cerebral protective agent, and if there are common functions in the mechanism of antiepileptic drugs and that of cerebral protective agents. We found that there is a relationship between the type of anticonvulsant, its potency, and its cerebral protective action.[5,6]

TABLE 3. Effects of ZNS on the time to R.R. and S.M. after cerebral trauma

	Righting reflex (R.R.)time (sec ±SE)	Spontaneous movement (S.M.) time (sec ±SE)
vehicle control (n=8)	20.3±7.7	54.3±15.4
ZNS 50 mg/kg p.o. (n=8)	9.7±2.7**	12.4±2.9**

** Significantly different from the value control ($p<0.01$)

TABLE 4. Effects of ZNS on delayed neuronal death

	Dose mg/kg p.o.	Cell density of CA1 subfield mm^2 ± S.E.
sham control (n=6)	—	3549±281
vehicle control (n=6)	—	250±64
ZNS (n=6)	30	2713±228**
	100	3326±49 **

** Significantly different from the value of vehicle control ($p<0.01$)

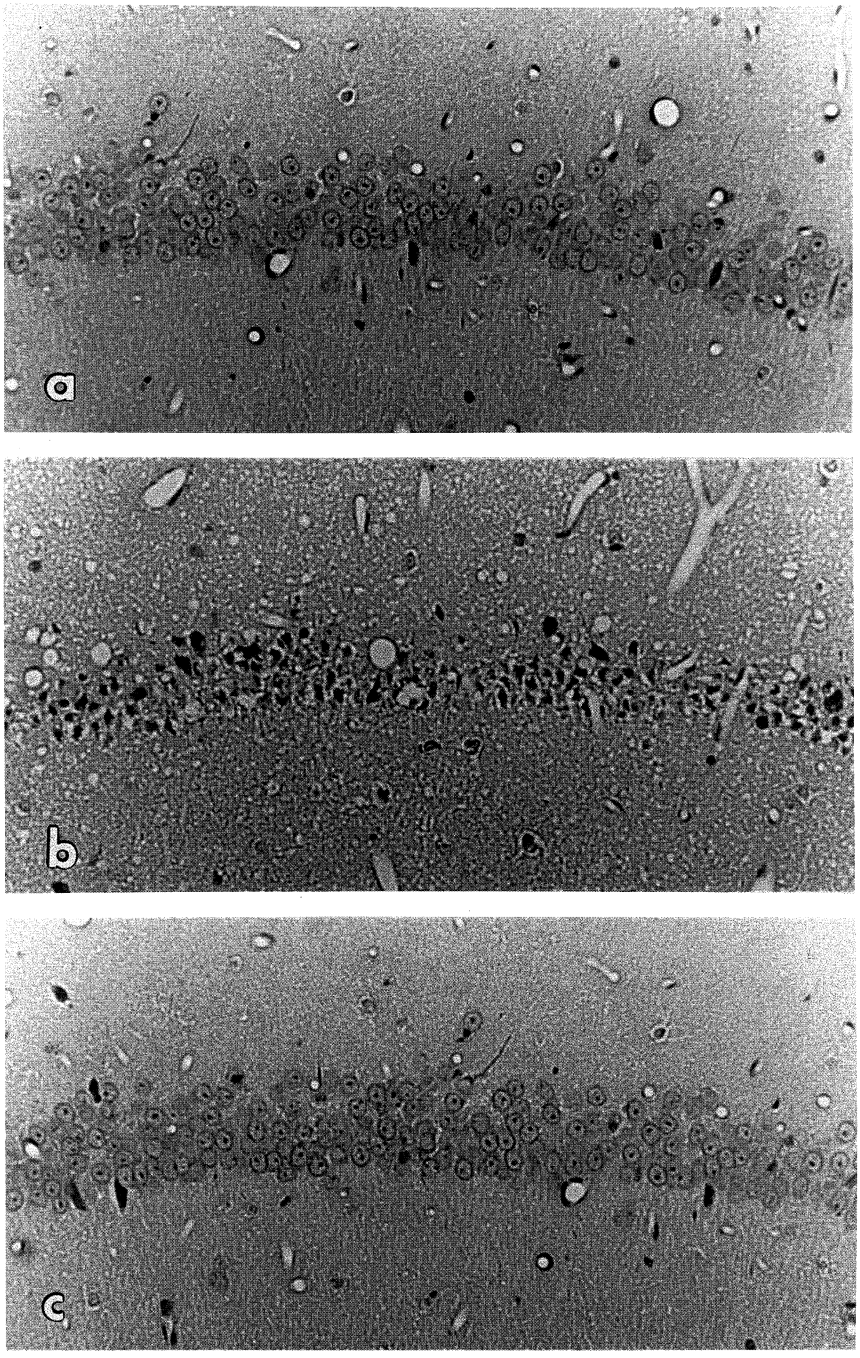

Fig. 4. Photomicrographs of neurocyte density of the CA1 sector (×200)

ZNS inhibited the decrease in the neurocyte density of the hippocampal CA1 field caused by transient ischemia.

a : normal gerbils, b : ischemic gerbils with vehicle
c : ischemic gerbils with ZNS (30 mg/kg, p.o.)

We studied the cerebral protective effect of a new anticonvulsant, ZNS (1, 2-benzisoxazole-3-methanesulfonamide), which is thought to effectively inhibit the tonic extensor component of seizures induced by maximum electric shock and pentylenetetrazol, and which is further thought to be effective in treating refractory epilepsy. The pharmacological action of ZNS is characteristic in that it shows a strong inhibitory action on the maximum convulsion in mice induced by electrical and chemical stimuli. The pharmacological profile[1,2] of ZNS resembles that of PHT and carbamazepine (CBZ). The mechanism of the anticonvulsant action of ZNS is attributed to the inhibition of seizure spreading.[7] The neurotoxicity of ZNS is weak[1,3] — about 1/2 to 1/10 that of PHT, CBZ and PB.

The results of our experiment on hypoxia also showed that ZNS had a cerebral protective action. The PC_{30} value calculated from the relationship between the survival time and the brain ZNS concentration was similar to the anticonvulsive concentration. Gasping appears as a rapid change in cerebral energy metabolism caused by decapitation — which is the end of anaerobic metabolism — and the duration time closely corresponds to the time in which the EEG becomes flat and cerebral ATP and phosphocreatinine are rapidly consumed. In complete cerebral ischemia by decapitation, ZNS prolonged the gasping duration time.

These facts show that there is a closer relationship between the intensity of the anticonvulsant action and the cerebral protective action of the anticonvulsant. The RR time in the traumatic model is thought to indicate coma, mainly brain stem damage, and the SM time indicates stupor and cerebral hemisphere damage. Accordingly, the fact that ZNS shortened the RR and SM times indicates prevention of damage to the brain stem and cerebral hemisphere, and further suggests the protective cerebral action of this agent.

In the model of the five minute forebrain ischemia gerbil, it is known that most CA1 pyramidal cells are destroyed on the fourth day.[8] The results of our experiment proved that the remaining neurocyte density markedly decreased on the fourth day after ischemia,[9] and ZNS was effective in maintaining the remaining neurocyte density. In the mechanism of delayed neuronal death in the hippocampal CA1 field, excitatory amino acids play a large role.[10] In other words, Ca^{2+} influx into cells through NMDA receptors is thought to be an intracellular mechanism whereby excitatory amino acids demonstrate toxicity to neurocytes. The total amount of Ca in the hippocampal CA1 field increases,[11] corresponding to the degree of cell necrosis. Accordingly, our experimental results may suggest that the protective effect of neurocytes appears as a result of direct or indirect participation of excitatory amino acids in the mechanisms of delayed neuronal death. Clinically, this agent has a wide paroxysmal spectrum, and is especially effective for partial epilepsy and generalized tonic-clonic seizures.

Thus, ZNS shows a cerebral protective effect and it may also be effective in treating acute phases of various cerebral lesions.

CONCLUSIONS

In our animal study, ZNS was found to have a cerebral protective effect. ZNS is believed both to prevent convulsions and to provide cerebral

protection, which is useful in treating the acute phases of various cerebral lesions.

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