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Influence of oral adsorbent AST-120 on anticonvulsive effect of zonisamide in rats

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Abstract

The influence of oral adsorbent AST-120 (Kremezin) on the anticonvulsive effect and pharmacokinetics of zonisamide was investigated. Oral administration of zonisamide (50 mg/kg) blocked the appearance of the tonic extension induced by maximal electroshock seizure. This effect of zonisamide was inhibited by the oral coadministration of AST-120 (5 g/kg). In pharmacokinetics study, the serum zonisamide concentration after coadministration of zonisamide and AST-120 was significantly lower than that of single administration of zonisamide. However, the anticonvulsive effect of zonisamide was not affected by the administration of AST-120 1.5 h after zonisamide administration. In this condition, the serum zonisamide concentration was not changed. In the in vitro study, AST-120 completely adsorbed zonisamide. These findings suggest that when AST-120 is administered concurrently with zonisamide, a significant inhibition of the anticonvulsive effect of zonisamide occurs, and the decrease in serum zonisamide concentration by the adsorption effect of AST-120 is related to this phenomenon.

Keywords: AST-120; Zonisamide; Adsorption; Anticonvulsive effect; Blood concentration

1. Introduction

AST-120 (Kremezin) is an orally administered adsorbent that consists of porous spherical particles composed mainly of carbon (Honda and Nakano, 1997). It is used to inhibit the progression of renal failure by adsorbing the uremic toxins secreted or produced in the gastrointestinal tract (Sanaka et al., 1988; Yamazaki et al., 1980). AST-120 is quite efficacious against chronic renal failure in patients during the predialytic stage. It results in the improvement of uremic manifestations and delays the need to introduce dialysis (Sanaka et al., 1988; Takara et al., 1985). AST-120 is adsorbent to combination drugs (Honda and Nakano, 1997). When other drugs are administered in combination with AST-120, the quantity of these drugs may be influenced by the adsorption effect of AST-120. Namely, because of the adsorptive effect of AST-120, it is possible to attenuate the clinical effect of combination drugs.

In general, existing anticonvulsants have been reported to cause various central adverse effects, by narrowing the therapeutic range between anticonvulsant and neurotoxic blood concentration (Baumel et al., 1973; Nishiguchi et al., 1992). It is necessary to keep the blood concentration of anticonvulsants within the therapeutic range to provide adequate anticonvulsive effects with the use of therapeutic drug monitoring (Levy et al., 1992; Tange et al., 1994; Tozer and Winter, 1992; Walson, 1994).

Zonisamide is a sulfonamide derivative that is effective in treating patients with generalized tonic-clonic seizure, secondary generalized seizures, complex partial seizures and simple partial seizure (Sackellares et al., 1985). It was also reported that zonisamide effectively treated electroconvulsive seizure in rodents (Masuda et al., 1980a,b). The therapeutic range of the blood zonisamide concentration is narrow as with other anticonvulsants (Nishiguchi et al., 1990, 1992). If it is assumed that zonisamide is adsorbed by AST-120 in the digestive tract, epileptic patients taking

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zonisamide would experience a seizure by decreasing the serum zonisamide concentration. However, few attempts have been made to examine the adsorption of anticonvulsants, including zonisamide. We found one case that zonisamide was prescribed for an epileptic patient who had been taken AST-120. Therefore, we think that the investigation of the influence of the oral adsorbent AST-120 on the anticonvulsive effect of anticonvulsants is important information for the pharmaceutical care and risk management of epileptic patients.

In the present study, we first examined the effect of AST-120 on the in vitro adsorption of zonisamide. Then, we investigated the influence of oral administration of AST-120 on the anticonvulsive effects of zonisamide to maximal electroshock seizure and measured the concentration of zonisamide in the blood of rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River, Yokohama, Japan), 310–400 g, were used. They were housed in groups of four animals per cage under controlled conditions of light (from 7:00 a.m. to 7:00 p.m.), temperature (23 ± 1 °C) and relative humidity (approximately 60%). The animals were allowed free access to standard laboratory food and tap water.

2.2. Drugs

The following drugs were used: AST-120 (Kremezin) was obtained from Kurehakagaku (Tokyo, Japan), and zonisamide was obtained from Dainippon Pharmaceutical (Osaka, Japan).

2.3. Adsorption of zonisamide by AST-120 in vitro

Pure zonisamide powder was dissolved in distilled water (100 μg/ml), and AST-120 was added to the solution at five different AST-120/zonisamide ratios (1:1, 10:1, 20:1, 40:1 and 100:1). After shaking for 30 min at room temperature using a shaker from Ikemoto Rika (Tokyo, Japan), AST-120 was removed using a 0.45-μm membrane filter (Millex-HU Millipore, Bedford, MA, USA). The zonisamide concentration in the filtrate was measured using a spectrophotometer (228:Hitachi, Tokyo, Japan) at a 284-nm wavelength. Similarly, the influence of shaking time was examined every 5 min between 0 and 30 min after zonisamide addition. The influence of pH also was examined in distilled water (pH 7.0), 0.1 N HCl (pH 1.2) and 0.05 M phosphate buffer (pH 6.8).

2.4. Procedure for maximal electroshock seizure

The animals were administered maximal electroshock, a 60 Hz AC current of 100 mA for 0.4 s through correal electrodes using the Woodbury and Davenport's (1952) apparatus. The incidence of tonic hindlimb extension following maximal electroshock was measured. Zonisamide was given 2 h before observation. AST-120 was administrated at 30 min and 2 h before observations. All drugs were suspended in 0.5% methylcellulose on the day of testing, and zonisamide and AST-120 were administrated in a volume of 2 and 20 ml/kg body weight, respectively.

2.5. Determination of the zonisamide concentration in serum

Blood samples were collected 2 h after zonisamide administration from the tail vein. After being centrifuged at 5400 × *g* for 5 min, the serum obtained was used for the determination of the serum concentration of zonisamide. The 20-μl serum samples plus 10 μg of *N*,*N*-dimethylzonisamide, as the internal standard, were passed through a Bond Elut Cartridge (C18 Varian, Harbor, CA, USA). The serum protein was removed in 8% methanol. A 30-μl aliquot of the elute was injected into a high-performance liquid chromatography system (Waters, Tokyo, Japan) with UV spectrophotometric detection (Lambada-Max Model 418, Waters). Zonisamide was separated on a reversed phase column LiChroCART (Superspher RP (e) 4 μm). The detection of zonisamide was performed at 284 nm. The mobile phase was 40% methanol and the flow rate was 1 ml/min.

2.6. Statistics

Values are expressed as group means and S.E.M. The serum zonisamide concentration measurements were analyzed by one-way analysis of variance (ANOVA), and the

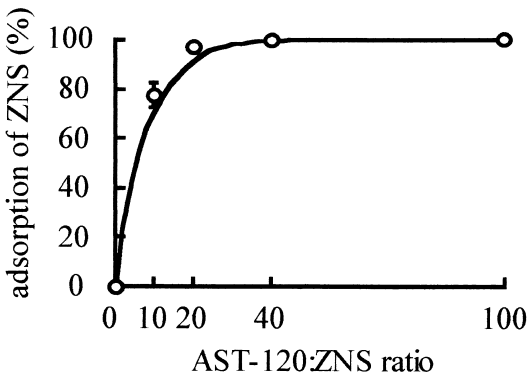

Fig. 1. Effect of adsorption of zonisamide by AST-120 at different AST-120/zonisamide ratios in vitro. Pure zonisamide (100 μg/ml:ZNS) was dissolved in distilled water. AST-120 was added to the solution at five different AST-120/zonisamide ratios (1:1, 10:1, 20:1. 40:1 and 100:1). AST-120 was removed after shaking for 30 min at room temperature. The zonisamide concentration in the filtrate was measured. The adsorption of zonisamide is expressed as the mean ± S.E.M. for five separate experiments.

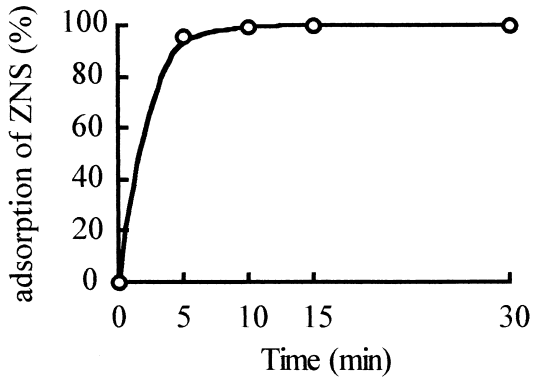

Fig. 2. Effect of adsorption of zonisamide by AST-120 at different shaking times in vitro. Pure zonisamide (100 μg/ml:ZNS) was dissolved in distilled water. AST-120 was added to the solution at an AST-120/zonisamide ratio of 100:1. AST-120 was removed after shaking for 0 to 30 min at room temperature. The zonisamide concentration in the filtrate was measured. The adsorption of zonisamide is expressed as the mean ± S.E.M. for five separate experiments.

group means were compared by Dunnett's test for multiple comparisons. The incidence of the tonic extensor induced by maximal electroshock seizure was analyzed by the χ^2 test.

3. Results

3.1. Effect of adsorption of zonisamide to AST-120 in vitro

Fig. 1 shows the effects of adsorption of zonisamide to AST-120 at different AST-120/zonisamide ratios in vitro. Zonisamide was very effectively bound by AST-120 in vitro, even at low AST-120/zonisamide ratios. For example, at the AST-120/zonisamide ratio of 20:1, about 97% of zonisamide was bound to AST-120. At the AST-120/zonisamide ratio of 100:1, AST-120 adsorbed 96% of zonisamide after mixing for 5 min (Fig. 2). Table 1 shows that the adsorbing effect of AST-120 was not changed in different pH's between 1.2 and 7 using the AST-120/zonisamide ratio of 100:1.

Table 1
Influence of adsorption of zonisamide by AST-120 at different pHs in vitro

Solvent	pH	Adsorption of ZNS (%)
Distilled water	7.0	100 ± 0.0004
0.1 N HCl	1.2	99.6 ± 0.0009
0.05 M phosphate buffer	6.8	100.0 ± 0.0006

Pure zonisamide (100 μg/ml) was dissolved in distilled water, 0.1 HCl and 0.05 M phosphate buffer. AST-120 was added to the solution at the AST-120/zonisamide ratio of 100:1. AST-120 was removed after shaking for 30 min at room temperature. The adsorption of zonisamide is expressed as the mean ± S.E.M. for five separate experiments.

Table 2
Effect of coadministration of AST-120 and zonisamide on the incidence of tonic hindlimb extension induced by maximal electroshock in rats

Drugs	Dose	Incidence of tonic hindlimb extension
Vehicle	–	6/6
Zonisamide	50 (mg/kg)	0/6**
Zonisamide + AST-120	50 (mg/kg) 1 (g/kg)	1/6
Zonisamide + AST-120	50 (mg/kg) 5 (g/kg)	4/6*

Zonisamide (50 mg/kg) and AST-120 (1 g/kg, 5 g/kg) were coadministrated 2 h before the observation. Values are expressed as the incidence of tonic hindlimb extension after maximal electroshock for six rats.

* $P < .05$, significantly different from the administration of zonisamide alone group.

** $P < .01$, significantly different from vehicle.

3.2. Effect of AST-120 on the anticonvulsive action of zonisamide in rats

The incidence of maximal electroshock-induced tonic hindlimb extension was perfectly suppressed by zonisamide at a dose of 50 mg/kg, po. However, the suppressive effect of zonisamide on maximal electroshock-induced tonic hindlimb extension was dose-dependently and significantly inhibited by coadministration of AST-120 (5 g/kg, po) (Table 2). When AST-120 was administered 1.5 h after the zonisamide administration, the inhibitory effect of zonisamide on maximal electroshock-induced tonic hindlimb extension was not changed (Table 3).

3.3. Effect of AST-120 on the serum concentration of zonisamide in rats

Fig. 3 shows the effect of AST-120 on the serum concentration of zonisamide in rats. The serum concentration of zonisamide was 48 μg/ml in control rats, with nonadministration of AST-120. The serum concentration of zonisamide was significantly lower ($P < .01$) at 2 h after coadministration of AST-120. However, when AST-120 was

Table 3
Effect of administration of AST-120 1.5 h after zonisamide on the incidence of tonic hindlimb extension induced by maximal electroshock in rats

Drugs	Dose	Incidence of tonic hindlimb extension
Vehicle	–	6/6
Zonisamide	50 (mg/kg)	0/6**
Zonisamide + AST-120	50 (mg/kg) 5 (g/kg)	0/6**

Zonisamide (50 mg/kg) and AST-120 (5 g/kg) were administered 2 h and 30 min before the observation, respectively. Values are expressed as the incidence of tonic hindlimb extension after maximal electroshock for six rats.

** $P < .01$ significantly different from vehicle.

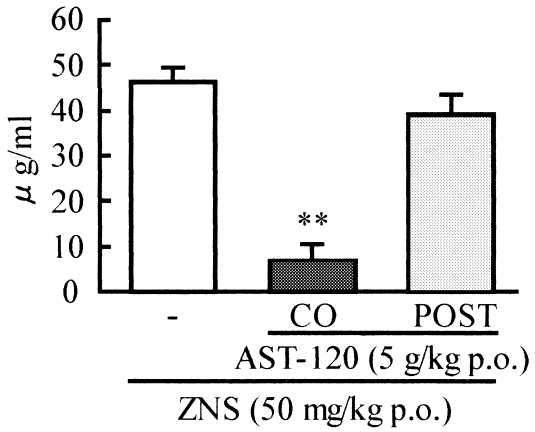

Fig. 3. Effect of coadministration of zonisamide and AST-120 on serum zonisamide concentration in rats. Zonisamide (50 mg/kg:ZNS) and AST-120 (5 g/kg) were coadministrated (CO) 2 h before collecting blood. AST-120 (5 g/kg) was administrated 1.5 h (POST) after administration of zonisamide at a dose of 50 mg/kg. Blood collection was performed 2 h after the administration of zonisamide. Each column is expressed as the mean ± S.E.M. for six rats. ** $P < .01$ significantly different from the administration of zonisamide alone group.

administrated 1.5 h after zonisamide, it was not changed compared to a single administration of zonisamide.

4. Discussion

The effect of the adsorptive capacity of AST-120 on zonisamide was examined. AST-120 adsorbed zonisamide in vitro by almost 100% in concentrations 40-fold higher than those of zonisamide, and after only 5 min of shaking. The adsorptive effect of AST-120 on zonisamide was not affected by pH level.

Honda and Nakano (1997) reported that AST-120 had an adsorptive capacity in the coadministration of other drugs, and this capacity depended on the molecular weight of the adsorbates. It was reported that AST-120 possessed an adsorptive capacity ranging in molecular weights from about 100 to 1000 (Honda and Nakano, 1997). Zonisamide's molecular weight is 212.23 (Seino et al., 1991), and it appears to be a good candidate for adsorption by AST-120. The present result suggests that other drugs also must be influenced when they are administered in combination with AST-120.

The influence of the coadministration of AST-120 and zonisamide on the anticonvulsive effect of zonisamide in rats was investigated. It was confirmed that the incidence of tonic hindlimb extension was significantly and completely (100%) suppressed by zonisamide at a dose of 50 mg/kg. However, this suppressive effect of zonisamide (50 mg/kg) was significantly (33.3%) inhibited by coadministration of AST-120 at a dose of 5 g/kg. The AST-120/zonisamide ratio was 100:1 in this in vivo condition. AST-120 adsorbed zonisamide in vitro by almost 100% at an AST-120/zonisamide ratio of 100:1. Although there were differences between the in vivo and in vitro conditions, the inhibitory effect of AST-120 on the anticonvulsive effect of zonisamide in the present study appeared to be induced by the adsorptive capacity of AST-120.

It has been shown that the anticonvulsive effect is correlated with the blood concentration of the anticonvulsant (Kinoshita, 1986). It was suggested that this inhibition of the anticonvulsive effect of zonisamide was due to a decrease in the zonisamide blood concentration induced by the administration of AST-120. The serum concentration of zonisamide was 48 μg/ml in the single administration of zonisamide at a dose of 50 mg/kg. However, the serum concentration of zonisamide was 7 μg/ml in the coadministration of zonisamide (50 mg/kg) and AST-120 (5 g/kg). It was suggested that coadministration of AST-120 inhibited the effect of zonisamide on maximal electroshock-induced tonic hindlimb extension by decreasing the serum concentration of zonisamide by adsorption. In fact, when AST-120 (5 g/kg) was administrated 1.5 h after zonisamide (50 mg/kg) administration, the inhibitory effect of zonisamide on maximal electroshock-induced tonic hindlimb extension was not affected. At this time, the serum concentration of zonisamide was not decreased compared to a single administration of zonisamide. Namely, since zonisamide was absorbed well at 1.5 h after administration, the anticonvulsive effect of zonisamide was not affected by the delayed administration of AST-120.

In summary, AST-120 adsorbed zonisamide completely at concentrations 40-fold greater than those of zonisamide, after 5 min of shaking, and this effect was not affected by pH. The anticonvulsive effect of zonisamide was markedly inhibited by coadministration of AST-120, and this phenomenon was related to the decrease in the serum zonisamide concentration by the adsorbing effect of AST-120. These findings suggest that epileptic patients who receive antiepileptic drugs should be monitored carefully for decreasing serum concentrations under AST-120 administration.

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