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The Effects of Exposure to Cigarette Smoke on the Pharmacokinetics and Pharmacodynamics of Zonisamide in Rats

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The effects of exposure to cigarette smoke on the pharmacokinetics and pharmacodynamics of zonisamide, an antiepileptic drug, were investigated in rats. Absorption of oral zonisamide was significantly inhibited by exposure to cigarette smoke. The Cmax, $t_{1/2}$ and the area under the plasma concentration-time curve 0-24 values in the cigarette smoke exposure group were significantly lower than those in the control group. Although tonic extension (TE) induced by maximal electroshock was completely blocked by the administration of zonisamide in the control group, 50% of rats showed TE in the cigarette smoke exposure group. Exposure to cigarette smoke influences both the pharmacokinetics and antiepileptic effects of zonisamide. The effects of smoking on epileptic patients using zonisamide warrants further attention.

Key words: cigarette smoking, nicotine, absorption, convulsion, zonisamide

The pharmacological effects of drugs are usually most significantly regulated by the drug-concentration and sensitivity of receptors towards ligands at the site of action. The effects of drugs are also influenced by the dosage form, dosage, administration method *etc.*, and the patients condition, for example, age, sex, clinical history, *etc.*. Other factors such as emotional stress, temperature and smoking can also significantly influence drug effects. For most drugs, the effects of many of these other factors have not been fully investigated.

The effects of exposure to cigarette smoke on drug therapy have not been extensively investigated (1, 2). We have previously reported that the pharmacokinetics of nicorandil (3), isosorbide (4), theophylline (5-7), cimetidine (5, 8) and indomethacin (9, 10) are influenced by exposure to cigarette smoke.

Zonisamide (1,2-benzisoxazole-3-methanesulfonamide) is a new antiepileptic drug, which lacks the ureide structure and is used for the treatment of both juvenile and adult epileptics experiencing simple partial seizures, refractory complex partial seizures and generalized tonic clonic seizures of partial origin (11). Zonisamide has a long plasma half-life and the therapeutic range of the serum concentration of this drug during dosing is rather narrow (10-30 μg/ml). High doses often cause side effects such as ataxia, nystagmus, diplopia, drowsiness, and loss of appetite (12).

The objective of this study was to determine the effect of exposure to cigarette smoke on the pharmacokinetics and pharmacodynamics of zonisamide.

Materials and Methods

Animals. Eight-week-old male Wistar rats (Charles River Japan Inc., Yokohama, Japan) were used. Rats were housed 3-4 to a cage in 26 × 36 × 25 cm plastic-walled cages and were maintained on a 12 h light-dark cycle (lights on from 7:00 to 19:00) at a room temperature of 22 ± 2 °C and a relative humidity of approximately 50-60 %. Food and water was given *ad libitum*.

Drugs. Zonisamide (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) was suspended in a 0.5 % sodium carboxymethyl cellulose solution (0.5 % CMC) and given orally at 1 ml/kg by gastric sonde. The dosage of zonisamide was fixed at 35 mg/kg.

Cigarettes and apparatus for cigarette smoking. The cigarettes used in the present study were "Peace longs filter cigarettes" supplied by Japan

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Tobacco Inc., Tokyo. The Hamburg II Smoking Machine (Borgwaldt GMBH, Hamburg, Germany) was used for exposing the animals to cigarette smoke. The method of inhalation was as reported in our previous study (7, 8). Fifteen cigarettes were lit initially and the remaining 15 cigarettes were lit after the first 15 cigarettes had burned out. The inhalation duration was 2 sec and the frequency was 15 inhalations/min. All animals in the smoking and non-smoking groups were confined in smoking machines for equal durations.

Blood collection. The animals in the cigarette smoke exposure group were exposed for 15 min to cigarette smoke. Immediately after exposure to cigarette smoke, zonisamide at a dose of 35 mg/kg was administered per os (p. o.). Zonisamide was also administered to the control animals. Blood samples were repeatedly collected from the tail vein for measuring plasma concentrations of zonisamide by high-performance liquid chromatography (HPLC). For the measurement of plasma concentration of zonisamide, the proximal part of the tail vein of each rat was slightly anesthetized with Xylocaine Jelly, incised, and blood samples collected. After administration of zonisamide, blood samples were collected from the tail vein in capillary tubes (60 μl, Miles-Sankyo Co., Ltd., Tokyo) at 15 min intervals until the 2 h point, after which samples were collected at 4, 6, 8, 12 and 24 h. Samples were centrifuged for 3 min at 5,400 $\times$ g using a hematocrit centrifuge (Compur M 1100, Miles-Sankyo Co., Ltd.). The plasma (20 μl) obtained was used for the determination of drug plasma concentrations. Zonisamide and the internal standard (IS) N,N-dimethyl-zonisamide (Dainippon Pharmaceutical Co., Ltd.) were separated from plasma using a Bond-Elut PH cartridge (Analytichem International, CA, USA) containing phenyl silica (C/PH column). The plasma (20 μl) containing zonisamide and IS (50 μl of 10 μg/ml solution) were passed through the Bond-Elut PH cartridge, which had previously been washed twice with 1 ml of methanol and twice with 1 ml of distilled water. Then, zonisamide and IS were eluated with 250 μl of methanol. The elute (40 μl) was used for HPLC assay.

Determination of zonisamide plasma concentration by HPLC. Zonisamide plasma concentrations were determined according to a modified HPLC method previously reported by Furuno *et al.* (13). Thirty μl of the eluate was injected into the HPLC-system (Waters division of Millipore, MA, USA; pump-Type 510 with UV detector). A stainless steel column packed with octadecyl silica (LiChro CART RP-18(e), Kantokagaku Co., Ltd., Tokyo, 4 μm) was kept at room temperature. The sample was injected with an automatic sample processor (Type WISP 710B, Waters division of Millipore). Zonisamide and the IS; N, N-dimethyl-zonisamide were detected at 246 nm using a UV detector (Type L-4000, Hitachi Co., Ltd., Tokyo). The areas of the peaks were calculated using a Data Module (Lambda-Max Model 481, Waters division of Millipore). The mobile phase was methanol/distilled water (40/60), and the flow rate was 1.2 ml/min.

Pharmacokinetic parameters. The area under the plasma concentration-time curve (AUC) was obtained from the zonisamide plasma concentration-time data for each animal using a personal computer program for model independent analysis. The AUC values were determined according to the trapezoidal rule from 0 to 24 h for oral administration.

Experimental procedure. The inhibitory effect of zonisamide on convulsions induced by maximal electroshock (MES test) were examined according to a previous study (14). To induce MES convulsions, a sinussoidal A.C. current of 60 Hz and 50 mA was applied to the head of animals through corneal electrodes, using the Woodbury and Davenport apparatus (15). Decreases in the tonic extension (TE) phase of MES-induced convulsion were used as an index of the anticonvulsant effect.

Statistics. Statistical analysis of plasma concentration of zonisamide and the pharmacokinetic parameters were performed with the repeated measure two-way ANOVA test and the two-tailed Student's unpaired t-test, respectively. The incidence of TE induced by maximal electroshock was analyzed with the χ^2-test.

Results and Discussion

The time courses of plasma zonisamide concentrations in both the cigarette smoke exposure and the control group after oral administration of zonisamide at a dose of 35 mg/kg are shown in Fig. 1. Plasma zonisamide concentrations in the control group increased to approximately 20 μg/ml 4 h after drug administration. The concentration in the cigarette smoke exposure group reached approximately 15 μg/ml 8 h after drug administration. The plasma concentration of zonisamide in the cigarette smoke exposure group during the absorption phase was lower than in the control group. Pharmacokinetic parameters of zonisamide in the 2 groups are shown in Table 1.

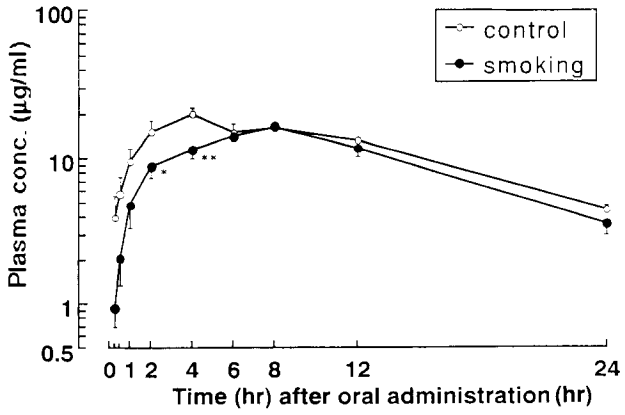

Fig. 1 Influence of exposure to cigarette smoke on plasma zonisamide concentrations in rats. Immediately after exposure to cigarette smoke, zonisamide at a dose of 35 mg/kg was administered p.o.. Values represent the mean ± S.E.M. of 5 rats per group. Conc.: Concentration.

Cmax, $t_{1/2}$ and AUC_{0-24} values of the cigarette smoke exposure group were significantly lower than those in the control group.

MES was performed 4 h after exposure to cigarette smoke and zonisamide administration. In the cigarette smoke exposure group, the plasma concentration of zonisamide was lower than in the control group. Although the incidence of TE was completely blocked by zonisamide in the control group, 50 % of rats showed TE in the cigarette smoke exposure group (Table 2).

It has been reported that cigarette smoking influences the pharmacokinetics of theophylline (5, 7), phenacetin (16), antypirine (17), lidocaine (18), pentazocine (19) and phenothiazines (20). It is very important to know whether cigarette smoking has an influence on drug pharmacokinetics, especially for drugs having a narrow therapeutic range. For example, cigarette smoke has been shown to have the following effects on theophylline metabolism: It is eliminated more rapidly in smokers than in non-smokers; exposure to smoke shortens the half-life of this drug; and cigarette smoke more rapidly reduces the blood levels of this drug (5, 7).

In the present experiment, the plasma concentration of zonisamide during the absorption phase in the cigarette smoke exposure group was lower than that in non-smoking control group. Cmax, $t_{1/2}$ and AUC_{0-24} values in the cigarette smoke exposure group were significantly lower than those in the non-smoking control group. These results indicate that a single exposure to cigarette smoke may influence the gastrointestinal absorption of

Table 1 Influence of exposure to cigarette smoke on pharmacokinetic parameters of zonisamide in rats

Parameters	Control	Smoking
Ka (h^{-1})	0.76 ± 0.31	0.22 ± 0.03
Tmax (h)	4.38 ± 0.90	6.26 ± 0.41
Cmax (μg/ml)	18.82 ± 1.43	11.51 ± 1.77*
$t_{1/2}$ (h)	8.40 ± 0.70	6.03 ± 0.37*
AUC_{0-24} (μg·h/ml)	284.83 ± 12.24	202.28 ± 23.20*

Values represent the mean ± S.E.M. of 5 rats per group.
*$P < 0.05$, significantly different from control.
AUC: Area under the plasma concentration-time curve.

Table 2 Effect of exposure to cigarette smoke on the incidence of tonic extension induced by maximal electroshock in zonisamide administered rats

Groups	Plasma zonisamide concentration (μg/ml)	Incidence
Control	18.30 ± 1.38	0/6
Smoking	11.90 ± 2.13	3/6*

Values represent the mean ± S.E.M. of 6 rats per group.
*$P < 0.05$ significantly different from control.

zonisamide. It has been reported that exposure to cigarette smoke inhibits the secretion of pancreatic juice and bicarbonate from the pancreas, resulting in a decrease in pH in the intestine (21).

The anticonvulsive effect of zonisamide on MES-induced TE convulsions was inhibited by cigarette smoke. The MES tests were performed 4 h after exposure to cigarette smoke and zonisamide administration, when the plasma concentration of zonisamide in the cigarette smoke exposure group was lower than in the control group. In a previous study, the anticonvulsive effect of zonisamide on MES-induced TE convulsions was heightened on mice undergoing immobilization stress. Plasma concentrations of zonisamide were not affected by stress level (in preparation). The reason why stress facilitated the anticonvulsive effect of zonisamide is not yet known, but it is guessed that hormonal changes may be related to this phenomenon. In fact, ACTH and TRH, which are released in conditions of stress, have an antiepileptic effect in spontaneous epileptic rats (22–24). On the other hand, it has been reported that nicotine and related constituents of cigarette smoke inhibit selected P450 enzymes in the glucocorticoid and sex steroid synthetic pathway. Aldosterone synthesis is also cytochrome P450 dependent, meaning that tobacco compounds including nicotine can cause direct and specific inhibition of aldosterone synthe-

sis (25). In addition to these facts, the plasma concentration of zonisamide was lower in the control group than in the cigarette smoke exposure group. Therefore, it is assumed that cigarette smoke exposure has a facilitating effect on MES-induced tonic convulsions.

In general, exposure to cigarette smoke influences the central nervous system. Nicotine, thought to be one of the most chemically active chemical constituents of cigarette smoke, also has an excitatory effect on the central nervous system. It is well known that it induces tremors and convulsions in both small and large doses (26). Exposure to cigarette smoke influences pharmacokinetics as well as the central nervous system, and reduces the antiepileptic effects of zonisamide. The effects of smoking on the efficacy of zonisamide warrant further attention.

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