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Effects of Tandospirone Citrate and Oxazolam on Pharmacokinetics of Valproic Acid Following Oral Administration of Sodium Valproate to Rats

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The drug interactions between tandospirone citrate, a non-benzodiazepine antianxiety drug, and oxazolam, a minor benzodiazepine tranquilizer and antianxiety agent, and valpronic acid (VPA), an anti-convulsant, were studied in rats. When tandospirone citrate or citric acid was administered orally immediately following oral administration (p.o.) of sodium valproate (VPA-Na), the plasma VPA levels were significantly lower than those in the control. In addition, pharmacokinetic parameters such as plasma VPA concentration and area under the plasma concentration-time curve up to 3 hr (AUC) were significantly decreased. However, when tandospirone citrate was administered intraperitonally, or both it and oxazolam were administered orally with VPA-Na, the VPA pharmacokinetic parameters were unchanged. These results suggest that decreases in plasma VPA concentrations (including those for AUC) may be due to a reduction in VPA-Na adsorption from the intestinal tract due to tandospirone citrate.

Key words —— sodium valproate, tandospirone, tandospirone citrate, citric acid, oxazolam

Introduction

Previously, we examined the interaction of a carbapenem antibiotic drug, meropenem, with β-blockers, carvedilol[1] and sodium valproate (VPA-Na)[2], as well as the interactions between panipenem/betamipron[3] or imipenem/cilastin sodium[4] and VPA-Na.

A branched-chain fatty acid, VPA-Na, is administered to treat epilepsy attacks and myoclonus of the skeletal muscle. This agent is routinely combined with other anticonvulsants or anti-epileptic drugs[5]. A survey on prescriptions of anti-epileptic drugs showed that they were combined with antianxiety agents[6,7]. Also, antianxiety agents are prescribed in patients taking VPA-Na. We have reported that effect of salicylate[8], rizatriptan benzoate and bezoic acid[9] on the pharmacokinetics of VPA after oral administration of VPA-Na in rats. In that paper, the plasma VPA concentrations including maximum plasma concentration (C_{max}) and area under the plasma concentration-time curve up to 3 h (AUC) were significantly decreased compared to those in the control group, and these results suggest that may be related to reduction of VPA absorption.

On the other hand, oxazolam[10] is a benzodiazepine preparation for antianxiety, which is metabolized and produces mainly N-desmethyl-diazepam, and tandospirone citrate[11], a non-benzodiazepine antianxiety drug with which CYP 3 A 4 and CYP 2 D 6 of liver microsomal P-450 participate for metabolism. And, some epileptic patients receiving long-term anticonvulsant therapy occasionally require coadministration with other medicines, such as non-benzodiazepine antianxiety drug.

A pharmacokinetic interaction between VPA and tandospirone citrate has not been reported, therefore, we investigated a possible drug interaction between tandospirone citrate and VPA in this study.

Materials and Methods

1. Chemicals

VPA-Na was prepared in the form of Depakene syrup (5 % VPA-Na) which was donated from Kyowa Hakko Kogyo

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Co. Ltd., Tokyo, Japan. Tandospirone citrate and tandospirone were prepared in the form of powder samples for external (non-clinical) investigation from Sumitomo Pharmaceuticals Co., Ltd., Tokyo Japan.

Oxazolam was prepared in the form of Serenal tablets (10 mg oxazolam, Sankyo Co. Ltd., Tokyo, Japan). Citric acid and sodium carboxymethylcellulose (CMC-Na) were purchased from Nacalai Tesque (Kyoto, Japan) and Maruishi Pharmaceuticals (Osaka, Japan).

2. Animal experiments

This study was performed in accordance with the Guidelines for Animal Experimentation of the University of Ryukyus, and was approved by the Animal Care and Use Committee of this institution (Permission number 3497).

Male Sprague-Dawley rats weighing 230 to 300 g were obtained from the Seac Yoshitomi, Ltd. (Fukuoka, Japan) and acclimatized for at least one week prior to the experiments. The rats were maintained in aluminum rat cages and housed in animal care facilities with a 12 hr light/dark cycle, a temperature of 23－25℃ a humidity of 50±15% and free access to food and water.

The rats were fasted from 19 : 00 on the previous day with free access to water and used in the experiments 12 hr later. Under light ether anesthesia, VPA-Na at dose of 100 mg/kg body weight (b.w.) (5 mL/kg) was administered orally (p.o.), using a polyethylene catheter, to all rats. Tandospirone citrate and oxazolam at a doses of 60 mg/kg and 50 mg/kg (5 mL/kg) b. w. suspended in 5% CMC-Na (12 mg/mL and 10 mg/mL/200 g b. w.) solution were administered orally immediately following oral administration of VPA-Na. Tandospirone at a dose of 53 mg/kg (10.6 mg/mL) b. w. higher than 40 mg/kg b. w. and citric acid at a dose of 20 mg/kg (4 mg/mL) b. w., which are equal to one mole of tandospirone citrate 60 mg/kg, resolved in distilled water were administered orally to the rats. Also, citric acid (100 mg/kg b. w.) at five time the dose of tandospirone citrate 60 mg/kg (12 mg/mL) b. w. resolved in distilled water was administered orally to the rats. In another rat group, tandospirone citrate at a dose of 60 mg/kg (5 mL/kg) b. w. was administered intraperitoneally (i.p.) immediately following oral administration of VPA-Na. Therefore, in control cases of p.o. administration of tandospirone citrate and tandospirone, 5% CMC-Na (un-dissolved in water) solution at a dose of 5 mL/kg b. w. was administered to the control rats. About 0.15 mL blood samples were collected at 0.25, 0.5, 1.0, 2.0 and 3.0 hr from the tail vein into a microcapillary and centrifuged (model CT 12, Hitach, Tokyo, Japan) at 12,000 rpm for 5 min to separate the plasma.

3. Determination of VPA

The quantitative analysis of valproic acid (VPA) concentration in plasma samples was conducted using an automated fluorescence polarization immunoassay (TDX, Abbott Laboratories, Abbott Park, USA).

4. Pharmacokinetic analysis

The pharmacokinetic parameters were obtained from VPA concentrations. Maximum plasma concentration (C_{max}) of VPA and time to reach C_{max} (T_{max}) were estimated from the actual measurements. The elimination rate constant (Kel) was determined by a linear least square regression analysis using the plasma concentrations of VPA during the elimination phase. The $t_{1/2}$ of VPA was calculated from 0.693/Kel. AUC values were calculated using the linear trapezoidal rule.

5. Statistical analysis

Data are expressed as the mean±SD. In the **Table 1**, the effect of the various treatments on the pharmacokinetic parameters was analyzed using a one way Analysis of Variance (ANOVA), and individual differences between the treatments was evaluated using the Tukey-Kramer test. In the **Fig. 1**, the results was analyzed using a repeated measures ANOVA followed by Dunnett's test. The statistical significance in the **Table 2** and **Fig. 2** were analyzed using the unpaired Student's t-test. A significant difference was defined as $p<0.05$.

Results

The time courses of plasma VPA concentrations following oral administration of VPA-Na followed by oral administration of tandospirone citrate at a dose of 60 mg/kg, tandospirone at a dose of 53 mg/kg and oxazolam at a dose of 50 mg/kg b. w. are shown in Fig. 1. Plasma VPA concentrations following VPA-Na p.o. were significantly lower in the tandospirone citrate 60 mg/kg p. o. group than in the control group. However, the VPA concentrations were unchanged in the tandospirone 53 mg/kg and oxazolam 50 mg/kg p.o. group. Pharmacokinetic parameters of plasma VPA following VPA-Na p.o. are represented in Table 1. The oral administration of tandospirone citrate at a dose of 60 mg/kg significantly decreased the AUC values of VPA by 52%, when the control value was considered to be 100%, respectively. The plasma concentration-time curves of VPA up to 3.0 hr following i.p. administration of tandospirone citrate and p.o. administration of tandospirone to rats did not change significantly. Pharmacokinetic parameters of plasma VPA following oral administration of VPA-Na with the i.p. tandospirone citrate (Fig. 2, Table 2) and p.o. tandospirone (Fig.1, Table 1) administration did not significantly decrease the values of Cmax and AUC compared with the control values.

The plasma VPA concentration-time curves following oral administration of VPA-Na followed by oral administration of citric acid at a dose of 100 mg/kg and 20 mg/kg are shown in Fig.1. Plasma VPA concentrations following VPA-Na p.o. were significantly lower in the citric acid group at a dose of 100 mg/kg p.o. than in the control group. The oral administration of citric acid at a dose of 20 mg/kg did not significantly decreased plasma VPA concentrations follow-

Table 1. Pharmacokinetic Parameters of Plasma VPA Following oral Administration (p.o.) of 100 mg/kg Sodium Valproate Alone (control), Followed by p.o. of Tandospirone Citrate 60 mg/kg or by p.o. of Tandospirone 53 mg/kg or by p.o. of Oxazolam 50 mg/kg or by p.o. of Citric Acid 100 and 20 mg/kg to Rats.

Treatment	n	Tmax (hr)	Cmax (μg/mL)	$t_{1/2}$ (hr)	AUC (μg·hr/mL)
Sodium valproate with 0.5% CMC-Na (control)	4	0.25 ±0.00	210.4 ±14.7	0.66 ±0.13	221.7 ±11.6
Sodium valproate with tandospirone citrate(60 mg/kg)	5	0.31 ±0.14	107.3 ±54.3	0.87 ±0.75	117.7 ±56.6*
Sodium valproate with tandospirone (53 mg/kg)	4	0.31 ±0.13	193.8 ±60.2	0.83 ±0.6	214.1 ±57.2
Sodium valprate with oxazolam (50 mg/kg)	5	0.35 ±0.14	265.2 ±84.9	0.84 ±0.5	200.3 ±82.0
Sodium valproate with citric acid (100 mg/kg)	5	0.40 ±0.14	76.6 ±16.4*	0.83 ±0.19	94.6 ±18.6*
Sodum valproate with citric acid (20 mg/kg)	4	0.44 ±0.13	186.0 ±13.3	0.62 ±0.11	203.4 ±6.9

Each value is the mean±SD. T_{max} : time to reach C_{max}. C_{max} : maximum plasma concentration. $t_{1/2}$: apparent elimination half-life. AUC : area under the plasma concentration-time curve. An asterisk (*)denotes a significant difference ($p<0.05$) vs control group.

ing VPA-Na p.o. compared with control values.

Table 1 represents the pharmacokinetic parameters of plasma VPA following VPA-Na p.o. The oral administration of citric acid at a dose of 100 mg/kg sig-nificantly decreased the AUC and C_{max} values of VPA by 36% and 44 %, respectively. Citric acid of 20 mg/kg p.o. decreased the value of AUC and Cmax of VPA by 88% and 91% respectively, but did not show the significant decrease.

Discussion

The metabolism of VPA remains unclear. There are many metabolism courses, and the route of a metabolism process differs slightly by researcher. More than 96% of administered VPA was metabolized in the liver, and was excreted. Drug- metabolizing enzyme activities of the liver are important in VPA excretion. In these enzymes, β-oxidation in mitocondrial fractions, metabolism by P-450 (CYP 2 D 6, CYP 2 C 9, CYP 2 C 19, CYP 1 A 2) and conjugation with glucuronic acid by UDP-glucuronyl transferases in microsomal franctions[12] are important; however, there is no report of metabolism by drug-metabolizing enzymes in the intestinal tract.

It is reported that tandospirone citrate, a non-benzodiazepine antianxiety drug, is absorbed following oral administration, almost completely metabolized, and excreted in urine. It was confirmed that CYP 3 A 4 and CYP 2 D 6 participated in its metabolism by *in vitro* examination with human liver microsomes[11]. Moreover, oxazolam is widely used as antianxiety agent and is a benzodiazepine derivative. It was reported that it was excreted in feces mainly via bile excretion after oral administration in rats[13]. Its possible metabolism in the liver was examined along with its interaction with VPA-Na.

The effective plasma concentration of VPA ranges from 50 to 100 μg/mL[14]. The dosage of tandospirone citrate and oxazolam in animal experiments ranges from 20 to 100 mg/kg[15] and 20 to 320 mg/kg for a condition avoidance response[16]. In this study, we used doses of VPA-Na, tandospirone citrate and oxazolam, of 100 mg/kg, 60 mg/kg and 50

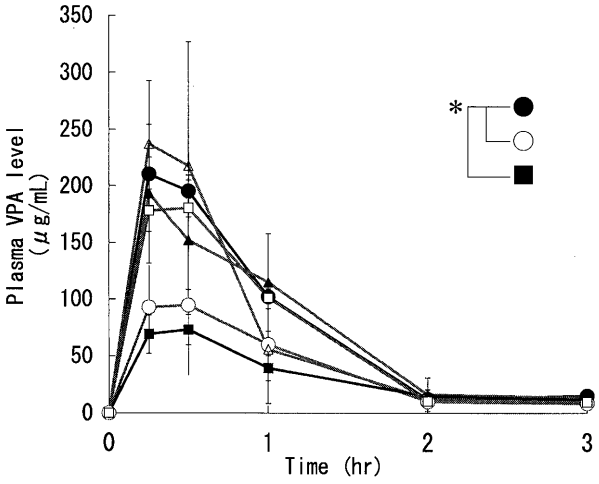

Fig. 1. Time Course of Plasma VPA Concentrations after oral Adminisration (p.o.) of VPA-Na (100 mg/kg) alone, Following by p.o. of Tandospirone Citrate (60 mg/kg), by an Tandospirone (53 mg/kg), by Oxazolam (50 mg/kg) and Citric acid (100 mg and 20 mg/kg) to Rats.
Results are expressed as mean±SD for 4–5 rats. An asterisk (*) denotes the significant difference ($p<0.05$) between control groups. Vertical lines indicate SD of the mean.
—●—control (0.5% CMC-Na solution) p.o. ; —○—tandospirone citrate 60 mg/kg ; —▲—tandospirone 53 mg/kg ; —△—oxazolam 50 mg/kg ; —■—citric acid 100 mg/kg ; —□—citric acid 20 mg/kg

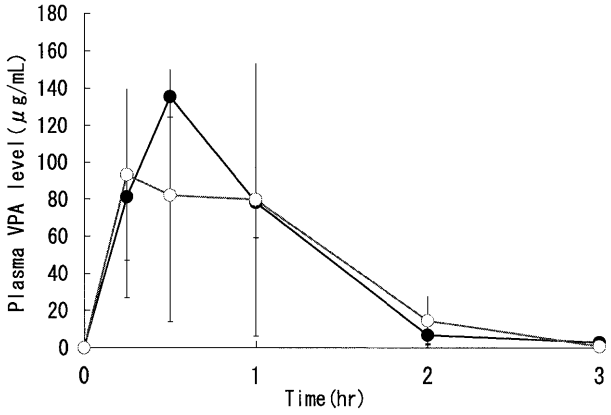

Fig. 2. Time course of plasma VPA Concentration after oral Administration (p.o.) of VPA-Na (100 mg/kg), Followed by i.p. of 0.5% CMC-Na Solution and Tandospirone Citrate (60 mg/kg). Result are expressed as mean±SD for 4–5 rats Vertical lines indicate SD of the mean. —●— control (0.5% CMC-Na solution) p.o. ; —○— tandospirone citrate (60 mg/kg) i.p.

mg/kg, respectively.

In this experiment, when tandospirone, prepared by removing citric acid from tandospirone citrate, and oxazolam, which were metabolized in the liver, were administered p.o. with VPA-Na, respectively, there were no changes in any pharmacokinetic parameters. It is thought that the plasma VPA concentration will increase if tandospirone causes a metabolism inhibition of VPA in the drug- metabolizing enzyme CYP 2 D 6 of the intestinal tract or liver ; however, as shown in Fig. 1 and Table 1, plasma VPA concentrations did not significantly increase after simultaneous oral administration of 100 mg/kg VPA-Na with 53 mg/kg tandospirone. Therefore, we think that drug-metabolizing enzymes of the intestinal tract are not related with VPA metabolism. In addition, the combination of paroxetine hydrochloride hydrate (selective serotonin reuptake inhibitors)[17] of inorganic acid, which does not have a mono-carboxylic group, did not change the values of plasma VPA concentration and AUC for VPA.

When administering tandospirone citrate immediately after

Table 2. Pharmacokinetic Parameters of Plasma VPA Following oral Administration (p.o.) of 100 mg/kg Sodium Valproate Alone (control), Followed Intraperitonal Administration (i.p.) of Tandspirone Citrate (60 mg/kg) to Rats.

Treatment	n	Tmax (hr)	Cmax (μg/mL)	$t_{1/2}$ (hr)	AUC (μg·hr/mL)
Sodium valproate alone p.o. (control)	5	0.45 ±0.11	138.8 ±6.1	0.73 ±0.23	138.0 ±6.5
Sodium valproate p.o. with tandospirone citrate i.p.	4	0.38 ±0.14	99.7 ±65.6	0.37 ±0.23	119.1 ±109.7

Each value is the mean±SE. T_{max} : time to reach C_{max}. C_{max} : maximum plasma concentration. $t_{1/2}$: apparent elimination half-life.
AUC : area under the plasma concentration-time curve.

the administration of VPA-Na, the plasma VPA concentration, AUC were significantly decreased (Fig. 1, Table 1). In this experiment, the degree of mean AUC of VPA-Na decreased by about 50% in coadministered rats. VPA is used in the treatment of epileptic seizures and it has been reported that the therapeutic range of VPA in plasma was from 50 to 100μg/mL. If this interaction took place in a patient whose epileptic seizures were well-controlled, the predicted concentration of VPA-Na would be lower than the therapeutic range, and seizures might occur[18]. As mentioned above, we reported a similar effect of salicylic acid[8], benzoic acid[9] and rizatriptan benzoate[9] on the pharmacokinetics of the oral administration of VPA-Na in rats. In this paper, salicylic acid, benzoic acid and rizatriptan benzoate were administered orally after VPA-Na p.o., and the plasma VPA concentrations including C_{max} and AUC were significantly decreased compared to those in the control group. Not only pH-partition theory-related active diffusion, but also a carrier-mediated mechanism may be involved in the absorption of VPA-Na and a mono-carboxylic acid, salicylic acid, in the intestinal tract[19]. Concerning the latter mechanism, an *in vitro* experiment demonstrated that salicylic acid and benzoic acid were transported via the mono-carboxylic acid transport (MCTs) of Caco-2 cells[20]. In addition, in another *in vitro* experiment on cell membrane permeability using salicylic acid and benzoic acid as substrates in Caco-2 cells, VPA significantly inhibited the cellular permeability of salicylic acid and benzoic acid[21]; however, no study has examined VPA as a substrate. In an *in vivo* experiment on combination therapy with salicylic acid and VPA-Na, salicylic acid inhibited the absorption of VPA-Na, significantly decreasing the plasma concentration of VPA. AUC was also significantly decreased, which may have been related to the inhibition of VPA-Na absorption[8]. Furthermore, VPA-Na is highly water-soluble, and its nonspecific cell membrane permeability in simple diffusion may be low; however, the small intestine may be the main site of absorption after oral administration. Therefore, competitive inhibition of VPA and a tricarboxylic acid, tandospirone citrate, which are transported by MCTs, may have reduced the absorption of VPA-Na. In this study, to eliminate the influence of tandospirone citrate on the digestive tract absorption of VPA-Na, the intraperitoneal administration of tandospirone citrate was performed. This agent did not influence the T_{max}, C_{max}, $t_{1/2}$, or AUC values of VPA. In addition, to eliminate the influence of a tricarboxylic acid, citric acid, on the digestive tract absorption of VPA-Na, we orally administered tandospirone, which was prepared by removing citric acid from tandospirone citrate, and there were no changes in any pharmacokinetic parameters.

On the other hand, M. Nakamura et al.[22] reported that they recognized a remarkable rise of the gastric juice acidity when 200 mg/kg of tandspirone citrate was administered to rats. The administration of tandspirone citrate (60 mg/kg b.w.) and citric acid (20 mg/kg b.w.) was equivalently molar; therefore the administration of citric acid (20 mg/kg b.w.) did not have pharmacologic action on the pH fall of the gastric juice with tandspirone citrate, and the fall of plasma VPA concentration was weak. When citric acid 100 mg/kg b.w., which is five times the molar concentration of tandspirone citrate 60 mg/kg b.w., was coadministered with VPA, the plasma VPA concentration and AUC for VPA were significantly decreased compared to the control values in the same pattern of tandspirone citrate 60 mg/kg b.w. administration.

Oral administration of citric acid significantly decreased the C_{max} and AUC values of VPA. These results suggest competitive inhibition of citric acid and VPA-Na, considering that citric acid has a carboxylic structure and decreased the plasma VAP concentration (including C_{max}).

We must now confirm whether any other transporters participate in the cellular permeability of VPA in a future study.

Simultaneous administration of VPA-Na and tandospirone citrate should be carefully performed. No negative influence of the simultaneous administration of VPA-Na and oxazolam was recognized.

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