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Effects of Carnitine on Valproic Acid Pharmacokinetics in Rats

Hirokazu Katayama*, Kohki Mizukami, Manami Yasuda, Tomomi Hatae

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Fukuyama, Hiroshima, Japan

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ABSTRACT

The long-term administration of valproic acid (VPA) may decrease the plasma concentrations of L-carnitine in epileptic patients. L-Carnitine is essential for the β-oxidation of fatty acids. The aim of this study is to determine whether endogenous L-carnitine affects the pharmacokinetics of VPA in L-carnitine-deficient (CD) rats. An L-carnitine deficiency was induced in rats using sodium pivalate. The pharmacokinetics of VPA were examined following its intravenous or oral administration to rats. The plasma and urine concentrations of VPA and its metabolites were determined using gas chromatography-mass spectrometry methods. Plasma VPA concentrations were slightly higher in CD rats than in control rats, whereas no significant differences were observed in the area under the curve or mean residence times of VPA between the 2 groups. After i.v. administration, the slope of the elimination phase (k) was significantly higher in CD rats than in control rats ($p < 0.01$). Some of the β-oxidation metabolites of VPA in plasma and urine decreased, while the glucuronide metabolites of VPA in urine increased complementarily in CD rats. Based on these results, it was concluded that hypocarnitinemia could affect the pharmacokinetics of VPA.

Introduction

Valproic acid (VPA, 2-propylpentanoic acid) is a branched-chain fatty acid and is commonly used as an antiepileptic drug with a wide spectrum of activity. VPA is mainly metabolized by mitochondrial β-oxidation or glucuronidation (Fig. 1), with the metabolites produced being excreted into the urine. VPA-glucuronide (VPA-Glu) undergoes enterohepatic recirculation. VPA is completely absorbed after its oral administration, strongly binds to plasma proteins, and its therapeutic plasma range is relatively narrow (50-100 μg/mL).[1] VPA may be used alone or as a supplementary medication, and therapeutic drug monitoring is needed for a large inter-subject variance in plasma concentrations due to age,[2,3] drug interactions,[4,5] or changes in plasma protein binding.[6,7]

Carnitine is an endogenous compound that is essential for the β-oxidation of fatty acids. Hypocarnitinemia is occasionally induced by the chronic administration of VPA.[8,9] Although L-carnitine undergoes glomerular filtration, 98% is reabsorbed from the renal tubular site, such that only a small amount of L-carnitine is excreted into the urine. Previous findings suggested that valproyl-carnitine formed in the body cannot be reabsorbed by the kidney, thereby resulting in decreases in L-carnitine concentrations.[10] L-Carnitine plays an essential role not only in the mitochondrial β-oxidation of long-chain fatty acids, but also in enhancing the removal of xenobiotic carboxylic acids by forming conjugates with them. L-Carnitine supplementation was previously reported to be effective for reducing VPA-associated hypocarnitinemia and hyperammonemia.[11] However, the effects of L-carnitine on the pharmacokinetics of VPA have not yet been examined in detail.

Therefore, the aim of this study was to determine whether the pharmacokinetics of VPA are affected in L-carnitine-deficient rats (CD rats).

Materials and Methods

Materials

Sodium valproate, undecylenic acid, *N*-methyl-*N*-trimethylsilyltrifluoroacetamide, and pivalic acid were purchased from Wako Pure Chemicals Company, Ltd. (Osaka, Japan). 2-en valproic acid (2-en VPA), 3-en valproic acid (3-en VPA), 4-en valproic acid (4-en VPA), 3-OH valproic acid (3-OH VPA), 4-OH valproic acid (4-OH VPA), and 3-keto valproic acid (3-keto VPA) were synthesized by Chemical Soft R&D, Inc. (Kyoto, Japan). Total Carnitine "Kainos" and

* *Correspondence to:* Hirokazu Katayama (Telephone: +81-0849362111; Fax: +81-0849362024).
E-mail address: katayama@fupharm.fukuyama-u.ac.jp (H. Katayama).

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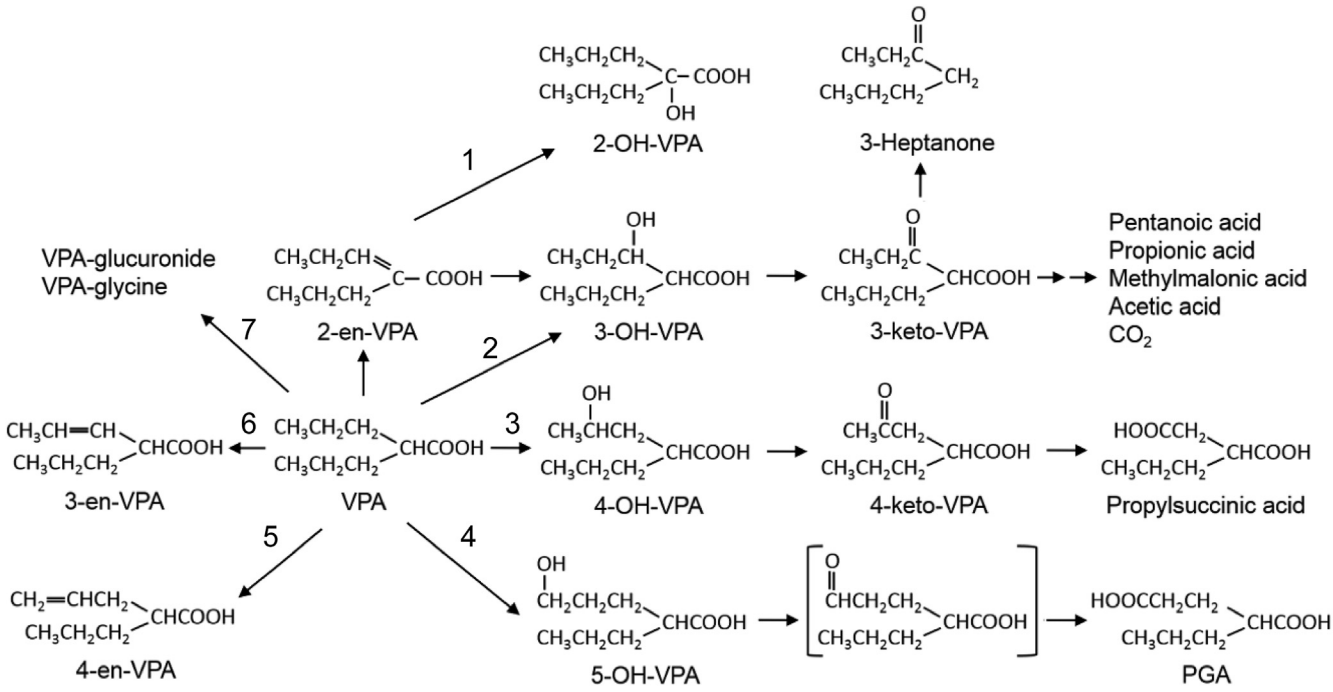

Figure 1. Metabolic pathways of VPA. 1, β-oxidation; 2, ω-2-oxidation; 3, ω-1-oxidation; 4, ω-oxidation; 5, δ-dehydrogenation; 6, γ-dehydrogenation; 7, conjugation.

free Carnitine "Kainos" were obtained from Asahi Kasei Pharma (Tokyo, Japan). All other chemicals and solvents used were of analytical reagent grade.

Animals

Male Wistar rats (Japan SLC, Shizuoka, Japan) weighing 230-320 g were fasted overnight before the experiment, but had free access to water. The animal experimental plan was approved by the Committee of the Laboratory Animal Center, and conformed to the Guiding Principles for the Care and Use of Experimental Animals in Fukuyama University.

CD Rats Induced by the Administration of Pivalic Acid

CD rats were prepared according to the method of Okudaira, with slight modifications.[12] The pivalic acid dosing solution (8 mg/mL) neutralized with NaOH was orally administered to rats (1 mL/kg, 8 mg/kg) twice a day at 10 AM and 6 PM for 4 days. On the 4th day at 2 PM after the 7th administration (4th day at 10 AM) of pivalic acid, rats were killed under deep anesthesia by a peritoneal injection of sodium pentobarbital to determine plasma carnitine concentrations.

VPA Administration Study

In total, 32 mg/kg of VPA was administered to obtain the VPA plasma concentrations of 50-100 μg/mL (the effective concentrations of VPA in human) following oral or i.v. administration. On the 4th day at 2 PM after the seventh administration (4th day at 10 AM) of pivalic acid, VPA (32 mg/kg) was administered intravenously or orally to CD rats. Control rats not administered with pivalic acid were given 32 mg/kg of the VPA solution. VPA was intravenously administered and blood was drawn from the jugular vein under pentobarbital anesthesia. In some experiments, rats were kept in metabolic cages to collect urine for 24 h.

Determination of VPA and Its Metabolites

The concentrations of VPA, 2-en VPA, 3-en VPA, 4-en VPA, 3-OH VPA, 4-OH VPA, and 3-keto VPA were determined in plasma and urine using gas chromatography-mass spectrometry with selected-ion monitoring (QP-5050; Shimadzu, Kyoto, Japan). Extraction and trimethylsilyl derivatization procedures were similar to those published by Tatsuhara et al.,[13] although ethyl acetate was used as the extraction solvent and undecylenic acid as the internal standard. The following ions were selected: m/z 199 (2-en, 3-en, 4-en, 4-OH VPA), m/z 201 (VPA), m/z 242 (internal standard), m/z 275 (3-OH VPA), and m/z 287 (3-keto VPA). The accuracy and precision were within 8% of the tested concentration range. The lower limit of quantification of VPA was 0.5 μg/mL, and its metabolite between 0.01 and 0.1 μg/mL, respectively. All compounds, stored at −40°C, were stable in plasma and urine for at least 10 days.

In the assay to determine total VPA (free plus conjugate), urine was hydrolyzed to VPA with NaOH. VPA-Glu concentrations were obtained by subtracting the free VPA concentration from the total VPA concentration. The concentrations of other glucuronides (such as 2-en-Glu and 3-en-Glu) in urine were determined using the same method.

Stability of Valproylcarnitine in Plasma

Fresh rat blood obtained from the abdominal aorta using a heparinized syringe was centrifuged. Plasma (4 mL) and sodium valproate aqueous solution (80 μL, 5.9 mg as VPA) were mixed and incubated at 37°C for 15, 30, or 60 min. Total and free carnitine concentrations were obtained using the spectrophotometric method (at 405 nm).