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A rapid and simple HPLC–MS/MS method for the simultaneous quantification of valproic acid and its five metabolites in human plasma and application to study pharmacokinetic interaction in Chinese epilepsy patients

Dingsheng Wen[a,b,1], Ziyi Chen[c,1], Chao Yang[c], Huanbin Liu[a,b], Hongliang Li[a,b], Juan Chen[a,b], Qiling Dai[c], Guoping Zhong[a,b], Jiaming Qin[c], Guanzhong Ni[c], Min Huang[a,b], Liemin Zhou[c,**], Xueding Wang[a,*]

[a] Laboratory of Drug Metabolism and Pharmacokinetics, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou City, Guangdong Province 510080, China
[b] Institute of Clinical Pharmacology, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou City, Guangdong Province 510080, China
[c] Department of Neurology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou City, Guangdong Province 510080, China

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ABSTRACT

Valproic acid(VPA) is a classic drug that used to treat epilepsy in monotherapy or combination with other anticonvulsant drugs such as lamotrigine (LTG). Although it was reported that VPA could increase lamotrigine trough concentration in clinical practice, there was no report about the effect of LTG on the trough concentration of VPA and its main metabolites, such as 4-ene-VPA, 3-OH-VPA, 4-OH-VPA, 3-keto-VPA, 2-PGA which are related to the therapeutic and toxic effects of VPA. In this study, a simple and rapid method for the simultaneous determination of VPA and its five metabolites in human plasma using HPLC–MS/MS was developed for the first time. Benzoic acid was used as an internal standard (IS). Separation was performed on a Hypersil GOLD C_{18} column by isocratic elution using acetonitrile: 10 mM ammonium acetate solution (90:10, v/v) as mobile phase at a flow rate of 0.3 mL/min. A triple quadrupole mass spectrometer operating in the negative ion-switching, electron spray ionization mode with selection reaction monitoring (SRM) was employed to determine transitions of *m/z* 143.183 → 143.183, 157.033 → 113.165, 173.017 → 129.074, 159.058 → 101.082, 140.870 → 140. 870, 159.049 → 123.076, 121.035 → 77.136 for VPA, 2-PGA, 3-keto-VPA, 3-OH-VPA, 4-ene-VPA, 4-OH-VPA and IS, respectively. The method also afforded satisfactory results in terms of sensitivity, specificity, precision (intra- and inter-batch), accuracy, recovery, matrix effect and stability. This method was successfully applied to evaluate the effect of LTG on the trough concentration of VPA, 2-PGA, 3-keto-VPA, 3-OH-VPA, 4-ene-VPA, 4-OH-VPA in Chinese epilepsy patients. The result showed that there was no significant difference in the concentration of VPA and its five metabolites between patients in VPA monotherapy and patients in therapy combining VPA with LTG.

Abbreviations: VPA, valproic acid; UGT, uridine diphosphate glucuronosyltransferases; HPLC, MS/MS high performance liquid chromatography tandem mass spectrometry; HPLC, high-performance liquid chromatography; GC–MS, gas chromatograph tandem mass spectrometry; SRM, selection reaction monitoring; QC, quality control; LQC, low quality control; MQC, medium quality control; HQC, high quality control; 3-OH-VPA, 3-hydroxy-2-propyopentanoic acid; 4-OH-VPA, 4-hydroxy-2-propyopentanoic acid; 4-ene-VPA, 2-propyl-4-pentenoic acid; 3-keto-VPA, 3-oxo-2-propylpentanoic acid; 2-PGA, 2-propylglutaric acid; LTG, lamotrigine.

* Corresponding authors.
** Corresponding author.
E-mail addresses: dswen168@163.com (D. Wen), chenziy@126.com (Z. Chen), Pedro_y@126.com (C. Yang), arlbenny@126.com (H. Liu), analyst369@163.com (H. Li), juanke526@163.com (J. Chen), Dai_qilin@126.com (Q. Dai), zhonggp@mail.sysu.edu.cn (G. Zhong), qinjiamin2005@163.com (J. Qin), gzni_neuro@163.com (G. Ni), huangmin@mail.sysu.edu.cn (M. Huang), lmzhou56@163.com (L. Zhou), wangxd@mail.sysu.edu.cn (X. Wang).
[1] These authors contributed equally to this work.

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1. Introduction

Valproic acid (VPA) is a branched-chain carboxylic acid with multiple pharmacological activity and is used to treat epilepsy, bipolar disorder and tumor in clinic [1–3]. It is almost entirely metabolized by the liver in three major metabolic pathways including UGT, mitochondrial oxidation and the CYP450 which are accounting for 50, 40 and 10% of a dose, respectively [4]. The metabolites in the UGT pathway are valproic acid β-O-glucuronide which eliminated by urine is considered to have no activity. Especially, 4-ene-VPA which is formed in the CYP450 pathway by CYP2C9, 2A6 and 2B6 may be associated with VPA-associated hepatotoxicity and teratogenicity [4,5]. Besides, 3-OH-VPA, 3-keto-VPA, 4-OH-VPA and 2-PGA involved in oxidation pathway show the anticonvulsant activity and can get to the cerebrospinal fluid [6,7]. however, as a coin has two sides, the metabolites in this pathway also show toxicity in vivo and vitro, 3-OH-VPA inhibited the activity of 3-methylcrotonyl-CoA carboxylase (3MCC) which is associated with the skin rash and hair loss [8]; 2-PGA showed the inhibitory effect on glucose synthesis which is related to hepatotoxicity [9]; 4-OH-VPA was toxic in rat hepatocyte cultures system [7]. Therefore, any drugs which could change the concentration of VPA and its metabolites may affect the efficacy and adverse reactions. It is necessary to detect the metabolites as well as VPA in clinical drug therapeutic monitor base on these facts above.

In the last 40 years, there were many methods to assay VPA and its metabolites using GC, HPLC, immunological, GC–MS and LC–MS/MS methods. Chen ZJ used the HPLC method to simultaneously detect the concentrations of VPA and 4-ene VPA in human plasma using 2,4′-dibromoacetophenone as the derivatization reagent, but it was a time consuming method for 21 min per sample [10]. Although the GC–MS method was employed to simultaneous quantification of VPA and all its metabolites and showed good sensitivity, the sample pretreatment was time consuming [11–13]. Furthermore, several LC–MS/MS methods were used to determine VPA [14,15]. While simultaneous detection of VPA, 3-OH-VPA, 4-ene-VPA and 5-OH-VPA was also carried out, the volume of plasma was up to 200 μL and the solid-phase extraction was employed in sample pretreatment [16]. What's more, to the best of our knowledge, there was no report about simultaneous quantification of VPA, 4-ene-VPA, 2-PGA, 3-OH-VPA, 4-OH-VPA and 3-keto-VPA. Thus, it is necessary to develop a more efficient and sensitive method to detect VPA and its metabolites simultaneously.

Although VPA monotherapy showed effectivity in the treatment of epilepsy, it is more commonly administered in combination with other anticonvulsants such as lamotrigine. It was reported that the valproic acid could increase the concentration of lamotrigine in clinic [17], there was no report about the effect of lamotrigine on 4-ene-VPA, 2-PGA, 3-OH-VPA, 4-OH-VPA and 3-keto-VPA which are associated with the VPA related adverse reactions and anticonvulsant activity. Therefore, in this study, a simple and rapid high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS) method for simultaneous quantification of VPA, 4-ene-VPA, 2-PGA, 3-OH-VPA, 4-OH-VPA and 3-keto-VPA was developed, validated and successfully applied to evaluate the effects of lamotrigine on the trough concentration of VPA and the main metabolites when VPA was combined with LTG in Chinese epilepsy patients.

2. Materials and methods

2.1. Chemicals and reagents

Valproic acid, 4-OH-VPA, 3-OH-VPA, 4-ene-VPA, 3-keto-VPA, 2-PGA and benzoic acid (IS) were purchased from Toronto Research Chemicals company Inc. (Toronto, Canada); HPLC grade acetonitrile, formic acid and ethyl acetate, dichloromethane, methyl *tert*-butyl ether and *n*-hexane were provided by Tedia Company Inc. (Fairfield, OH, USA). Ammonium acetate was from sigma. Ultrapure water (18.2 MΩ cm at 25 °C) was obtained from a Millipore Direct-Q® ultrapure water system (Billerica, MA, USA). Blank human plasma was obtained from healthy people with heparin as anticoagulant. Heparin was from the first affiliated hospital of Sun Yat-sen University.

2.2. Instruments and analytical conditions

The HPLC (Thermo Fisher Scientific Inc., Boston, USA) system consisting of Ultimate 3000 RSLC system with binary pumps and S surveyor autosampler (Thermo Scientific Inc., San Jose, CA, USA) coupled with a TSQ Ultra triple-quadrupole mass spectrometer was used to quantify valproic acid and its five metabolites in the plasma of Chinese epilepsy patients.

Samples were separated on an Hypersil GOLD C_{18} column (50 mm × 2.1 mm, 5 μm; Thermo Fisher Scientific Inc., Boston, USA). The column temperature was set at 40 °C. The mobile phase was acetonitrile: 10 mM ammonium acetate solution (90:10, v/v) as an isocratic mobile phase with a flow rate of 0.3 mL min^{-1} and total run time was 2.0 min.

Mass spectrometric detection was performed on a TSQ Quantum Ultra triple-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface. All compounds in this assay were monitored under negative ion-switching ESI conditions and quantified in selection reaction monitoring (SRM) mode with different transitions showed in Table 1. The source-dependent parameters for all compounds were as following: Spray Voltage, 3000 V; Vaporizer Temperature, 280 °C; Sheath Gas Pressure: 35 Psi; Ion Sweep Gas Pressure: 0; Aux Gas Pressure: 15 Psi; Capillary Temperature: 350 °C; Collision gas pressure 1.5 m Torr; Tube Lens offset and collision energy (CE) are showed in Table 1.

2.3. Preparation of stock and working solutions, calibrations standards and quality control samples

Stock solutions of VPA (10 mg/mL), 4-ene-VPA(2 mg/mL), 2-PGA (2 mg/mL), 3-OH-VPA (2 mg/mL), 3-keto-VPA (2 mg/mL), 4-OH-VPA (2 mg/mL) and IS (2 mg/mL) were prepared and dissolved in acetonitrile. The IS working solution was diluted with acetonitrile to 40 μg/mL. The stock solutions of compounds were then serially diluted in acetonitrile: water = 1:1 (v/v) to prepare standard working solutions at concentrations 10.0, 20.0, 50.0, 100.0, 250.0, 500.0, 1000.0, 2000.0 μg/mL for VPA, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0, 100.0, 200.00 μg/mL for 4-ene-VPA, 2.0, 4.0, 10.0, 20.0, 50.0, 100.0, 200.0, 400.0 μg/mL for 3-keto-VPA, 100.0, 200.0, 500.0, 1000.0, 2500.0, 5000.0, 10000.0, 20000.0 ng/mL for 2-PGA, 3-OH-VPA and 4-OH-VPA, respectively.

The calibration standards solutions were made by spiking 10 μL standard working solution with 90 μL human blank plasma at the concentration of 1.0–200.0 μg/mL for VPA, 0.1–20.0 μg/mL for 4-ene-VPA, 0.2–40.0 μg/mL for 3-keto-VPA, 10.0-2000.0 ng/mL for

Table 1
The mass spectrum condition of VPA, 4-ene-VPA, 2-PGA, 3-OH-VPA, 4-OH-VPA, 3-keto-VPA and IS.

Analyte	Parent ion	Product ion	Collision Energy	Tube lens
VPA	143.183	143.183	0	64
2-PGA	157.033	113.165	13	64
3-keto-VPA	173.017	129.074	16	64
3-OH-VPA	159.058	101.082	14	64
4-ene-VPA	140.870	140.870	0	64
4-OH-VPA	159.049	123.076	18	64
IS	121.035	77.136	14	64