AMINO ACID CONJUGATES: METABOLITES OF 2-PROPYLPENTANOIC ACID (VALPROIC ACID) IN EPILEPTIC PATIENTS

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ABSTRACT:

In this study, spectroscopic and chromatographic evidence is presented for the identification and characterization of the metabolites, valproyl glutamate (2-propylpentanoyl glutamate, VPA-GLU) and valproyl glutamine (2-propylpentanoyl glutamine, VPA-GLN) in the urine, serum, and cerebrospinal fluid (CSF) of patients on valproic acid (VPA) therapy. Moreover, the identification of valproyl glycine (2-propylpentanoyl glycine, VPA-GLY) in the serum and urine of patients on VPA, albeit in trace concentrations, is also reported here. The three amino acid conjugates excreted in urine accounted for about 1% of the VPA dose in four patients who were on VPA therapy chronically and had reached steady state. VPA-GLU was quantitatively the most prominent metabolite (0.66–13.1 μg/mg creatinine) compared with VPA-GLN (0.78–9.93 μg/mg creatinine) and VPA-GLY (trace-1.0 μg/mg creatinine) in overnight urine samples of all patients studied (n = 29). The relatively low serum concentrations of the three amino acid conjugates of VPA in six patients suggest that the metabolites are readily excreted once formed. In contrast, whereas VPA GLY was absent in the CSF of one patient on VPA, the concentrations of VPA-GLU and VPA-GLN in this CSF sample were 9 and 5 times, respectively, their corresponding serum concentrations.

The antiepileptic drug, valproic acid (VPA³), is primarily used for the management of generalized and absence seizures in children. Concerns over its teratogenicity and hepatotoxicity side effect have encouraged the need to develop analogs of VPA devoid of the adverse effects. However, this task is complicated by the fact that the mechanism of action of the drug itself remains to be understood.

A large body of evidence has emerged to link the hepatotoxicity of VPA to its metabolism and has been reviewed over the years (Eadie et al., 1988; Abbott and Anari, 1999; Radatz and Nau, 1999). Moreover, a review of mass-balance studies indicated that the total recovery of a VPA dose cannot be completely accounted for in humans (Bailie and Sheffels, 1995), suggesting the existence of yet unknown metabolites. For all these reasons, despite the extensive work that has been conducted on the metabolism of VPA, the subject remains an area that warrants further investigation.

To date, the role of the amino acid conjugation pathway in the biotransformation of VPA, particularly in humans, has remained relatively unexplored. 2-Propylpentanoyl glycine (VPA-GLY) is the only amino acid conjugate of the drug that has been detected and studied in rats (Granneman et al., 1984a,b). The role of amino acid conjugation of VPA in human is significant owing to the potential for CoA consumption. The depletion of mitochondrial CoA with VPA therapy is one of several hypotheses put forth to explain VPA hepatotoxicity (Turnbull et al., 1983; Harris et al., 1991). Furthermore, the conjugation of VPA with amino acids known to be neurotransmitters involved in epilepsy also need to be explored as a potential to further understand the mechanism of action of VPA.

Although in general, bile acids form conjugates with glycine and taurine by the action of microsomal enzymes, amino acid conjugation of xenobiotics is believed to be a mitochondrial reaction occurring primarily in the liver or the kidney (Williams, 1989). Conjugation of organic acids is initiated by the obligatory activation of the acid to a CoA ester followed by the acyl transfer to an amino acid residue with concurrent release of CoA. The last step is catalyzed by N-acyl transferase specific for each amino acid and defines the structural requirement for amino acid conjugation of xenobiotics (Hutt and Caldwell, 1990). Glycine-N-acyltransferase (Tishler and Goldman, 1970; Mawal et al., 1997) and glutamine-N-phenylacetyltransferase...
(Webster et al., 1976) have been isolated from human liver mitochondria.

In this study, we looked for evidence of the conjugation of VPA with the amino acid neurotransmitters glycine, glutamine, glutamic acid, and aspartic acid, as well as non-neurotransmitters, taurine and alanine in the biological fluids of humans by liquid-chromatography tandem mass-spectrometry (LC-MS/MS) and gas-chromatography mass-spectrometry (GC-MS). We describe here the identification and profiling of amino acid conjugates of VPA in the urine, serum, and CSF of patients on VPA therapy using LC-MS/MS and GC-MS.

Materials and Methods

Materials. 1-Bromo-2,3,4,5,6-pentafluorotoluene (PFBBr), diisopropylethylamine (DIEA), trifluoroacetic acid (TFA), 2-fluoro-2-propylpentanoic acid were purchased from Aldrich Chemical Co. (Milwaukee, WI); disodium L-glycine were purchased from Aldrich Chemical Co. (Milwaukee, WI); diisopropylethylamine, L-glutamine, L-glutamic acid, and L-aspartic acid were purchased from BDH Chemicals (Toronto, ON) or Caledon Laboratories Ltd. (Edmonton, AB). Trifluoroacetic acid (TFA) was purchased from Sigma-Aldrich (St. Louis, MO). Polyethylene glycol PE-10 was purchased from Clay Adams (Parsippany, NJ).

Instrumentation and Analytical Conditions. 1 H NMR spectra were obtained on a Bruker WH-200 spectrometer (Bruker, Newark, DE) or a Bruker WH-400 spectrometer (Department of Chemistry, University of British Columbia).

Gas chromatography in negative ion chemical ionization (GC-MS NICI) was performed on a Hewlett Packard (Avondale, PA) 5890 mass spectrometer (MS) engine coupled to a 5890 series II gas chromatograph (GC) fitted with a J & W Scientific (Folsom, CA) DB1701 column, (30 m × 0.32 mm, 0.25 μm). The oven temperature was programmed as follows: 100 to 200°C at 20°C/min, held for 5 min; 150 to 300°C at 15°C/min and held for 5 min. Other GC-MS conditions were identical to those reported in other studies. The ionization energy was 120 eV. For purposes of identification and characterization of the amino acid conjugates of VPA, both the scan and selected ion monitoring modes were used.

LC-MS/MS experiments were performed on a Micromass Quattro triple quadrupole mass spectrometer (Micromass, Montreal, QC) interfaced to a Hewlett Packard 1090 II liquid chromatograph. Chromatography was conducted on a Phenomenex (Torrance, CA) C8 column, (100 mm × 2.1 mm, 5 μm). Three HPLC methods were used in this study.

Method 1. Mobile phase A consisted of methanol/water (43:57, 0.05% TFA), and mobile phase B consisted of methanol. At time 0, mobile phase A was pumped isocratically for 30 min. Mobile phase B was gradually increased from 0 to 100% at time 30.1 min and held for 5 min, followed by a sharp gradient increase of mobile phase A to 100% at 35.1 min. This method was used initially for the identification of the amino acid conjugates of VPA in human urine. Flow rate was at 0.1 ml/min.

Method 2. Mobile phase A consisted of acetonitrile/water (30:70, 0.05% TFA) and mobile phase B consisted of acetonitrile. Mobile phase A was pumped for 25 min followed by a gradient increase of mobile phase B to 100% at 26 min, held for 5 min and reverted to 100% A at 30.1 min. Flow rate was at 0.1 ml/min. This method was used to detect the amino acid conjugates of VPA in urine where a longer run time program was required to separate the peaks of interest from interfering chromatographic peaks.

Method 3. Mobile phase A consisted of acetonitrile/water (40:60, 0.05% TFA), and mobile phase B consisted of a gradient program that allowed 100% of mobile phase A to be pumped for 14 min at a flow rate of 0.1 ml/min. Mobile phase B was then increased to 100% at a flow rate of 0.2 ml/min at 15 min and held for 5 min at 0.2 ml/min. Subsequently, mobile phase A was increased to 100% at time 20 min and at 0.1 ml/min. This method was used for the profiling of amino acid conjugates in human serum and CSF samples.

The ionization energy for LC-MS/MS experiments was in positive electrospray (ES +). The HPLC eluent was introduced to the stainless steel capillary sprayer held at 3.5 kV. The mass spectrometer was operated in either precursor ion scanning, product ion scanning or multiple reaction monitoring (MRM) mode. Multipliers 1 and 2 were set at 650 V for all three experiments; cone voltage was set at 30 V with skimmer offset by 5 V. The low and high mass resolution was set at 12.5 for both precursor ion scanning and product ion scanning and at 5 for MRM. The product ion scanning experiment determined the product ions of MH +, and the precursor ion scanning confirmed the pseudomolecular ion or the protonated molecular ion (MH +) of the metabolite of interest. Argon, the target gas, was set at a pressure of 3.0 × 10 -3 mbar for collision-induced dissociation (CID) and with a collision energy of 40 eV. During MRM, the pseudomolecular ion for each analyte was selected in the first quadrupole and subjected to CID with the target gas and collision energy set at 50 eV. Specific fragment ions were then selected by the third quadrupole. For all the experiments, the source temperature was at 115°C. Data analyses were performed using Mass Lynx software (Micromass).

Human Studies. Overnight urine samples (n = 29), 24-h urine samples (n = 4), serum samples (n = 6), and one CSF sample were obtained from epileptic and pediatric patients who have been on VPA therapy for more than 6 months and have reached steady state. The patients were attending the Seizure Clinic at Vancouver Children’s Hospital, British Columbia, Canada. The average dose of VPA received by each patient was 23.3 ± 12.6 mg/kg, the average age of the patients was 12.6 ± 5.9 years. Control urine and serum samples were obtained from healthy volunteers (n = 4). Control CSF were obtained from nonepileptic subjects (n = 10), who were either suffering from multiple sclerosis and attending the University of British Columbia Hospital, Canada or were healthy volunteers. Approval for all human studies was issued by the U.B.C. Human Ethics Committee (U.B.C. C95-0412).

Chemical Synthesis of Amino Acid Conjugates of VPA. The amino acid conjugates of VPA were synthesized according to the procedure described for 2-fluoro VPA-GLN (FVPA-GLN) (Tang et al., 1997). VPA was converted to the corresponding N-hydroxysuccinimide ester in the presence of dicyclohexylcarbodiimide followed by coupling with t-glycine, t-glutamine, t-glutamic acid, or t-aspartic acid to form 2-propylpentanoic acid (VPA-GLN), VPA-GLY, 2-propylpentanoyl glutamate (VPA-GLU), or valproyl aspartate (VPA-ASP), respectively.

VPA-GLN. LC-MS/MS spectrum: m/z (%): 273 (MH +), 100, 57 (75), 99 (35), 127 (25), 147 (20); 1 H NMR (200 MHz): δ 9.0 (t, 6H, 2xCH 3 ), 1.10 (s, 6H, 6xCH 2 ); GC-MS NICI spectrum of the PFB derivative: m/z (%) 271 (MH +), 100.

VPA-GLU. LC-MS/MS spectrum: m/z (%) 274 (MH +), 100, 57 (100), 99 (40), 127 (35), 148 (20); 1 H NMR (200 MHz): δ 9.0 (t, 6H, 2xCH 3 ), 1.15–2.50 (m, 12H, 6xCH 2 ); 4.45 (d, 1H, NHCH 2 ); GC-MS spectrum of the di-PFB derivative: m/z (%) 452 (MH +), 100.

VPA-GLY. LC-MS/MS spectrum: m/z (%) 202 (MH +), 100, 77 (70), 99 (15), 127 (15); 1 H NMR (400 MHz): δ 9.00 (1H, 6H, 2xCH 3 ), 1.10–1.40 (m, 5H, 4xCH 2 ), 2.0–2.1 (1H, CH 2 CH 2 CH 2 ); 4.1 (d, 2H, NHCH 2 ); GC-MS NICI spectrum of the PFB derivative: m/z (%) 200 (MH +), 100.

VPA-ASP. LC-MS/MS spectrum: m/z (%) 260 (MH +), 100, 57 (100), 99 (40), 127 (35), 134 (15); 1 H NMR (200 MHz): δ 9.00 (2H, 6H, 2xCH 3 ); 1.10–1.40 (m, 8H, 4xCH 2 ), 2.0–2.1 (CH 2 CH 2 CH 2 ); 4.80 (dd, 2H, NHCH 2 ); GC-MS NICI spectrum of the di-PFB derivative: m/z (%) 438 (MH +), 100.

Derivatization of Amino Acid Conjugates of VPA for NICI Analysis. Ten microliters of 40% PFBBr in ethyl acetate and 10 μl of DIEA were added to 2.50 ml of the corresponding N-hydroxysuccinimide ester.
to 10 μl of a solution (0.01 mg/ml) of VPA-GLU, VPA-GLN, VPA-GLY, and VPA-ASP in ethyl acetate and allowed to react at 50°C for 1 h.

Preparation of Urine Samples for the Identification and Profiling of Amino Acid Conjugates of VPA by Solid Phase Extraction. One milliliter of urine sample was adjusted to pH 3 with HCl and applied to a C₂ Bond-Elut cartridge that was preconditioned with water and methanol. The cartridges were washed once with water/methanol (95:5) and water. The amino acid conjugates of VPA were eluted with 6 ml of methanol. The organic layers were
For the quantitation of VPA-GLU, VPA-GLN, and VPA-GLY in human urine, 1 ml of each sample was spiked with 25 μl of 0.08 mg/ml of FVPA-GLN (IS) and VPA-ASP (IS). The samples were prepared and analyzed with a set of calibration standards ranging from 0.10 to 5.0 μg/ml. Calibration standards and all samples of patients were extracted as described above. The LC-MS/MS MRM transitions monitored were as follows: m/z 291 → 130 for FVPA-GLN; m/z 274 → 148 for VPA-GLU; m/z 273 → 147 for VPA-GLN, VPA-GLY, and VPA-ASP, respectively. At 1.0 μg/ml, the intra-assay variation was less than 10% for all three metabolites. The larger variation observed at the lower limit of quantitation was acceptable in view of the fact that the metabolites were detected at concentrations well above the lowest standard (0.1 ng/ml). At both concentrations, each metabolite was measured within 83 to 120% of their expected values.

The concentration of each amino acid conjugate of VPA measured in overnight urine samples was normalized to creatinine. The % recovery of VPA as amino acid conjugates of VPA was calculated for 24-h urine samples (n = 4). Creatinine was measured in urine according to the routine Jaffé method (1886).

**Preparation of Serum Samples for the Identification and Quantitation of the Amino Acid Conjugates of VPA.** The amino acid conjugates of VPA were extracted from the serum samples of human by liquid-liquid extraction. Two milliliters of each specimen and control serum was adjusted to pH 3 with HCl and extracted with 5 ml of ethyl acetate, twice. The organic layers were separated and evaporated, and the residue was reconstituted in 200 μl of 0.08 mg/ml of FVPA-GLN (IS) and VPA-ASP (IS). The assay for the simultaneous analysis of amino acid conjugates of VPA by LC-MS/MS was linear over a range of 0.1 to 5.0 μg/ml for all three conjugates. The coefficients of determination (r²) of the calibration curves were 0.99 or better.

The interassay variation was assessed from the slopes of five calibration curves run consecutively and was less than 7% for each identified metabolite. The intra-assay variation was assessed by three replicate analyses of two samples. At 0.12 μg/ml, the intra-assay variation was 11.4, 28.8, 18.5% for VPA-GLU, VPA-GLN, and VPA-GLY, respectively. At 1.0 μg/ml, the intra-assay variation was less than 10% for all three metabolites. The larger variation observed at the lower limit of quantitation was acceptable in view of the fact that the metabolites were detected at concentrations well above the lowest standard (0.1 ng/ml). At both concentrations, each metabolite was measured within 83 to 120% of their expected values.

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260, respectively. Upon CID of the [MH]\(^+\) of each metabolite, the resulting product ion spectra showed that the most abundant fragment at m/z 57 was shared by all four conjugates as illustrated in Fig. 1. This fragment is believed to be the protonated n-butyl group of VPA following cleavage on two sides of the tertiary carbon (C₂) causing the loss of the formyl amino acid group and the n-propyl group. The other product ions included m/z 99 resulting from fragmentation at the carbonyl carbon (C₁) causing the neutral loss of the n-formyl amino acid group leaving the protonated n-heptanyl chain of VPA. Cleavage of the amide bond gave rise to the protonated 2-propylpentanal fragment at m/z 127 for each conjugate.

The product ion spectra of each compound (described in Fig. 1) following CID of their [MH]\(^+\) are shown in Fig. 2. The corresponding protonated amino acid moiety was also observed at m/z 148 for VPA-GLU or m/z 147 for VPA-GLN or m/z 134 for VPA-ASP but not for VPA-GLY. The subsequent loss of a water molecule afforded a product ion at m/z 129 from the protonated glutamine and m/z 130 from the protonated glutamic acid. In contrast, the product ion spectrum of [MH]\(^+\) of FVPA-GLN had a different fragmentation pattern compared with the nonfluorinated analog and was consistent with previously reported data (Tang et al., 1997).

GC-MS NICI analysis of the PFB esters or di-esters of the conjugates served to complement the structural characterization of the compounds. The GC-MS NICI spectrum of each derivatized conjugate was characterized by a single base peak corresponding to the [M-pentafluorobenzyl]\(^-\) fragment anion of the di-PFB ester of VPA-GLU (m/z 452) and VPA-ASP (m/z 438) or the PFB ester of VPA-GLN (m/z 271), VPA-GLY (m/z 200) and FVPA-GLN (m/z 289). Elution times under our GC-MS NICI experimental conditions are shown in Table 1.

### Identification of Amino Acid Conjugates of VPA in Urine Samples of Patients

The amino acid conjugates of VPA eluted between 22.97 and 30.30 min by LC-MS/MS method 1, 14.70 and 23.99 min by method 2, and 7.86 and 11.99 min by method 3 as described in Table 2.

In all the urine samples (n = 33) studied, all product ions of [MH]\(^+\) of VPA-GLU, VPA-GLN, and VPA-GLY were detected at the same retention times and in the same area ratio as those observed for synthetic standards of the conjugates by two mobile phase systems (water/acetonitrile and water/methanol) in MRM mode. None of the product ions were detected in the control samples. A full product ion spectrum of VPA-GLU and VPA-GLN identical to those of reference compounds shown in Fig. 2 were obtained in four patients’ urine extracts. MRM experiments failed to detect any product ions of the protonated VPA-ASP in any of the patients’ samples studied. The search for VPA taurine and VPA alanine was approached by MRM of the corresponding transition [MH]\(^+\) to m/z 57, the most abundant product ion shared by the identified amino acid conjugates of VPA. Neither of the two conjugates were detected in the urine extracts analyzed.

In the patients’ urine samples analyzed, VPA-GLU was found at a concentration range of 0.66 to 13.1 μg/mg creatinine; VPA-GLN was found at lower concentration range of 0.78–9.93 μg/mg creatinine, whereas VPA-GLY was the minor metabolite at a concentration range of trace-1.0 μg/mg creatinine (Table 3). The total recoveries of the three conjugates, VPA-GLU, VPA-GLN, and VPA-GLY in urine samples of patients on VPA therapy were calculated for a 24-h period and found to range from 0.53 to 1.45% (0.75 ± 0.41) of a VPA dose (Table 4). The urinary VPA concentration was available for three of the patients, and the mean concentration was found to be 420 μg/mg of creatinine.

### Identification of Amino Acid Conjugates of VPA in Serum Samples of Patients

Analysis of serum samples by LC-MS/MS provided strong evidence for the identification of VPA-GLU, VPA-GLN, and VPA-GLY in the serum extracts of all six patients but not in any control serum. The retention times and area ratio of all product ions of [MH]\(^+\) of the metabolites were authenticated by comparison with synthetic standards. The mean serum concentration of VPA-GLU in six patients was 8.0 ± 2.0 ng/ml. VPA-GLN was estimated at 5.0 ± 3.0 ng/ml, and VPA-GLY was detected but was below the limit of quantitation of the assay.

### Identification of Amino Acid Conjugates of VPA in Human CSF

The retention times and area ratio of all the product ions of [MH]\(^+\) of VPA-GLU (Fig. 3) and VPA-GLN in a human CSF sample were the same as those observed for both synthesized amino acid conjugates of VPA by LC-MS/MS. The identification of VPA-GLU was further investigated by GC-MS under NICI by monitoring for the [M-181]\(^-\) fragment carboxylate anion of the di-PFB derivative of the conjugate at m/z 452. The total ion current of the PFB-derivatized CSF extract (Fig. 4) and its corresponding PFB-derivatized serum extract showed the elution of VPA-GLU at tᵣₐ = 16.36 min, the identical retention time observed for the synthetic standard. The peak was absent in the PFB-derivatized control CSF and serum extract.

The quantitation of VPA-GLU, and VPA-GLN in CSF by LC-MS/MS was relatively facile because no extraction procedure was required. The assay was designed to monitor one characteristic MRM transition for each amino acid conjugate of VPA identified in human and using VPA-ASP as the IS. A relatively high volume (60 μl) of sample was introduced onto the HPLC column, but the resulting slight broadening of the peaks was not chromatographically significant. The concentration of VPA-GLU in the CSF of one patient was 60 ng/ml, and that of VPA-GLN was 34 ng/ml of CSF. The corresponding serum concentrations of VPA-GLU and VPA-GLN of the same patient were 7.0 ng/ml and 6.0 ng/ml, respectively. The concentration of CSF VPA of the patient was less than 15 μg/ml of CSF.

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**TABLE 4**

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<th>Patient No.</th>
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<th>VPA GLN (μg/ml)</th>
<th>VPA GLY (μg/ml)</th>
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Mean ± S.D. 0.75 ± 0.41
Discussion

We report here the identification of valproyl glutamate and valproyl glutamine, both conjugates of VPA in humans. Valproyl glycine was also identified in the biological samples of patients on VPA. Preliminary accounts of these findings were reported (Gopaul et al., 1997a,b).

The discovery of VPA-GLU, in the biological fluids of humans, is intriguing due to its extreme rarity. To the best of our knowledge, the glutamate conjugate of benzoic acid in the Indian fruit bat (Idle et al., 1975) and p-nitrobenzoic acid in spiders (Smith, 1962) are the only two glutamate conjugates of a xenobiotic carboxylic acid reported in the literature. Conjugates of amino acids that are normally found are species-dependent and vary according to the structure of the carboxylic acid involved.

In humans, the conjugation of glycine, glutamine, taurine, and ornithine with carboxylic acids are known (Caldwell et al., 1976; Webster et al., 1976; Hutt and Caldwell, 1990; Shirley et al., 1994; Kasuya et al., 1999). Reports in the literature indicate that glycine and to a lesser extent glutamine are the most likely amino acids to be involved in the conjugation reactions of carboxylic acids (Hutt and Caldwell, 1990). It should also be added that recently the conjugation of xenobiotic amines with glutamate has also been reported (Mutlib et al., 2000; 2002).

In this study, quantitative analysis of the conjugates in human urine, serum, and CSF samples revealed that VPA-GLU was consistently the predominant amino acid conjugate compared with VPA-GLN, and VPA-GLY was a minor conjugate. The more extensive conjugation of VPA with glutamic acid than either glutamine or glycine is also an unprecedented observation for the human species.

The higher abundance of the amino acid conjugates in urine samples relative to their serum concentrations suggests that the conjugates are readily excreted upon formation in the liver. These metabolites can also be formed at other sites, such as the kidney. For example, in vitro studies support the conjugation of benzoic acid with glycine in human liver and kidney tissues (Caldwell et al., 1976; Temellini et al., 1993). Alternatively, a portion of the conjugates formed may be excreted into the intestinal lumen (via biliary excretion or exsorption) and excreted in feces.

Amino acid conjugation of an organic acid involves the activation of the acid to its CoA derivative prior to coupling with an amino acid by the respective amino acid transferase (Hutt and Caldwell, 1990). The mean observed recovery of amino acid conjugates of VPA in the urine samples of four patients was 0.75 ± 0.41% of the VPA dose. By comparison, the β-oxidation metabolites of VPA, namely 2-ene VPA and 3-keto VPA, that are also initiated by the activation of VPA to its CoA ester, account for more than 15% of a VPA dose in humans (Pollack et al., 1986). This would imply that the VPA CoA ester is more susceptible to β-oxidation than amino acid conjugation in mitochondria. However, whether the amino acid conjugation of VPA is a mitochondrial or microsomal-based reaction remains to be confirmed.

The identification of VPA-GLU and VPA-GLN in the CSF sample stood as the most interesting finding in this study. The availability of human CSF of patients on VPA therapy is rare, and as part of our efforts to support this human evidence, we reported the results of a preliminary study that confirmed unequivocally the identification of VPA-GLU as the only amino acid conjugate of VPA in the CSF of...
the CSF VPA level (15/11021VPA-GLU and VPA-GLN in the patient in this study were/110211104/110111104/1102/110111104/1102111021VPA-GLU is probably the direct result of the conjugation of VPA with glutamic acid and is not primarily the hydrolyzed product of VPA-GLN although this needs to be confirmed.

The CSF results of the patient in this study are consistent with the levels of VPA metabolites that are usually found at concentrations <10% of VPA in the CSF (Lössher et al., 1988) or <15% of VPA in the human brain (Adkinson et al., 1995). The CSF concentrations of VPA-GLU and VPA-GLN in the patient in this study were <1% of the CSF VPA level (15 μg/ml of CSF). Because, the concentrations of VPA and unsaturated VPA metabolites in the CSF are believed to correspond to those in the brain (Vajda et al., 1981; Lössher et al., 1988), it would be reasonable to suggest that the levels of the CSF VPA-GLU and VPA-GLN reflect their respective brain tissue concentrations. However, this would be inconsistent with a recent report from Scism et al. (2000), which suggests that VPA may be concentrated in the intracellular compartments of the brain parenchyma.

The CSF concentrations of VPA-GLU, and VPA-GLN were 9 and 5 times, respectively, their corresponding serum concentrations in the human subject, suggesting that the metabolites could be formed in the brain itself as a result of the conjugation of VPA with glutamic acid or its precursor, glutamine. If so, the detection of VPA-GLU and VPA-GLN in the CSF of human and rabbit provides some grounds to propose that the mechanism of action of VPA could in part be the result of the conjugation of VPA with the excitatory neurotransmitter glutamic acid believed to be responsible for seizure activity or its precursor glutamine. In support of this theory, significantly elevated concentrations of GLU are found in human epileptogenic foci (Perry et al., 1976) and in the CSF of newly diagnosed epileptic patients untreated with anticonvulsants (Kalviainen et al., 1993). Although the concentrations of human CSF VPA-GLU with respect to the concentration of VPA itself (~15 μg/ml) is low, it is consistent with the concentration of CSF glutamate that is usually found in comparatively low concentrations in the CSF of control subjects (Kalviainen et al., 1993; Jimenez-Jiminez et al., 1998). In light of the implication of VPA metabolism in the mechanism of action of VPA and the induction of hepatotoxicity, the significance of this new metabolic pathway, namely the glutamic acid conjugation with VPA in humans, needs to be pursued.

Conclusion

LC-MS/MS was used for the identification and quantitation of the novel VPA-GLU and VPA-GLN conjugates in the urine, serum, and CSF samples of patients on VPA. These amino acid conjugates of VPA in urine account for about 1% of a VPA dose. The identification of VPA-GLU and VPA-GLN in the CSF of a human subject stands as the most interesting finding with the detection of amino acid conjugates of VPA in the CSF of humans. In our opinion, the significance of these conjugates in the CNS requires further investigation. A report on the studies of the amino acid conjugates of VPA in animals is to follow.

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References
