Apparent autoinduction of valproate β -oxidation in humans

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Aims The study aimed to show whether autoinduction of valproate (VPA) along its β -oxidation pathway occurred upon chronic dosing in humans.

Methods Twelve young volunteers without active illness took sodium valproate (NaVPA) 200 mg orally 12 hourly for 3 weeks. On days 7 and 21, serial blood samples and all urine passed over an interdosing interval from 08.00 to 20.00 h were collected for analysis of VPA and certain metabolites.

Results Plasma AUC(0,12 h) of VPA was significantly lower on day 21 than on day 7 (2.40 vs 2.84 $\mu\text{mol ml}^{-1} \text{ h}$, 95% CI for the difference 0.13–0.81 $\mu\text{mol ml}^{-1} \text{ h}$). Significant differences in plasma AUC(0,12 h) of the β -oxidation metabolites E-2-en-VPA and 3-oxo-VPA were not found. However, formation clearances of plasma VPA to urinary E-2-en-VPA and 3-oxo-VPA were significantly increased from day 7 to day 21 (0.010 vs 0.024 and 2.57 vs 3.60 $\text{ml kg}^{-1} \text{ h}^{-1}$, respectively, 95% CI for the differences –0.025 to –0.004 and –1.72 to –0.34 $\text{ml kg}^{-1} \text{ h}^{-1}$, respectively). Formation clearances to VPA-glucuronide (0.534 vs 0.505 $\text{ml kg}^{-1} \text{ h}^{-1}$) and 4-OH-VPA (0.112 vs 0.110 $\text{ml kg}^{-1} \text{ h}^{-1}$) were not significantly different.

Conclusions Regular low dose VPA intake in humans over a period of 3 weeks appears to be associated with a small induction of its metabolism by the β -oxidation pathway, but not by glucuronidation or 4-hydroxylation.

Keywords: anticonvulsants, autoinduction, β -oxidation, glucuronidation, valproate

Introduction

Valproic acid (VPA, 2-propylpentanoic acid) is an effective, commonly prescribed antiepileptic agent [1] finding increasing use for indications such as migraine prophylaxis [2] and for bipolar affective disorder in psychiatry [3]. As a branched, short-chain fatty acid, it is structurally unrelated to other anticonvulsant agents, and its metabolism is complex [4–6]. However, two pathways clearly predominate (Figure 1). β -Oxidation (mitochondrial and peroxisomal), analogous to the oxidative degradation of dietary/endogenous fatty acids, leads sequentially through the E-2-en-, 3-OH- and 3-oxo-derivatives of VPA before yielding metabolites indistinguishable from the products of intermediary metabolism. The other major pathway is glucuronidation (in the

endoplasmic reticulum) of the carboxy function of VPA, which yields the acyl glucuronide. Several other pathways are initiated by cytochrome P450-catalysed oxidations (e.g. to 4-OH-VPA, Figure 1) [7].

Metabolism of VPA is dose/concentration dependent. Whereas β -oxidation may be quantitatively more important at low doses, glucuronidation dominates at higher (including usual therapeutic) doses [8, 9]. Whereas some other antiepileptic drugs (e.g. phenobarbitone, carbamazepine, phenytoin) are well known to induce hepatic mono-oxygenases, and, certainly in the case of carbamazepine, its own metabolism, the situation with VPA is less clear. In rats, chronic high dose administration of VPA caused enhanced glucuronidation and β -oxidation of the drug [10]. Rat liver and kidney peroxisomal β -oxidation was also increased by chronic VPA administration [11]. Others have reported VPA to induce certain cytochrome P450 monooxygenase activities in cultured rat hepatocytes [12]. Comparable reports of VPA induction of metabolizing enzymes in humans are lacking, though recently

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Received 7 June 1999, accepted 2 February 2000.

Figure 1 Valproic acid (VPA) and partial metabolic pathways via β -oxidation, glucuronidation and ω -1 oxidation.

Anderson *et al.* [13] reported increased formation clearances to VPA-glucuronide, E-2-en-VPA and 4-OH-VPA in patients with traumatic brain injury. These increases were observed in the second week of VPA therapy, and were attributed to trauma-induced non-specific induction of hepatic enzymes. However, the question of whether altered dietary patterns in persons recovering from states of depressed consciousness may have altered β -oxidation was not considered.

During a study of the mechanism of the interaction which occurs between felbamate and VPA in humans [14], it was noted that, in a small control group of four subjects who took no drugs apart from sodium valproate (NaVPA) in constant dosage (200 mg 12 hourly) for 3 weeks, the plasma clearance of VPA appeared to increase between day 7 and day 21 of the study. This was associated with presumptive evidence of increased β -oxidation of the drug. This finding in such a small group of subjects seemed potentially important enough to warrant a larger scale study. Such a study has been carried out and is described below.

Methods

Subjects

The study was carried out in 12 young adult volunteers

without active illness (11 males, 1 female), age range 18–29 years (mean 23 years), weight range 58–100 kg (mean 74 kg), who had no history of alcohol or substance abuse, and who gave written informed consent after the aims and procedures of the study protocol were explained in full. Their health status was checked via medical history and physical examination. Routine biochemical, haematological and serological screenings were carried out prior to commencement of the study, which was approved by the Medical Research Ethics Committee of the University of Queensland. Biochemistry and haematology tests were also performed on exit from the study.

Study design

On day 1 of the study at about 08.00 h, the subjects began taking oral NaVPA (a 200 mg enteric-coated tablet, ValproTM, Alphapharm Pty Ltd) 12 hourly, and continued doing so for 3 weeks. On days 7 and 21 whilst continuing to receive this medication, they participated in a 12 h interdosing interval pharmacokinetic and metabolite excretion study. On these days prior to the morning dose, the subjects were fitted with a forearm venous cannula. They emptied their bladders, a predose blood sample (10 ml) was drawn, and the 08.00 h dose taken with a glass of water. No breakfast was taken, and subjects refrained from eating until a standard lunch was provided

4 h after dosing. Blood samples (7–10 ml) at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 h postdose were collected into tubes containing lithium heparin. The plasma was separated as soon as practicable by centrifugation, and frozen (-20°C) until analysis. All urine passed between 08.00 h and 20.00 h (when the subjects emptied their bladders again) was collected. The volume was recorded, the pH adjusted to 5.0 with 4 M hydrochloric acid, and two 20 ml samples frozen (-20°C) until analysis.

Four subjects were taking regular medication throughout the study period (oral contraceptive steroid – 1 subject, salbutamol – 2 subjects, salbutamol and budesonide – 1 subject). Three subjects took one or two single doses of paracetamol, but not on study days 7 and 21 nor on the preceding days (6 and 20). Four subjects missed a single dose of NaVPA (the morning dose on day 4 for two subjects, and the evening dose on day 19 for two subjects). In the opinion of the investigators, the missed doses and medications detailed above were not sufficiently serious to warrant exclusion of these subjects from the study.

Analytical methods

VPA, E-2-en-VPA and 3-oxo-VPA in plasma and urine, and additionally 4-OH-VPA and VPA-glucuronide in urine, were determined with validated assay methods using gas chromatography with mass spectrometric detection (GC/MS), with minor modifications of methods described earlier [9, 14].

In brief, nonconjugated VPA, E-2-en-VPA and 3-oxo-VPA in plasma were measured by adding to 0.5 ml aliquots of plasma samples 100 μl of internal standard solution (500 $\mu\text{g ml}^{-1}$ nonanoic acid in 0.04 M sodium hydroxide solution) and 100 μl of 4 M hydrochloric acid solution. After equilibration with 5 ml 1-chlorobutane, the organic layer was separated and equilibrated with 0.5 ml of 0.5 M sodium hydroxide solution. The aqueous layer was then separated, acidified with 1.0 ml of 1 M hydrochloric acid/4 M sodium chloride solution, and the sealed vial heated at 60°C for 1 h (these conditions decarboxylate 3-oxo-VPA into 3-heptanone and dehydrate 4-OH-VPA into 4-OH-VPA- γ -lactone). The mixture was cooled and equilibrated with chloroform (800 μl). A sample (200 μl) of the chloroform layer was transferred to an autoinjector vial, and 0.5 μl injected into the GC/MS.

In urine, assays for nonconjugated VPA and for total (nonconjugated plus conjugated) VPA, E-2-en-VPA, 3-oxo-VPA and 4-OH-VPA were carried out. For totals, 100 μl samples of urine were added to 100 μl of internal standard solution (500 $\mu\text{g ml}^{-1}$ nonanoic acid in 0.04 M sodium hydroxide solution) and 200 μl of 0.5 M sodium hydroxide solution. After mixing, the sealed vials were heated at 60°C for 60 min (causing alkaline

hydrolysis of glucuronide conjugates). After cooling, 250 μl of 1 M hydrochloric acid/4 M sodium chloride solution was added, and the resealed vials heated again at 60°C for 60 min (giving decarboxylation of 3-oxo-VPA and dehydration of 4-OH-VPA as noted above). After cooling, the samples were processed for GC/MS analysis as described above for plasma analyses. For analysis of nonconjugated VPA in urine, the alkaline hydrolysis step was omitted. In order to achieve measurable concentrations of total urinary E-2-en-VPA, an optimized procedure was used. This involved alkaline hydrolysis of 500 μl urine samples by heating at 60°C for 60 min in a sealed vial with 100 μl of 0.1 M sodium hydroxide solution (or E-2-en-VPA standards in 0.1 M sodium hydroxide solution), 100 μl of internal standard solution (50 $\mu\text{g ml}^{-1}$ nonanoic acid in 0.04 M sodium hydroxide solution) and 100 μl 2 M sodium hydroxide solution. After cooling, 200 μl 2 M hydrochloric acid and 5 ml 1-chlorobutane were added. After equilibration, the organic layer was separated and equilibrated with 500 μl of 0.5 M sodium hydroxide solution. The aqueous layer was then separated, acidified with 1.0 ml of 1 M hydrochloric acid/4 M sodium chloride solution, and equilibrated with 100 μl chloroform. A sample (2.5 μl) was injected into the GC/MS.

The Hewlett-Packard GC/MS instrument comprised a model 7963 autoinjector, a model 5890 Series II gas chromatograph, and a model 5971 mass spectrometer with PC-based HP-MS Chemstation G1034C software. The column was a HP-FFAP capillary column (25 m $\times$ 0.20 mm ID, with 0.3 μm film). Helium carrier gas flow was 0.8 ml min^{-1} . The oven program was 80°C for 1 min, $50^{\circ}\text{C min}^{-1}$ to 130°C with hold for 0.5 min, $15^{\circ}\text{C min}^{-1}$ to 220°C . 3-Heptanone (from 3-oxo-VPA), 4-OH-VPA- γ -lactone (from 4-OH-VPA), VPA, E-2-en-VPA and nonanoic acid eluted at 2.3, 5.6, 6.6, 7.3 and 8.1 min, respectively. The MS was operated in selected ion mode, and verified for the precise and accurate quantification of VPA at 0.5–100 $\mu\text{g ml}^{-1}$ plasma, VPA metabolites at 0.1–10 $\mu\text{g ml}^{-1}$ plasma, VPA and 3-oxo-VPA at 1.0–500 $\mu\text{g ml}^{-1}$ urine, 4-OH-VPA at 2.0–200 $\mu\text{g ml}^{-1}$ urine and E-2-en-VPA (optimized method) at 0.01–2.0 $\mu\text{g ml}^{-1}$ urine. Precision and accuracy were determined for each analyte by replicate (usually 6) analyses of quality control samples at low, medium and high concentrations in each stated concentration range. In all cases, parameters for precision (relative standard deviation in a replicate set) and accuracy (mean relative difference between 'measured' and 'true' concentrations in a replicate set) were within $\pm 20\%$, and usually within $\pm 10\%$. The minimum quantified concentration was defined as the lowest concentration at which precision and accuracy were $\leq 20\%$, and this was equated with the lower limit of the concentration range for each analyte stated above. Sources of VPA, its reference

metabolites and nonanoic acid were as described earlier [14].

Data analysis

Plasma AUC(0,12 h) values for VPA, E-2-en-VPA and 3-oxo-VPA were calculated by trapezoidal rule integration. Steady-state plasma clearance (CL/F) of VPA was calculated as VPA dose/VPA AUC(0,12 h), and steady-state formation clearances of VPA or E-2-en-VPA to various metabolites in urine as the quotient of the amount of metabolite in urine and the AUC(0,12 h) for VPA or E-2-en-VPA, as appropriate. Confidence interval analysis (paired samples) was used to assess the statistical significance of findings.

Results

The profiles for plasma concentrations of VPA, E-2-en-VPA and 3-oxo-VPA across the 08.00–20.00 h interdosing interval on days 7 and 21 of the study are shown in Figure 2. The mean VPA concentrations at all time points were lower on day 21 than on day 7, with mean VPA AUC(0,12 h) falling from 2.84 to 2.40 $\mu\text{mol ml}^{-1} \text{h}$ over this period (Table 1). However, the mean AUC(0,12 h) values for plasma E-2-en-VPA and 3-oxo-VPA did not appreciably alter (Table 1), though on each day, plasma 3-oxo-VPA concentrations were lower at the end of the (daytime) interdosing interval (i.e. at 20.00 h) than at the beginning (unlike VPA itself and E-2-en-VPA, Figure 2). Plasma E-2-en-VPA to VPA AUC(0,12 h) ratios significantly increased from day 7 to day 21, whereas the ratios of 3-oxo-VPA to E-2-en-VPA were unaltered (Table 1).

Urinary recoveries of various VPA metabolites are shown in Table 2. The initial assays for nonconjugated VPA and total E-2-en-VPA gave peaks below the limit of quantification: in the case of total E-2-en-VPA, an optimized procedure was developed and used for reassay. There were no significant differences (95% CI analysis) in urinary recovery of any of the measured metabolites on days 7 and 21, though mean 3-oxo-VPA was higher and mean VPA-glucuronide lower on day 21.

Plasma clearance of VPA was significantly increased on day 21 (Table 3), as were formation clearances of VPA to the urinary β -oxidation metabolites E-2-en-VPA and 3-oxo-VPA (Table 3), and to their sum (not shown). The day 21 to day 7 ratios of plasma clearance of VPA and formation clearances to urinary E-2-en-VPA and 3-oxo-VPA were 1.19 ± 0.23 , 3.91 ± 2.79 and 1.41 ± 0.43 (mean $\pm$ s.d.), respectively. Formation clearances to urinary VPA-glucuronide and to 4-OH-VPA were not significantly altered between days 7 and 21. Mean clearance of plasma VPA to substances not measured in the study (estimated by difference between measured clearances to

Figure 2 Mean ($\pm$ s.e. mean, $n=12$) plasma concentrations of VPA, E-2-en-VPA and 3-oxo-VPA across an interdosing interval from 08.00 to 20.00 h on day 7 (open symbols) and day 21 (closed symbols) of 12 hourly 200 mg NaVPA dosing.

metabolites and plasma VPA clearance) were also not appreciably different (Table 3).

Clearance of plasma E-2-en-VPA to urinary 3-oxo-VPA appeared to increase from day 7 ($48.6 \pm 13.3 \text{ ml kg}^{-1} \text{h}^{-1}$) to day 21 ($57.0 \pm 19.6 \text{ ml kg}^{-1} \text{h}^{-1}$), though the difference did not achieve statistical significance at the 5% level of confidence (difference = -8.4 , 95% CI = -18.2 – $1.31 \text{ ml kg}^{-1} \text{h}^{-1}$).

Discussion

The study has shown that plasma VPA clearance increased between days 7 and 21 of regular intake of the drug in young adults who were receiving the relatively low oral dose of 200 mg NaVPA 12 hourly. This increase in plasma VPA clearance appears to be accounted for by an increase in clearance of the drug along its β -oxidation pathway as far as it was feasible to follow this pathway. There was no increase in clearance of the drug by conjugation to VPA-glucuronide or by ω -1 oxidation to 4-OH-VPA, nor of change in clearance to the unaccounted for portion of the dose.

These data would appear *prima facie* evidence that the early weeks of VPA intake in humans are associated with

Table 1 Plasma AUC(0,12 h) data on days 7 and 21 of twice daily oral dosing of 200 mg NaVPA.

AUC(0,12 h)	Day 7	Day 21	Mean difference	95% CI
VPA ($\mu\text{mol ml}^{-1} \text{ h}$)	2.84 ± 0.79	2.40 ± 0.54	0.438	0.128–0.811 *
E-2-en-VPA ($\mu\text{mol ml}^{-1} \text{ h}$)	0.149 ± 0.042	0.147 ± 0.040	0.002	–0.018–0.022
3-oxo-VPA ($\mu\text{mol h ml}^{-1}$)	0.491 ± 0.056	0.468 ± 0.076	0.023	–0.025–0.072
E-2-en-VPA/VPA	0.0534 ± 0.0149	0.0630 ± 0.0179	–0.0096	–0.146 to –0.0046 *
3-oxo-VPA/E-2-en-VPA	3.54 ± 1.11	3.32 ± 0.91	0.217	–0.544–0.979

AUC, area under the plasma concentration–time curve over a steady state interdosing interval from 08.00–20.00 h; mean $\pm$ s.d., $n=12$. *95% CI excludes zero.

autoinduction of VPA metabolism along its β -oxidation pathway, which is that of a branched chain fatty acid. This β -oxidation involves the conversion of VPA to its CoA thioester, then dehydrogenation to E-2-en-VPA.CoA, hydration of this unsaturated derivative to 3-OH-VPA.CoA, followed by its dehydration to 3-oxo-VPA.CoA (Figure 1). Considerable amounts of 3-oxo-VPA are excreted as such in urine, but the β -oxidation pathway continues through to propionic and other small acids which then enter the tricarboxylic acid cycle to be ultimately excreted as CO_2 and water. Using unlabelled VPA, it is impossible to trace metabolite flux along this pathway beyond the 3-oxo-VPA stage, because subsequent metabolites may have various metabolic origins.

We are not aware of any previous report that the β -oxidation metabolic pathway of VPA is autoinducible in healthy humans, though Fisher *et al.* [10] presented evidence that chronic high dose treatment of rats with VPA (200 mg kg^{-1} thrice daily for 6 weeks) caused a marked enhancement in its elimination rate, attributed mainly to increases in glucuronidation and β -oxidation. It is interesting that a recent report by Anderson *et al.* [13] found significant increases in formation clearance of VPA to VPA-glucuronide, E-2-en-VPA and 4-OH-VPA in the second week of VPA therapy in patients with traumatic brain injury. Parallel increases in the 6- β -hydroxycortisol to cortisol ratio suggested an induction of hepatic drug metabolizing enzymes attributable to the brain injury.

Various cytochrome P450-catalysed drug oxidations are known to be autoinducible, e.g. that of carbamazepine [15, 16]. In the present study, it was therefore necessary to

consider the possibility that the apparent autoinduction could be explained by an enhanced direct oxidation (e.g. by cytochrome P450 in the endoplasmic reticulum) of VPA to its 3-OH-metabolite (Figure 1), with this 3-OH-VPA then possibly entering mitochondria and there being dehydrogenated to 3-oxo-VPA, and passed along the branched chain fatty acid β -oxidation pathway. If this happened there seems no reason to expect the increased clearance of plasma VPA to urinary E-2-en-VPA found in the present study. Also, if VPA metabolism to E-2-en-VPA did not increase, and increased 3-oxo-VPA formation came from increased 3-OH-VPA formation via P450 oxidation, plasma E-2-en-VPA AUC(0,12 h) values should have fallen in parallel with plasma VPA AUC(0,12 h) values between days 7 and 21 of the study. This did not happen; instead the AUC(0,12 h) ratio of E-2-en-VPA to VPA increased to a statistically significant extent between days 7 and 21. Another possible explanation might be that the clearance of E-2-en-VPA to 3-oxo-VPA fell between days 7 and 21. However, the study showed that this tended to increase.

In the present study, total VPA concentrations in plasma were measured and used to calculate clearances, rather than measuring and using unbound VPA concentrations. However, this should not affect the conclusions, since VPA protein binding is reported to remain essentially constant at the relatively low VPA concentrations found in the study [17]. Overall, the findings of the study point consistently towards VPA β -oxidation undergoing autoinduction, and thus explaining the increased clearance of the drug.

Table 2 % recovery of the 200 mg NaVPA dose in urine as various metabolites.

Recovery	Day 7	Day 21	Mean difference	95% CI
VPA-glucuronide (%)	9.38 ± 3.75	7.55 ± 3.59	1.83	–0.53–4.18
E-2-en-VPA (%)	0.167 ± 0.166	0.310 ± 0.192	–0.143	–0.309–0.023
3-oxo-VPA (%)	42.7 ± 11.3	49.6 ± 13.9	–6.92	–17.3–3.43
4-OH-VPA (%)	2.00 ± 1.00	1.61 ± 1.21	0.40	–0.34–1.14
Total recovery (%)	54.2 ± 14.5	59.1 ± 17.0	–4.84	–17.6–7.89

Urinary recovery over a steady state interdosing interval from 08.00 to 20.00 h, mean $\pm$ s.d., $n=12$. E-2-en-VPA, 3-oxo-VPA and 4-OH-VPA are measured as the sum of nonconjugated and conjugated species.

Table 3 Plasma clearance (CL/F) of VPA and formation clearances to various urinary metabolites.

Clearance	Day 7	Day 21	Mean difference	95% CI
Plasma VPA (ml kg ⁻¹ h ⁻¹)	6.17 ± 1.50	7.30 ± 2.33	-1.13	-2.20 to -0.636 *
VPA-glucuronide (ml kg ⁻¹ h ⁻¹)	0.534 ± 0.145	0.505 ± 0.182	0.029	-0.062-0.121
E-2-en-VPA (ml kg ⁻¹ h ⁻¹)	0.010 ± 0.009	0.024 ± 0.016	-0.014	-0.025 to -0.004 *
3-oxo-VPA (ml kg ⁻¹ h ⁻¹)	2.57 ± 0.75	3.60 ± 1.47	-1.03	-1.72 to -0.339 *
4-OH-VPA (ml kg ⁻¹ h ⁻¹)	0.112 ± 0.046	0.110 ± 0.074	0.0021	-0.038-0.042
Unaccounted for (ml kg ⁻¹ h ⁻¹)	2.96 ± 1.50	3.07 ± 1.81	-0.108	-1.25-1.03

Clearances over a steady state interdosing interval from 08.00 to 20.00 h, mean ± s.d., n = 12. * 95% CI excludes zero.

The question may be raised as to why this apparent autoinduction phenomenon has not been noted previously, as it might at first sight be expected to result in a fall in plasma VPA concentrations under steady-state conditions early in the course of VPA therapy in epileptic patients. However, the autoinduction of VPA β -oxidation found in the present study has been demonstrated at VPA doses appreciably lower than those commonly used in the initiation of VPA therapy in adults. At such low doses β -oxidation is the dominant pathway of VPA metabolism [8, 9]. At higher doses, glucuronidation accounts for the majority of a VPA dose, and the usual use of such doses in practice may conceal the consequences of an increase in VPA β -oxidation early in the course of therapy.

The findings of the present study may be regarded simply as a novel biochemical peculiarity of little clinical consequence, though clearly its time course and extent need to be defined more fully in case most of the phenomenon has already occurred prior to day 7 of VPA intake (which was the baseline measurement in the present study). However, VPA-associated hepatotoxicity is accompanied by decreased β -oxidation of the drug, and is seen to occur most commonly in the first 6 months of treatment [18–20]. Therefore it is possible that abnormalities in autoinduction of VPA β -oxidation may play a role in determining those rare individuals who are vulnerable to potentially lethal VPA-associated hepatotoxicity.

This work was supported by a Project Grant from the National Health and Medical Research Council of Australia. We thank Prof Frank Abbott and Dr Reza Anari of the Faculty of Pharmaceutical Sciences, University of British Columbia, for interlaboratory analytical validation of valproate metabolites.

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