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Concentration-dependent disposition of glucuronide metabolite of valproate

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Abstract

The glucuronide conjugation metabolism of valproate (VPA) has been assessed to be non-linear within the therapeutic concentration range. However, disposition of its metabolite, valproic acid glucuronide (VPAG), in relation to VPA doses is unclear. The purpose of this study was to elucidate the characteristics of dose-related disposition of VPAG. Guinea-pigs were treated with an intravenous bolus dose of sodium valproate at 20, 100, 500 or 600 mg kg^{-1}. Plasma was sampled on a pre-selected time schedule, and bile and urine were collected. Concentrations of VPA and VPAG in plasma, bile and urine were determined by gas chromatography. The pharmacokinetics of VPA and VPAG both were dose-dependent. However, the plasma concentration–time profiles of VPAG and VPA were not parallel. At a usual dose of VPA (20 mg kg^{-1}), plasma VPAG declined with plasma VPA, whereas at a high dose of VPA ($>$ 500 mg kg^{-1}), plasma VPAG was elevated against the decline of plasma VPA, which suggested accumulation of plasma VPAG possibly owing to saturated elimination. The biliary and urinary clearances of VPA (vCL_b and vCL_u) were independent of dose. However, the clearances of plasma VPA (vCL_p), plasma VPAG (gCL_p), biliary and urinary VPAG (gCL_b and gCL_u) all were decreased against the increase in VPA doses. The dose-dependent decrease of gCL_u (from 3.19 to 1.12 mL min^{-1}) was less pronounced than that of gCL_p (from 6.72 to 0.86 mL min^{-1}) and the gCL_u turned to exceed the gCL_p at high doses of VPA ($>$ 500 mg kg^{-1}). These results suggest that the excess urinary VPAG might be produced in kidney. In conclusion, at a high dose of VPA, plasma VPAG is accumulated. The concentration-dependent biliary and urinary recovery of VPAG might be governed by a saturable elimination process rather than by saturable hepatic biotransformation rate. Glucuronide conjugation metabolism of VPA in kidney is speculated, which might be minor at low levels of plasma VPA, but more obvious after saturation of hepatic glucuronidation.

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Introduction

Valproic acid (VPA, 2-*n*-propylpentanoic acid) is an anti-epileptic agent. The major metabolite of VPA in most species is glucuronic acid conjugate (VPAG) (Dickinson et al 1979, 1989; Granneman et al 1984a; Yu & Shen 1996). The glucuronidation metabolite of VPA undergoes enterohepatic recycling (Dickinson et al 1979; Yu & Shen 1991; Yu 1993), which may serve as an extra dose of VPA. The metabolic glucuronidation rate of VPA is non-linear within the therapeutic concentration range (Yu & Shen 1996). It obeys Michaelis-Menten type kinetics (Yu et al 1993; Yu & Shen 1996), with the Michaelis-Menten constant (K_m) in the middle of the therapeutic concentration range (Yu & Shen 1996), and the maximum biotrans-

formation rate (V_{max}) approximating a high therapeutic dosing rate (Yu et al 1993). Non-linear biliary excretion (Dickinson et al 1979; Yu et al 1993) and urinary recovery (Granneman et al 1984b) of VPAG have been reported. Biliary VPA is equivalent to, but biliary VPAG decreases relative to, the liver concentration of VPA (Yu et al 1993). Urinary recovery of VPAG increases with an increase in VPA dose in rats (Dickinson et al 1979) and humans (Granneman et al 1984b). It was reported that plasma VPAG was too low to be detectable in humans, dogs and rats (Nau & Loscher 1984). The plasma concentration–time profile of VPAG has never been reported. Whether the dose-related excretory recovery of VPAG is governed by its metabolic formation rate or by its excretion rate per se is unclear. The concentration-dependent biotransformation and elimination rates of VPAG are therefore of great interest. Predicting drug/metabolite profiles and routes of excretion requires an understanding of the pharmacokinetics of a drug and its metabolites, as well as the rate-limiting steps governing these processes. Elucidation of the disposition of drugs and their metabolites in response to a wide dose range may provide a basic principle for prediction.

The purpose of the present study was to investigate the disposition of VPAG in association with various plasma concentrations of VPA including linear, non-linear and metabolic saturation levels, to elucidate the disposition of VPAG in relation to dose-dependent pharmacokinetics of VPA, and to examine the concentration-related plasma, biliary and urinary clearances. The results may clarify the rate-limiting steps governing the dose-dependent disposition of VPAG.

Materials and Methods

Animals and treatment

Male adult guinea-pigs (Experimental Animal Center, College of Medicine National Taiwan University), 230–280 g, were cannulated with polyethylene tubing under light ether anaesthesia and kept in restraining cages. After recovering from anaesthesia, animals received 20, 100, 500 or 600 mg kg^{-1} sodium valproate by intravenous bolus injection. Blood samples (approx. 0.2 mL) were collected via the carotid artery cannula on a preselected time schedule. Bile and urine were collected from the bile duct cannula and urethra cannula, respectively. Concentrations of VPA and its glucuronide metabolite VPAG were determined in plasma, bile and urine immediately after sampling.

Analytical methods

The concentration of VPA was determined by gas chromatography (GC) (Yu & Shih 1996). VPAG was hydrolysed to VPA and determined by GC as reported previously (Yu et al 1993). Briefly, VPA in collected samples was extracted under acidic conditions with chloroform containing octanoic acid as an internal standard. An aliquot of the chloroform layer was injected onto the GC apparatus for determination of VPA. VPAG in the aqueous layer was hydrolysed to VPA by alkaline hydrolysis and then extracted with chloroform under acidic conditions. The GC (Hewlett-Packard 5890) was equipped with a capillary column wall-coated with crossbonded carbowax, an FID and an integrator. The injector was set to split mode. The carrier gas was nitrogen. The temperatures for the injection port, oven and FID were 220, 190 and 240°C, respectively. The detection limit for VPA from plasma samples was 0.5 μg mL^{-1}. The linearity of the detector response was assessed with concentrations ranging from 1 to 200 μg mL^{-1}. For plasma samples containing higher concentrations of VPA, multiples of the chloroform volume were used in the extraction process (Yu 1981) to bring the drug concentrations within the linear range for GC analysis.

Data analysis

The pharmacokinetic parameters of VPA were analysed by model fitting of plasma concentrations using the PCNONLIN program (Statistical Consultants, Lexington, KY, USA). The AUC of VPAG was calculated by the trapezoidal method. Biliary clearance (CL_b) and urinary clearance (CL_u) of VPA (vCL_b and vCL_u) and VPAG (gCL_b and gCL_u) were estimated by the following equations:

$$vCL_b \text{ (or } vCL_u) = vM_b \text{ (or } vM_u)/vAUC_t \quad (1)$$

$$gCL_b \text{ (or } gCL_u) = gM_b \text{ (or } gM_u)/gAUC_t \quad (2)$$

where vM_b and vM_u represent the amount of VPA in the collected bile and urine, and gM_b and gM_u represent that of VPAG. The AUC_t is the area under the plasma concentration–time curve (AUC) within the sample-collecting period for VPA ($vAUC_t$) and VPAG ($gAUC_t$), respectively. Plasma clearance of VPAG (gCL_p) was calculated by equation 3.

$$gCL_p = gM_p/gAUC_t. \quad (3)$$

In equation 3, gM_p represents the total amount of metabolic VPAG produced within the time period t. The gM_p was estimated by equation 4, in which the metabolic parameters (V_{max} = 1.2 μmol min^{-1} kg^{-1};

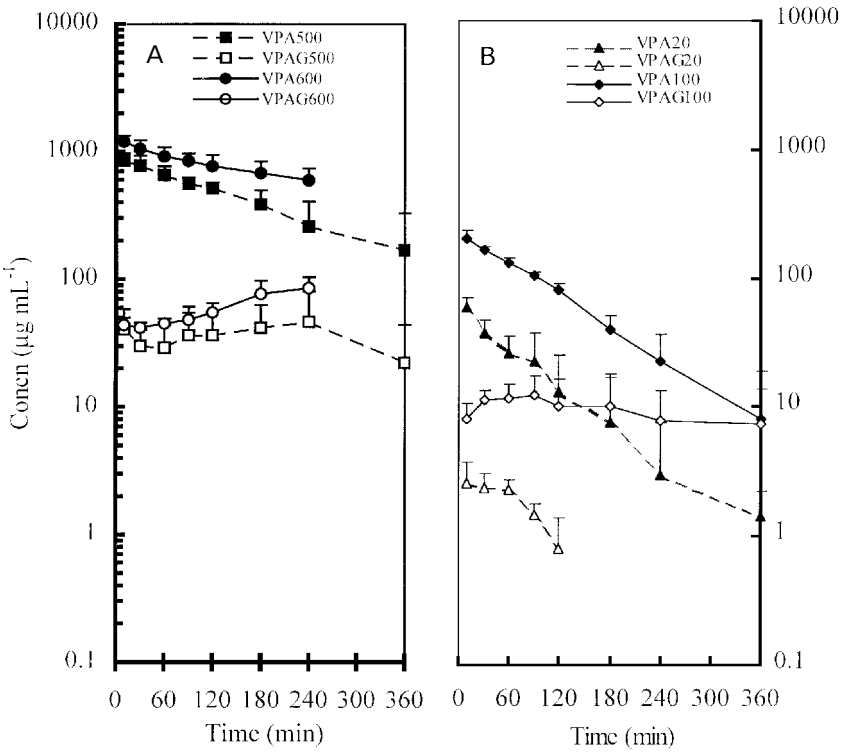

Figure 1 Plasma concentration–time profiles of valproate (VPA; closed symbols) and its glucuronide metabolite valproic acid glucuronide (VPAG; open symbols) in guinea-pigs after an intravenous dose of sodium valproate (mean±s.d., n = 5). A. 600 mg kg^{-1} (VPA600, VPAG600) and 500 mg kg^{-1} (VPA500, VPAG500) VPA dose; B. 100 mg kg^{-1} (VPA100, VPAG100) and 20 mg kg^{-1} (VPA20, VPAG20) VPA dose.

K_m = 0.16 μmol mL^{-1}) (Yu & Shen 1996) and plasma unbound fraction (fu) (Yu & Shen 1992) were derived from previous studies. The averaged plasma concentration (C′) of VPA during the experimental period (t) for each dose was calculated by equation 5.

$$gM_p = v \times t = [V_{max} \times fu \times C'/(K_m + fu \times C')] \times t \quad (4)$$

$$C' = vAUC_t/t. \quad (5)$$

The eliminated fraction of a dose (FD_{el}) up to the last blood sampling (the end of the experiment) was calculated by equation 6:

$$FD_{el} = vAUC_t/vAUC_\infty \quad (6)$$

where $vAUC_\infty$ is the AUC of VPA from time zero to infinity.

Data are presented as mean±s.d. Results were tested by analysis of variance. Tukey's test was further used to determine significant differences between two groups. The level of significance was set at $P < 0.05$.

Results

The plasma concentration–time profiles of VPA and VPAG are shown in Figure 1. Plasma VPAG and VPA concentration–time profiles were not parallel. The former revealed a trend of accumulation after high doses of VPA. In the 20 mg kg^{-1} dose group, the plasma VPAG showed a plateau at around 2.4 μg mL^{-1} for the initial 60 min, and then declined to undetectable levels (< 0.5 μg mL^{-1}) over the next 60 min (Figure 1B). In the 100 mg kg^{-1} dose group, the plasma VPAG remained at an almost steady level (over the range 7.3±8.2 to 12.5±3.5 μg mL^{-1}) up to the end of the experiment (360 min) (Figure 1B). In the 500 mg kg^{-1} dose group, the plasma VPAG increased slightly (from 30.0±7.9 to 46.2±27.6 μg mL^{-1}) in the initial 4 h, and then started to decline (Figure 1A). In the 600 mg kg^{-1} dose group, the plasma VPAG was elevated from 43.9±13.7 to 85.2±29.8 μg mL^{-1} throughout the experiment (240 min) against the decline of VPA (Figure 1A). Four out of five animals in this group (600 mg kg^{-1}) died

Table 1 Pharmacokinetic parameters of valproate in guinea-pigs.

Parameter	Valproate dose (mg kg^{-1})			
	20	100	500	600
Bodyweight (g)	250±12.3	249±19.2	265±21.9	258±6.5
Vd_{β} (mL kg^{-1})	261.4±29.9	397.9±39.1[a]	533.2±83.8[a,b]	493.7±84.5[a,b]
$K_{10}\times10^3$ (min^{-1})	16.6±4.9	9.0±0.8	4.6±1.3[a,b]	2.6±1.1[a,b]
AUC_{β} (min μg mL^{-1})	4859±2189	25233±2462[a]	213377±65375[a,b]	425791±55331[a,b]
$t_{1/2\beta}$ (min)	49.0±19.7	80.2±9.8	185.8±75.8[a,b]	273.4±32.4[a,b]
Co_{β} (μg mL^{-1})	67.7±8.0	220.8±21.0[a]	906.1±91.9[a,b]	1292.5±106.2[a,b]
CL_{β} (mL min^{-1} kg^{-1})	4.3±1.1	3.6±0.2	2.3±0.4[a]	1.3±0.2[a,b]
FD_{el} (%)	97±6.7	96±6.7	81±53.6	46±33.5[a,b]
$gAUC_t/vAUC_t$ (%)	7.5±3.1	10.0±0.6	8.3±2.7	8.0±3.6

Co_{β}, plasma concentration for β-phase at time zero. Data are mean±s.d. of five animals. [a]Significantly different ($P < 0.05$) compared with the 20 mg kg^{-1} dose group; [b]significantly different ($P < 0.05$) compared with the 100 mg kg^{-1} dose group.

Table 2 Biliary and urinary recovery (% of eliminated dose) of valproate and valproic acid glucuronide in guinea-pigs.

Recovery	Valproate dose (mg kg^{-1})			
	20	100	500	600
Biliary				
Valproate	2.4±2.2	2.8±1.3	4.5±2.7	8.0±2.9[a,b]
Valproic acid glucuronide	10.1±5.8[c]	16.8±12.7[c]	10.6±5.6[c]	7.1±4.9
Urinary				
Valproate	0.5±0.4	1.0±0.6	1.5±1.1[a]	2.8±1.5[a,b]
Valproic acid glucuronide	19.7±17.2[c]	13.5±4.0[c]	13.8±9.6[c]	10.4±3.8[c]

Data are mean±s.d. of five animals. [a]Significantly different ($P < 0.05$) compared with the 20 mg kg^{-1} dose group; [b]significantly different ($P < 0.05$) compared with the 100 mg kg^{-1} dose group; [c]significantly different ($P < 0.05$) compared with the recovery of valproate within the same group.

Table 3 Clearance (mL min^{-1}) of valproate and valproic acid glucuronide in guinea-pigs.

Clearance	Valproate dose (mg kg^{-1})			
	20	100	500	600
Valproate				
Plasma	1.1±0.2	0.9±0.0	0.6±0.2[a,b]	0.3±0.0[a,b]
Biliary	< 0.04	< 0.04	< 0.04	< 0.04
Urinary	< 0.04	< 0.04	< 0.04	< 0.04
Valproic acid glucuronide				
Plasma	6.7±3.3	3.1±0.4[a]	1.3±0.5[a,b]	0.9±0.4[a,b]
Biliary	1.8±0.9	1.9±1.6	1.0±1.0	0.3±0.3[a,b,c]
Urinary	3.2±2.0	1.4±0.4[a]	1.4±1.0[a]	1.1±0.9[a]

Data are mean±s.d. of five determinations. [a]Significantly different ($P < 0.05$) compared with the 20 mg kg^{-1} dose group; [b]significantly different ($P < 0.05$) compared with the 100 mg kg^{-1} dose group; [c]significantly different ($P < 0.05$) compared with the 500 mg kg^{-1} group.

before the sampling time of 360 min. The plasma elimination (K_{10}) and clearance (CL_β) of VPA significantly decreased, and elimination half-life ($t_{1/2\beta}$) and volume of distribution (Vd_β) significantly increased, with the increase in dose (Table 1). The AUC ratio of VPAG to VPA was not significantly different among the four groups (Table 1).

The urinary recovery of VPAG was significantly greater than that of unchanged VPA in all groups, as was the biliary recovery, except in the 600 mg kg^{-1} dose group (Table 2). Both biliary and urinary recovery of unchanged VPA, in terms of eliminated dose fraction, increased (biliary: from 2.4 to 8.0%; urinary: from 0.5 to 2.8%) with the increase in dose (Table 2). However, the vCL_b (0.02 to 0.04 mL min^{-1}) and the vCL_u (0.01 mL min^{-1}) were very low and were not significantly altered with different doses (Table 3). For VPAG, the biliary (10.1 → 7.1%) and the urinary (19.7 → 10.4%) recovery (Table 2) were not significantly altered, but the gCL_p (6.7 → 0.9 mL min^{-1}), gCL_b (1.8 → 0.3 mL min^{-1}) and gCL_u (3.2 → 1.1 mL min^{-1}) (Table 3) were significantly decreased with the increase in VPA dose.

Discussion

Our previous studies showed that the distribution phase of VPA is less than 10 min ($t_{1/2\alpha}$ 0.3–2.4 min), and the difference between total AUC and AUC_β, or total CL and CL_β, is less than 5% (Yu et al 1985, 1987). In order to minimize blood loss, the blood sampling for the distribution phase was omitted. The dose-dependent pharmacokinetics of VPA observed in the present experiment are in agreement with our previous study (Yu et al 1987). The pharmacokinetics of VPA are non-linear; the elimination rate decreases with an increase in the administered dose. The elimination rate of VPA could be adequately described by a biphasic Michaelis-Menten equation with two sets of parameters (V_{max1}, K_{m1} and V_{max2}, K_{m2}) (Yu et al 1993). The primary set, which describes glucuronidation rate, is non-linear within the therapeutic concentration range (K_{m1} value corresponding to approx. 78 μg mL^{-1} plasma level; Yu & Shen 1992), and approaches saturation when plasma concentrations achieve therapeutic upper levels (approx. 156 μg mL^{-1}) (Yu et al 1993; Yu & Shen 1996). The secondary set is linear over a very wide concentration range (K_{m2} = 11.7 μmol mL^{-1} = 1678 μg mL^{-1}). The apparent non-linear metabolic rate of VPA is mainly contributed to by the non-linear glucuronidation (Yu et al 1993; Yu & Shen 1996). The plasma concentration–time profile of VPA obtained from a single therapeutic dose (20 mg kg^{-1}) is in the linear glucuronidation region ($C_p < K_{m1}$), and that from a single dose of 100 mg kg^{-1} covers the non-linear region ($C_p \approx K_{m1}$), but below metabolic saturation of glucuronidation. This plasma concentration range is within the therapeutic level. In order to maintain metabolic saturation levels throughout the experimental period with a single dose, a very high dose of VPA (600 mg kg^{-1}) was used. However, this dose had to be reduced because most of the treated animals died within 360 min. A dose of 500 mg kg^{-1} VPA was found to be feasible.

Theoretically, the saturated metabolic production rate (V_{max}) of VPAG and the decreased elimination rate of VPA with high doses of VPA should result in a decreased gAUC/vAUC ratio. Nevertheless, the gAUC/vAUC ratio did not significantly decrease with high doses of VPA, which indicated a comparative increase in plasma VPAG. The increase in plasma VPAG with high doses of VPA may be attributed to its decreased elimination since the production of VPAG is constant (V_{max}) according to the Michaelis-Menten equation. This implies that the gCL_p is concentration-dependent and saturable. In the 100 mg kg^{-1} dose group, the plasma VPA in the terminal phase was far below metabolic saturation, and the formation rate of VPAG should decrease with the decline of plasma VPA. However, plasma VPAG did not decline with the decline of plasma VPA (even below the K_m value), but remained at an almost steady level. This suggested that the plasma elimination of VPAG might be slower than its formation. In the 600 mg kg^{-1} dose group, the plasma VPA throughout the experiment was high enough to attain V_{max}, and consequently the formation rate of VPAG should be constant ($= V_{max}$). However, a gradual elevation of plasma VPAG was observed, which demonstrated an accumulation of plasma VPAG, again supporting the hypothesis of concentration-dependent decrease or saturable elimination of plasma VPAG.

In the group receiving 600 mg kg^{-1} VPA, the sole surviving guinea-pig after 360 min showed a decrease of plasma VPA (653 → 521 μg mL^{-1}) and an increase of plasma VPAG (95 → 140 μg mL^{-1}) during the period of 240–360 min. This implied that in this group the plasma VPAG might be increasing further against the decrease of plasma VPA during this period. The guinea-pigs in this group died before 360 min, at which time the plasma VPA was declining ($< 605.1 \pm 92.9$ μg mL^{-1}), but VPAG was increasing to more than double that in the 500 mg kg^{-1} dose group ($> 85.2 \pm 29.9$ vs $< 43.2 \pm 24.2$ μg mL^{-1}). Thus, the high concentration of VPAG may be responsible, in part, for the death of the animals. Evidence of drug toxicity with the

accumulation of VPAG has been reported (Eadie et al 1988). For several xenobiotics, the glucuronide is more toxic than the parent compound (Osborne et al 1988). For example, the glucuronide of ethinylestradiol is cholestatic (Vore et al 1991), the glucuronide of zomepirac is immunogenic (Smith et al 1990), and suprofen acyl glucuronide covalently binds to kidney tissue (Smith & Liu 1995). VPAG is an acyl glucuronide. Acyl glucuronides are labile and may spontaneously break down (Watt et al 1991). VPAG undergoes systemic hydrolysis (Dickinson et al 1986). Its contribution to enterohepatic recycling is apparent (Dickinson et al 1979; Yu & Shen 1991; Yu 1993) and should also be considered.

The disproportional increase of VPA recovery in bile and urine with dose (Table 2) may be attributable to the increase of fu. The fu of VPA in-vivo could increase from 25 to 86% (3.5-fold) (Yu et al 1993) as the plasma VPA increased from 17 to 808 μg mL^{-1} (the averaged plasma concentration of VPA for the 20 and the 600 mg kg^{-1} dose groups, respectively). The increment of biliary and urinary recovery (dose percent) of VPA with dose is comparable with the increment of fu. Free VPA can traverse membranes by passive diffusion (Booth et al 1996).

At doses of 500 or 600 mg kg^{-1} VPA, the Cu of VPA greatly exceeds the K_m value so that the formation rate of VPAG should be limited to V_{max} regardless of the increasing dose. Thus, the production of metabolic VPAG would almost not increase with VPA dose, and subsequently, the dose percent of VPAG in bile and urine should be decreased. A trend of decreased urinary recovery of VPAG with an increase in VPA dose was observed, although it was not statistically significant owing to large standard deviations (Table 2). Nevertheless, the gCL_b and gCL_u significantly decreased with the high dose of VPA (Table 3). Saturable biliary excretion of VPAG was observed in our previous study (Yu et al 1993). Canalicular excretion of VPAG could be changed from a formation to an elimination rate-limited process in rats pretreated with phenobarbital (Booth et al 1996). It is possible that the biliary excretion of VPAG may be formation rate-limited at low plasma levels, but elimination rate-limited at high plasma levels. The pattern of dose-dependent biliary recovery of VPAG (Table 2) agrees with this hypothesis. At low plasma VPA (< 100 μg mL^{-1}), the biliary excretion of VPAG might be formation rate-limited, which increased with dose, whereas at high plasma VPA, the biliary excretion of VPAG might shift to elimination rate-limited, which decreased relative to VPA dose.

The glucuronidation reaction occurs mainly in the liver, but it may also occur in kidney. Glucuronidation of pranoprofen in mouse kidney (Arima 1990) and of morphine in human kidney (Pacifici & Rane 1982; Mazoit et al 1990) have been reported. The higher gCL_u than gCL_p in the 500 and 600 mg kg^{-1} dose groups (Table 3), suggests the possibility of VPA-glucuronidation in kidney, which might be minor at low levels of plasma VPA, but more obvious after saturation of hepatic glucuronidation.

The present study is the first report providing the dose-dependent plasma concentration–time profiles of VPAG and evidence of its dose-dependent elimination. Glucuronidation metabolism of VPA in kidney is speculated. High doses of VPA result in the accumulation of plasma VPAG, which may exert toxic effects. This becomes clinically important when the elimination of VPAG is impaired.

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