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Pharmacokinetics of Valproic Acid in Guinea-pigs with Biliary Abnormality

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Abstract—To explore whether biliary cannulation, biliary obstruction or gall bladder obstruction could alter the disposition of valproic acid, guinea-pigs were subjected to common bile duct cannulation or ligation, gall bladder-neck ligation or sham surgery as a control. They were then given an intravenous (i.v.) dose of sodium valproate (50 mg kg^{-1}) and the pharmacokinetics of valproic acid in each group were compared. In the cannulated group, significant decreases ($P < 0.05$) in the area under the elimination curve (AUC), the volume of distribution at steady-state (Vd_{ss}) and the mean residence time (MRT) were observed. Significant increases ($P < 0.05$) in the elimination rate constant (k_z) and total clearance (CL_{tot}) of valproic acid were noted. In the biliary obstructed guinea-pigs, the Vd_{ss} was significantly decreased ($P < 0.05$). In the gall bladder obstructed guinea-pigs, there was a secondary peak of valproate plasma concentration, and the k_z was significantly decreased. The biliary excretion of unchanged and conjugated valproic acid was 2.0 ± 0.7 (s.e.m.) and 19.7 ± 3.6 (s.e.m.) % of dose, respectively, and was almost completely reabsorbed in the enterohepatic recycling. Urinary excretion of unchanged and conjugated valproic acid, as well as non-conjugate metabolic clearance of valproic acid, were not significantly different among the four groups. The results suggest that the pharmacokinetics of valproic acid in guinea-pigs are particularly sensitive to interruption of the enterohepatic cycle. Biliary obstruction may elevate plasma concentrations owing to the decreased Vd_{ss} of valproic acid. Gall bladder obstruction may cause fluctuation of valproate plasma concentrations. The data indicate that the apparent total clearance of valproic acid is significantly less than the intrinsic clearance owing to enterohepatic recycling.

Valproic acid is an anticonvulsant (Wu et al 1984; Rall & Schleifer 1985) which undergoes enterohepatic recycling in rats (Dickinson et al 1979). Conjugation with glucuronic acid is a major metabolic route for the drug (Granneman et al 1984; Dickinson et al 1989). Other pathways include hydroxylation, dehydrogenation and isomerization. Some of the conjugated metabolite is excreted in the bile into the intestine, where the conjugate is hydrolysed and the liberated valproic acid is reabsorbed (Dickinson et al 1985). There are reports on the pharmacokinetics of other drugs subject to enterohepatic recycling (Pedersen & Miller 1980a, b; Ichikawa et al 1986) and several classic compartment models incorporating biliary transport have been developed (Colburn 1982, 1984; Semmes & Shen 1990). However, the effect of biliary abnormality on enterohepatic recycling of drugs has been rarely studied. The effects of phenytoin, phenobarbitone and carbamazepine on enterohepatic circulation of valproic acid in rats has been reported (Ogiso et al 1989). Biliary obstruction enhances the nephrotoxicity of gentamicin (Lucena et al 1989) and alters drug disposition of theophylline (Fruncillo et al 1982) and iopanoate (Cooke et al 1975) in rats. We believed that biliary abnormality may alter the pharmacokinetics of valproic acid. During long-term maintenance therapy for epilepsy, the disposition of valproic acid may be altered by biliary or gall bladder obstruction. Studies of enterohepatic recycling and the influence of biliary or gall-bladder obstruction on the drug disposition of valproic acid should provide some basis for interpretation of the observed plasma disposition in man. Guinea-pigs were used in these experiments because the physiology of their enterohepatic system is similar to that of man.

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Materials and Methods

Chemicals

Sodium valproate was a gift from Labaz (France). All other reagents, of analytical grade, were purchased from E. Merck (Germany).

Animal preparations

Male guinea-pigs, ca 250 g (Experimental Animal Center, National Taiwan University), were anaesthetized by ether inhalation. The jugular vein and the carotid artery were cannulated with PE-50 tubing (Clay Adams) which was passed subcutaneously to the animal's back, then exited between the scapulae. The urethra was also cannulated with PE-50 tubing. Guinea-pigs were divided into four groups, each of which had undergone an additional specific procedure: 1) sham operation (control), 2) common bile duct cannulation, 3) common bile duct ligation, or 4) gall bladder-neck ligation. A bolus dose of sodium valproate (50 mg kg^{-1} body weight), injected through the jugular vein cannula was followed by 0.3 mL of 0.9% NaCl (saline). The moment of saline flushing was designated as zero time. Blood specimens were collected from the carotid artery cannula at specified times. Plasma was separated by centrifugation (Model KM-15200, Kuboda, Japan), and the drug concentration was determined the same day. Urine was collected from 0 to 4, 4 to 8 and 8 to 24 h. Bile samples were collected from the cannulated guinea-pigs. The volumes of bile and urine, and the concentrations of unchanged and conjugated valproic acid in the bile and urine were determined. Faeces were

collected and analysed for unchanged and conjugated valproic acid.

Analytical method

The concentration of valproic acid was determined by gas chromatography (Yu 1981). In brief, a 0·1 mL portion of plasma or bile, or a 1 mL portion of urine was extracted with 0·1 mL of chloroform containing the internal standard (octanoic acid) after addition of 0·05 mL 3 M sulphuric acid. One μL of the chloroform layer was injected into the gas chromatograph (HP 5840A) which was equipped with a flame ionization detector and a column of 10% FFAP on Chromosorb-W AW-DMCS 80–100 mesh. Nitrogen carrier gas was used; oven temperature was maintained at 190°C.

Conjugated valproic acid was isolated by the method of Dickinson et al (1979) with slight modification. Briefly, the aqueous layer remaining after removal of free valproic acid was extracted once again with 0·1 mL of chloroform to assure complete removal of free valproic acid. An aliquot of the aqueous layer was made alkaline with 2·5 M sodium hydroxide, and placed in a shaking water bath at 80–85°C for 30 min. After cooling, the hydrolysed solution was acidified with 3 M sulphuric acid followed by chloroform extraction. One μL of the chloroform extract was then taken for analysis of valproic acid by gas chromatography. Faeces were homogenized with saline, then analysed for unchanged and conjugated valproic acid as described above.

Serum bilirubin was determined by a spectrophotometric method, using a reagent kit (Wako Chemicals, Japan).

Data analysis

The plasma valproate concentration data were analysed by the non-compartment method (Gibaldi 1984), applying the LAGRAN (Rocci & Jusco 1983) program, based on the following equations:

$$CL_{tot} = \text{dose}/AUC \quad (1)$$

$$Vd_{ss} = \text{Dose} * (AUMC)/(AUC)^2 \quad (2)$$

$$MRT = (AUMC)/(AUC) \quad (3)$$

where CL_{tot} is the total body clearance, AUC is the area under the plasma elimination curve, Vd_{ss} is the volume of distribution at steady state, AUMC is the area under the first moment curve and MRT is the mean residence time. The AUC up to the last sampling time was determined by using the trapezoidal method; the residual area beyond the last sampling time was estimated as C_t/k_z, where C_t is the concentration at the last sampling and k_z is the slope of the terminal linear phase calculated by linear regression analysis for plasma concentration data.

The urinary clearance (CL_r) or the biliary clearance (CL_b) of the unchanged and the conjugated valproic acid was calculated by dividing the amount of the drug in the total urine or bile sample by the $AUC_{0\to\infty}$ value. The metabolic clearance (CL_m) other than by conjugation was calculated by subtracting the CL_r and the CL_b from the CL_{tot}.

All means are presented with their standard error (mean ± s.e.m.). All data were compared with the control group by the unpaired *t*-test using $P < 0.05$ as statistically significant.

Results

The plasma concentration-time profiles of valproic acid in guinea-pigs undergoing different biliary treatments are shown in Fig. 1. The valproate concentration in the 24 h plasma samples of the control, the cannulated and the biliary obstructed guinea-pigs was less than 0·2 μg mL^{-1}. In the cannulated group the plasma level of valproic acid showed a monophasic decline and was undetectable after 6 h. In the other three groups the concentration of the drug in the 10 h plasma sample was still measurable. In the gall bladder obstructed guinea-pigs a secondary peak appeared at 8 h after valproate dosing, and the 10 h plasma concentration was significantly higher ($P < 0.05$) compared with the corresponding sample in the other groups. The pharmacokinetic parameters of valproic acid for each group are shown in Table 1.

The urinary excretion and the urinary clearance (CL_r) of valproic acid are shown in Tables 1 and 2. Neither the unchanged nor the conjugated valproic acid in the urine of any group was significantly different from that of the control group. In the collected urine about 20% of the dose was conjugated, and 1% was unchanged, valproic acid. Metabolic clearance (CL_m) of valproic acid was not significantly different among other groups (Table 2).

Biliary excretion of the unchanged and the conjugated valproate from the cannulated guinea-pigs were 2·0 ± 0·7 and 19·7 ± 7·8 (s.e.m.) % of dose, respectively. Biliary clearance of valproic acid is also shown in Table 2.

The total bilirubin was 0·4 ± 0·1 and 0·7 ± 0·2 s.e.m. mg dL^{-1} in the control and the biliary obstructed groups, respectively. The slight increase in bilirubin in the biliary obstructed group failed to reach statistical significance.

Neither unchanged nor conjugated valproic acid was detectable in the collected faeces.

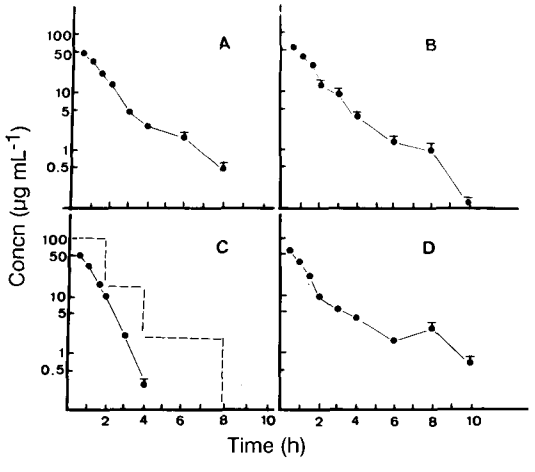

FIG. 1. Plasma concentration-time profiles of valproic acid following a 50 mg kg^{-1} i.v. dose. Each point with vertical bar represents the mean and s.e.m. of data from 5 guinea-pigs. A: control; B: with common bile duct ligation; C: with bile exteriorization; D: with gall bladder-neck ligation. The horizontal line in C represents the total concentrations of conjugated and unchanged valproic acid excreted in the bile.

Table 1. Pharmacokinetic parameters of valproic acid in guinea-pigs.

	Control	Biliary cannulated	Biliary obstruction	Gall bladder obstruction
AUC (μg h mL^{-1})	114·9 (3·77)	98·2 (2·93)*	130·0 (13·87)	147·3 (18·9)
AUMC (μg h^2 mL^{-1})	134·4 (11·2)	69·4 (4·2)*	157·6 (37·5)	428·4 (155·6)
CL_{tot} (mL h^{-1} kg^{-1})	379·0 (11·24)	443·1 (12·93)*	353·8 (47·07)	319·0 (40·4)
k_z (h^{-1})	0·41 (0·01)	1·42 (0·03)*	1·28 (0·27)	0·18 (0·01)*
$t_{\frac{1}{2}}$ (h)	1·69 (0·06)	0·49 (0·01)*	1·01 (0·25)	3·95 (0·31)
Vd_{ss} (mL kg^{-1})	438·4 (6·84)	293·7 (5·92)*	356·2 (24·17)*	699·6 (134·1)
MRT (h)	1·2 (0·06)	0·7 (0·02)*	1·1 (0·18)	2·6 (0·70)
Body wt (g)	241 (7·8)	243 (6·6)	236 (9·9)	239 (12·3)
Serum albumin (g dL^{-1})	3·30 (0·16)	3·28 (0·05)	3·18 (0·12)	3·31 (0·11)
Urinary excretion (% dose)				
Valproic acid				
0–4 h	1·2 (0·06)	1·9 (0·70)	0·9 (0·56)	0·8 (0·22)
4–8 h	0·3 (0·08)	0·6 (0·20)	0·1 (0·08)	0·2 (0·10)
8–24 h	0·1 (0·02)	0·1 (0·04)	0·1 (0·12)	0·1 (0·02)
Cumulative (0–24 h)	0·5 (0·12)	2·6 (0·66)	1·0 (0·52)	1·1 (0·26)
Conjugate				
0–4 h	13·2 (3·02)	12·1 (7·42)	14·5 (4·56)	14·5 (4·56)
4–8 h	3·9 (0·98)	6·1 (3·26)	3·6 (3·06)	1·7 (0·46)
8–24 h	2·2 (0·62)	1·6 (0·74)	1·4 (0·96)	1·1 (0·24)
Cumulative (0–24 h)	19·3 (4·20)	19·5 (7·30)	15·2 (3·90)	17·1 (0·90)

Data are means (s.e.m.) of five determinations. * $P < 0.05$; significantly different from the control.

Table 2. Renal and metabolic clearances of valproic acid in guinea-pigs.

	Control	Biliary cannulated	Biliary obstruction	Gall bladder obstruction
mL h^{-1} kg^{-1}				
CL_r (valproic acid)	5·3 (0·5)	11·2 (2·4)	2·5 (0·7)	3·4 (1·4)
CL_r (conjugate)	72·3 (15·3)	85·1 (15·3)	39·7 (12·6)	55·3 (16·4)
CL_m	300·8 (21·0)	262·2 (43·5)	298·8 (63·1)	239·6 (32·4)
CL_b (valproic acid)	—	8·0 (2·9)	—	—
CL_b (conjugate)	—	78·3 (12·3)	—	—

Data are means (s.e.m.) of five determinations.

Discussion

From a physiological point of view, guinea-pigs—with a gall bladder in the enterohepatic system—are preferable to rats as models for studying enterohepatic cycling of drugs and extrapolation of the results to man. A secondary valproate plasma peak owing to enterohepatic cycling is typical in rats (Dickinson et al 1979), but it was observed only in the gall bladder-obstructed guinea-pigs. The differences in the response profiles with respect to the presence or absence of a secondary peak owing to enterohepatic cycling after an i.v. dose depend on the time lag of reabsorption (Steimer et al 1982), as well as the relative magnitude of the reabsorption and elimination rate constants (Pederson & Miller 1980a, b; Steimer et al 1982). In the gall bladder-obstructed guinea-pigs, the secondary valproate plasma peak appeared to be due to a higher reabsorption rate than plasma elimination rate since the biliary excretion of the drug was directly to the intestine for reabsorption. In the control guinea-pigs, the biliary excreted drug was initially stored in the gall bladder and diluted by the bile in the gall bladder, with the reabsorption subsequent to the discharge of bile from the gall bladder. A slow transfer of valproate from plasma via bile to intestine, competing with elimination and distribution, resulted in an insignificant secondary peak.

There was a time lag for reabsorption of valproate in the enterohepatic cycling (Fig. 1): this represents the time required for successive steps of metabolic conjugation, biliary excretion, deconjugation in the gut and finally reabsorption of the parent drug. When a time lag was present, the pharmacokinetics after i.v. injection were insensitive to modifications in reabsorption until the lag time had elapsed (Steimer et al 1982). Influence of reabsorption was only apparent in the transient and terminal phase of the pharmacokinetic profile (Steimer et al 1982). The plasma elimination profiles up to 2 h were not significantly different among the four groups (Table 3).

The mechanism for the gastrointestinal absorption of valproic acid has not been reported. It most likely occurs through passive diffusion which depends on a concentration gradient. Glucuronide conjugate of valproic acid in bile was reabsorbed from the intestine mostly as free valproic acid after hydrolysis (Dickinson et al 1985). The concentration of total valproate (unchanged and conjugated) in the bile was greater than the valproate plasma concentration (Fig. 1C). This is a necessary condition for reabsorption by a diffusion

Table 3. Pharmacokinetic parameters of valproic acid in guinea-pigs estimated from the first two hour-plasma elimination profile.

Parameters	Control	Biliary cannulated	Biliary obstruction	Gall bladder obstruction
Vd_{ss} (mL kg^{-1})	637 (30)	602 (33)	625 (95)	522 (43)
k_{10} (h^{-1})	0·83 (0·03)	1·01 (0·04)	0·77 (0·09)	1·06 (0·03)
AUC (μg h mL^{-1})	92 (3·1)	83·1 (4·3)	112·7 (15·34)	97·6 (5·33)
$t_{\frac{1}{2}}$ (h)	0·8 (0·04)	0·7 (0·03)	0·9 (0·12)	0·7 (0·02)
C_o (μg mL^{-1})	75·9 (1·8)	83·8 (4·96)	82·3 (8·23)	92·5 (2·24)
CL (mL h^{-1} kg^{-1})	537 (35)	610 (40)	485 (102)	547 (45)

Data are means (s.e.m.) of five determinations.

mechanism; reabsorption would not occur unless the plasma level declined below the intestinal concentration. This may be another possible factor for the lag time of enterohepatic recycling.

The fraction of reabsorption (f_a) of a drug which is excreted through the bile into the gut can be estimated by the equation: $f_a = (1 - AUC/AUC^*)/f_b$ (Tse et al 1982) where AUC is a value for the bile-exteriorized animals, AUC* is a value for the bile-intact animals, and f_b is a fraction of the dose present in the bile. An estimate of f_a can be made by applying the appropriate values in Table 1: $f_a = 0{\cdot}7$ (control) and 1·5 (gall bladder obstructed). However, for a more precise estimate, a specific experimental design is necessary.

The significant decrease of Vd_{ss} in the biliary obstructed guinea-pigs was in agreement with the simulated results of an enterohepatic recycling model (Colburn 1982). Restriction of valproate distribution into bile and gut should reduce its Vd_{ss}. Although less than 2% of the unchanged valproic acid was found in the collected bile, biliary excretion of valproic acid into the primary bile might be much more than that found in the collected bile. This would be because valproic acid is a small organic anion and part of the biliary excreted drug might have been reabsorbed as the bile passed along the biliary tract (Clark et al 1971). The effect of biliary obstruction on theophylline was quite different in that the Vd_{ss} was unchanged but the CL_{tot} was significantly decreased (Fruncillo et al 1982). Biliary or gall bladder obstruction did not alter the AUC and the CL_{tot} of valproic acid, but showed a different shape and peak height in the plasma elimination curve from that of the control. The bile duct ligation can increase the biliary pressure, cause haemodynamic disturbance of liver perfusion, and decrease the biliary secretion rate of a drug, which might be followed by an increase in drug plasma concentration (Fleck & Braunlich 1984). The apparent AUC of valproic acid in guinea-pigs, resulting from biliary obstruction, was comparable with that from enterohepatic recycling, but the mechanisms for such results were different. However, interruption of the enterohepatic recycling by exteriorizing the bile flow significantly altered the pharmacokinetic parameters of valproic acid (Table 1).

Biliary obstruction can increase total bilirubin in plasma (Fruncillo et al 1982) which may compete with drugs for plasma protein binding and thus influence the pharmacokinetics of highly plasma protein bound drugs. However, in this study the slight increase of bilirubin in the biliary obstructed guinea-pigs would not affect the pharmacokinetics of valproic acid (Yu et al 1985).

The values of CL_{tot} obtained from the cannulated group represented the effective total clearance. In the control guinea-pigs, the biliary clearance was masked by reabsorption of the biliary excreted drug, and thus its apparent clearance represented the net clearance. Subtraction of the biliary clearance from the CL_{tot} for the cannulated group ($443{\cdot}1 - 86{\cdot}2 = 356{\cdot}9$ mL h^{-1} kg^{-1}) gave a value which is comparable with the apparent total clearance, i.e. net clearance of the control group (379·0 mL h^{-1} kg^{-1}). This result suggests that the apparent hepatic extraction ratio of valproic acid, usually estimated from apparent total clearance, was under-estimated since the effective total clearance is significantly higher than the apparent total clearance.

The proportion of the dose recovered in urine of guinea-pigs as valproate glucuronide is comparable with that in patients (Dickinson et al 1989).

In conclusion, enterohepatic cycling of valproic acid has been demonstrated in guinea-pigs. The pharmacokinetics of the drug are particularly sensitive to interruption of reabsorption of biliary excreted drug. Care should be paid to the possibility in clinical cases that enterohepatic recycling may be interrupted by decrease or inhibition of reabsorption resulting from the oral ingestion of ion exchange resins or adsorbants, or changes in the intestinal contents and microflora that decrease the hydrolysis of conjugated valproic acid. Gall bladder or common bile obstructions showed little influence on valproate elimination. The apparent hepatic clearance may not represent the effective hepatic activity when enterohepatic cycling is involved.

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