The Disposition of Valproyl Glycinamide and Valproyl Glycine in Rats

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Purpose. To investigate the disposition of valproyl glycinamide and valproyl glycine in rats and to compare it with that of valproic acid (VPA) and valpromide which were studied previously.

Methods. The study was carried out by monitoring the brain and liver levels of valproyl glycinamide and valproyl glycine (as a function of time after iv dosing) in addition to the regular pharmacokinetic (PK) monitoring of plasma and urine levels of these compounds.

Results. The following PK parameters were obtained for valproyl glycinamide and valproyl glycine, respectively: clearance, 7.1 and 16 ml/min/kg; volume of distribution (Vss), 0.78 and 0.41 l/kg; half-life, 1.1 and 0.37 h; and mean residence time, 1.8 and 0.4 h. The ratios of AUCs of valproyl glycinamide of liver to plasma and brain to plasma were 0.70 and 0.66, respectively. The ratios of the AUCs of valproyl glycine of liver to plasma and brain to plasma were 0.19 and 0.02, respectively.

Conclusions. Valproyl glycinamide distributes better in the brain than VPA, a fact which may contribute to its better anticonvulsant activity. Valproyl glycine was barely distributed in the brain, a fact which may explain its lack of anticonvulsant activity. In addition to the liver, the brain was found to be a minor metabolic site of the biotransformation of valproyl glycinamide to valproyl glycine.

KEY WORDS: valproyl glycinamide; valproyl glycine; pharmacokinetics; brain and liver distribution.

INTRODUCTION

Valproyl glycinamide (I—Fig. 1) was developed from structure-pharmacokinetic-pharmacodynamic relationship studies of a series of N-valproyl derivatives of GABA and glycine (1). The anticonvulsant activity of the drug was evaluated in the classical rodent models in collaboration with the NIH Epilepsy Branch. Following i.p. administration to mice, the drug had an ED_{50} of 151 mg/kg (maximal electroshock-MES test) and 131 mg/kg (subcutaneous metrazol-sc Met test), and its TD_{50} (minimal neurotoxicity), as assessed by the rotorod ataxia test, was 369 mg/kg (1). Following oral administration to rats, its ED_{50} in the MES test was 73 mg/kg and its TD_{50} was greater than 1000 mg/kg. Valproyl glycinamide was also effective in blocking seizures in corneal-kindled rats (ED_{50} = 162 mg/kg, i.p. administration). These data indicated that valproyl glycinamide has an anticonvulsant profile different from that of valproic acid (VPA) (1). Pharmacokinetic studies in seven dogs showed that valproyl glycinamide has a mean (±SD) clearance (CL) value of 2.9 ± 0.8 L/h, a volume of distribution (V_{ss}) of 12 ± 3.2 1, a half life ($t_{1/2}$) of 2.7 ± 0.5 h and oral availability (F) of 95 ± 34%. No detectable metabolic cleavage of valproyl glycinamide to VPA was observed in dogs. Less than 10% of the drug was excreted unchanged in the urine. Valproyl glycinamide was mainly biotransformed to an active metabolite valproyl glycine (II—Fig. 1), with a mean fraction metabolized (fm) of about 50%.

In clinical phase I studies, valproyl glycinamide was given in escalating doses of up to 4 g to different groups of eight (six on drug and 2 on placebo) healthy volunteers. Valproyl glycinamide (50 mg to 4 g) was considered to be safe and well tolerated (2). There were no clinically significant changes in laboratory safety, vital signs, ECG or neurological examination data during the study. Following oral administration of 1 g or 4 g of valproyl glycinamide to healthy subjects the pharmacokinetics of valproyl glycinamide was dose-independent with a mean $t_{1/2}$ of about 7 h and a mean oral clearance of (CL/F) of approximately 6.5 L/h. Overall, valproyl glycinamide seems to be a safe and promising new AED which acts as a drug on its own and not as a prodrug of VPA.

The purpose of the present study is to investigate the disposition of valproyl glycinamide and valproyl glycine in rats and to compare it with that of valproic acid (VPA) and valpromide (VPD) which was studied previously (3). This was done by monitoring brain and liver levels of both compounds (as a function of time after dosing), in addition to the regular PK monitoring of plasma and urine levels. Monitoring of valproyl glycinamide and valproyl glycine levels in the brain and liver (both as tissue homogenates and as organs taken out as a function of time from intact rats) will enable us to determine if the brain is capable of biotransforming valproyl glycinamide to valproyl glycine, or whether the liver is the primary metabolic site of both compounds. Brain-level monitoring will show whether the better anticonvulsant activity of valproyl glycinamide relative to VPA and the lack of anticonvulsant activity of valproyl glycine may be because of the better brain partitioning and PK profile of valproyl glycinamide or due to its better pharmacodynamic activity.

MATERIALS AND METHODS

Chemicals

Valproyl glycinamide, valproyl glycine and valproic acid were supplied by Teva Pharmaceutical Industries, Israel. All solvents used were analytical grade, and all chemicals were reagent grade.

Homogenate Studies

Liver and brain from male Sabra rats weighing 260–280 g were isolated under light ether anesthesia. Homogenates were obtained by homogenizing 1 g of tissue with 9 ml of phosphate-saline buffer [0.1 N (pH = 7.4)]. To each homogenate, valproyl

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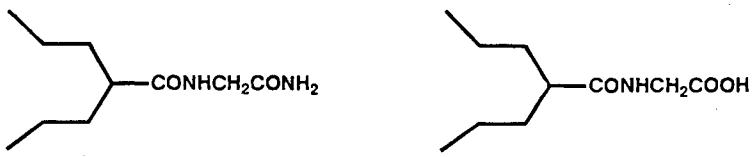

Valproyl glycinamide-(I) Valproyl glycine-(II)

Fig. 1. Chemical structures of valproyl glycinamide (I) and valproyl glycine (II).

glycinamide or valproyl glycine was added to create an initial concentration of 40 mg/l homogenate. The mixtures were placed in a shaker in a hot bath adjusted to 37 ± 2°C. Samples (0.5 ml) of the reaction mixture were taken at 10, 20, 30, 45, 60, 90, 120, 180, 300 and 720 min after the beginning of the incubation; 0.25 ml of 0.1 N HCl solution was added to stop the reaction, and the samples were kept at −20°C until analysis.

Pharmacokinetic (PK) Studies

Male Sabra rats weighing 260–280 g were divided into groups of five which were placed in different cages. The rats were kept in the laboratory 3 days for acclimatization, and food and water were provided ad libitum. At the beginning of the experiment, each rat was injected intravenously (dorsal penile vein) with valproyl glycinamide or valproyl glycine (27.8 mg/rat or 103 mg/kg) dissolved in 0.4 ml of propylene glycol-ethanol-saline mixture (6:3:1). All pharmacokinetic studies adhered to the "Principles of Laboratory Animal Care". Blood samples (8 ml) were collected from the ascending vena cava of an individual rat at 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 480, 600 and 720 min after dosing. Food was withheld after dosing, and water was supplied ad libitum. Five rats were sacrificed for each of the aforementioned time measurements, and the plasma or tissue levels obtained from each rat at each time point were averaged. Plasma was immediately separated by centrifugation at 3000 g for 15 min and stored at −20°C until analyzed. Brain and liver were quickly removed at the same time intervals under light ether anesthesia; the tissues were immersed in liquid nitrogen for a few minutes and kept at −70°C until analyzed. Ten rats were kept in metabolic cages, and the cumulative urine was collected from each group (five rats) 360 and 720 min after dosing. Urine, brain and liver levels of valproyl glycinamide and plasma and urine levels of valproyl glycine were assayed by GC assay. Plasma levels of valproyl glycinamide and brain or liver levels of valproyl glycine were determined by a HPLC assay. VPA plasma levels were assayed by a GC assay (3).

GC Assay for Valproyl Glycinamide and Valproyl Glycine

Rat urine (1 ml) or tissue (1 g) was spiked with known amounts of valproyl glycinamide or valproyl glycine and N-dimethyl valproyl glycinamide (internal standard). After short mixing, 0.5 ml of HCl 1 N was added and the mixture was extracted with 4 ml of $CHCl_3$ which was separated and kept at −20°C. The $CHCl_3$ was evaporated under nitrogen, the dry residue was dissolved in 100 μl of $CHCl_3$ and 1 μl was injected in to GC apparatus. GC Conditions: Temp: 180°C Inj; 250°C; Det. 300°C; column 5% biphenyl on polysiloxan. The concentration range for the calibration curve of valproyl glycinamide was 0.5 to 120 mg/L (urine) and 1 to 80 mg/kg (tissue). The interday coefficient of variation (%CV) among replicates ranged from 2.6 to 11% (urine) and 4.1 to 12% (tissue) with 20% at lowest limit of quantification (LOQ) of 0.5 mg/l (urine) and 23% at an LOQ of 1 mg/kg (tissue). This accuracy ranged from 2.3 to 12% (urine) and 4.3 to 15% (tissue) with an accuracy of 20% (urine) and 23% (tissue) at the LOQ. The concentration range for valproyl glycine in plasma or urine was 5 to 100 mg/L (plasma and urine) and the LOQ was 3 mg/L. Higher concentrations were diluted 1:1. The %CV was 2.2–9.1% with 19.6% at the LOQ. The accuracy ranged 2.4 to 11% with an accuracy of 11.6% at the LOQ. The recovery of valproyl glycinamide from urine was 90% and that from tissue was 78%. The recovery from plasma and urine of valproyl glycine was 70%.

HPLC Assay for Valproyl Glycinamide in Plasma

To 0.5 ml of plasma, 20 μl of internal standard solution (N-valproyl nipectoamide, 1 mg/ml in acetonitrile), and 200 μl of phosphoric acid 0.1 N were added. To the above mixture 4 ml of tert. butyl methyl ether was added followed by vortex for 30 seconds. The mixture was centrifuged for 10 min at 3000 g and the organic phase was separated and evaporated (using a vortex evaporator) to dryness. To the dry residue 120 μl of acetonitrile was added, and 20 μl was injected into the HPLC apparatus at a wavelength of 220 nm. HPLC Conditions: Column-RP-18 reverse phase column equipped with a precolumn. Mobile phase: acetonitrile 45%, bidistilled water 55%; and trifluoroacetic acid (TFA) 0.1%. The %CV and accuracy for the HPLC assay was similar to that of the above GC urine assay for valproyl glycinamide.

HPLC Assay of Valproyl Glycine in Liver or Brain Tissue

One gram of tissue (brain or liver) was homogenized with 3 ml of NaOH 0.1 N, the mixture was extracted with 4 ml of $CHCl_3$ and the organic phase was discarded. After centrifugation (10 min at 3000 g), 0.5 ml of $HClO_4$ 1 N was added to the dry residue and the mixture was extracted again with 4 ml of $CHCl_3$, separated by centrifugation (10 min at 3000 g) and evaporated to dryness. The residue was dissolved in 40 μl methanol and 20 μl was injected into HPLC. HPLC conditions and mobile phase were as described above. The concentration range of valproyl glycine was 1 to 60 mg/kg. The interday %CV ranged from 2.6–11.8 mg/kg, with 21% at the LOQ. The accuracy ranged from 2.1–14.5% with an accuracy of 22 at the LOQ. The recovery from brain and liver tissue was 86%.

Data Analysis

The linear terminal slope (β) of ln C (valproyl glycinamide or valproyl glycine plasma, liver or brain concentration) vs. t

(time) was calculated by the method of least squares. The terminal half-life of each compound was calculated from the quotient: (0.69)/(terminal slope). The AUC was calculated by using the trapezoidal rule with extrapolation to infinity by dividing the last concentration by the terminal slope (4). The clearance (CL) of valproyl glycinamide or valproyl glycine was calculated from the quotient of the intravenous dose and the plasma AUC. Vβ was calculated from the ratio of the clearance and the plasma linear terminal slope. The volume of distribution at steady state (Vss) and the mean residence time (MRT) were calculated by using classical methods (4–7).

The fraction of CL of valproyl glycinamide that furnished the metabolite, valproyl glycine (fm) was calculated by using eq. 1 (8), whereas $(AUCm)_D$ is the AUC of valproyl glycine obtained as a metabolite of valproyl glycinamide after intravenous administration of valproyl glycinamide, and AUCm is the AUC of its valproyl glycine obtained after its intravenous administration, D and Dm are the intravenous doses of valproyl glycinamide and valproyl glycine, and CL and CL(m) are the clearance of valproyl glycinamide and valproyl glycine, respectively. The metabolic intrinsic clearance (CLint) of valproyl glycinamide was calculated by using eq. 2 where fu is the unbound fraction (fu) in plasma (9). The fraction excreted unchanged in the urine (fe) of valproyl glycinamide or valproyl glycine was calculated from the quotient of the cumulative amount excreted intact in the urine (U) and the dose (D). Previous studies showed that the fu of valproyl glycinamide is 39% and the fraction of the dose of valproyl glycinamide excreted in the urine as galproyl glycine (Mu) was calculated for the quotient Mu and D.

$$fm = \frac{(AUCm)_D}{D} \cdot \frac{Dm}{AUCm} = \frac{(AUCm)_D}{AUC} \cdot \frac{CL(m)}{CL} \quad (1)$$

$$CLint = \frac{CL(1 - fe)}{fu(1 - E)} \quad (2)$$

The blood/plasma concentration ratio (10) of valproyl glycinamide and valproyl glycine and VPA (partition study) was taken from previous studies (1).

The liver extraction ratio (E) of valproyl glycinamide was calculated from the quotient of (blood) clearance and hepatic blood flow (Q). Valproyl glycinamide is evenly distributed between blood and plasma (1) therefore its blood CL is equal to its plasma CL (10). Rat liver blood flow was taken from literature data (11).

RESULTS

Figures 2–4 show the levels of valproyl glycinamide, valproyl glycine and valproyl glycine as a metabolite of valproyl glycinamide in the plasma, liver and brain, respectively, after their intravenous administration (103 mg/kg) to rats. Each data point in Figs. 2–4 represents a mean value obtained from five rats at each time measurement. The % CV among five replicates of the investigated compounds in the plasma, brain and liver ranged from 4–14% at all measured concentrations. The plasma and tissue levels of valproyl glycinamide and valproyl glycine declined in a biphasic manner and a biotransformation of valproyl glycinamide to valproyl glycine was observed. The metabolite valproyl glycine appeared within 15 min in the plasma at its maximal concentration of 66 mg/l. Although no valproyl glycine levels could be detected 2.5 h after its dosing, plasma levels of valproyl glycinamide were detected and quantified up to 8 h after dosing. Table I depicts the PK parameters of the two compounds. In the liver and brain peak levels of valproyl glycinamide and valproyl glycine (as a preadministered drug and as a metabolite of valproyl glycinamide) were obtained rather quickly (15–30 min after dosing). The mean liver levels of valproyl glycinamide and valproyl glycine were higher than their respective brain levels. The volume of 1 g of brain or liver homogenates was 1 ml, giving a specific gravity of about unity. Thus, brain and liver concentrations expressed in mg/kg could be compared with plasma concentrations expressed in mg/l. Analysis of liver homogenates showed that about 30% of valproyl glycinamide was biotransformed to valproyl glycine after 5 h of incubation. A similar analysis of brain homogenate showed that about 8% of valproyl glycinamide was biotransformed to valproyl glycine after 12 h of incubation. This may suggest that although the liver is the major metabolic site of the valproyl glycinamide—valproyl glycine biotransformation, the brain is an additional site for this metabolic process. No VPA was found in homogenate analysis of valproyl glycinamide and valproyl glycine. Following i.v. administration of valproyl glycinamide to rats only very low VPA plasma levels of 0.5 to 1 mg/l were detected.

DISCUSSION

In rats, as in dogs, valproyl glycinamide and valproyl glycine have different PK parameters. In comparison to valproyl glycinamide, valproyl glycine has a larger CL and smaller Vss (or Vβ) values. Therefore, its $t_{1/2}$ and MRT values were 3–4 times shorter than the respective values of valproyl glycinamide. Valproyl glycine was mainly excreted unchanged in the urine (fe = 85 ± 25%). The fe value of valproyl glycinamide was only 13 ± 2%, which is similar to the value obtained in dogs and humans. fm calculations, using plasma data (eq. 1) showed that, in rats, valproyl glycinamide is mainly and almost completely eliminated by biotransformation to valproyl glycine. The fraction of the valproyl glycinamide dose which was excreted in the urine as valproyl glycine was 25 ± 5%, indicating that valproyl glycine which is formed in the liver as a metabolite of valproyl glycine undergoes subsequent hepatic metabolism. However, when valproyl glycine is given intravenously it is mainly excreted unchanged due to its poor partitioning into liver, as it is mainly ionized at physiological pH.

As the liver is the major metabolic site of the biotransformation of valproyl glycinamide to valproyl glycine, and the metabolic (blood) clearance of valproyl glycinamide is only 11% of the rat liver blood flow (6.2 ml/min/kg) it can be concluded that the hepatic metabolism of valproyl glycinamide has low E (extraction ratio) value. Therefore, valproyl glycinamide will not undergo liver first-pass effect upon oral administration to rats and its metabolic clearance is similar to its intrinsic clearance (CL_mint).

The ratio of AUCs of valproyl glycinamide between liver to plasma and brain to plasma was 0.70 and 0.66, respectively. The ratio of the AUCs of valproyl glycine between liver to plasma and brain to plasma was 0.19 and 0.02, respectively, indicating that valproyl glycine is distributed in the liver and brain to a much lesser extent than valproyl glycinamide. The

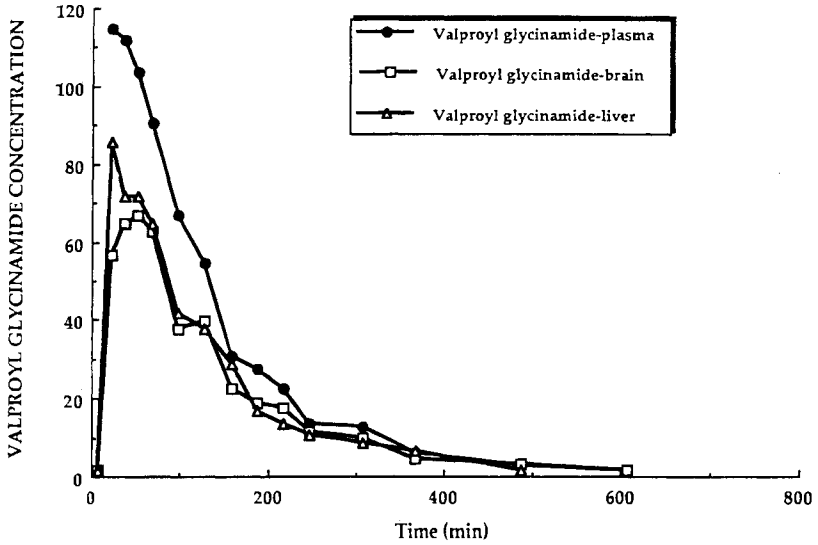

Fig. 2. Plasma (mg/l), brain (mg/kg) and liver (mg/kg) concentrations of valproyl glycinamide obtained after intravenous administration (103 mg/kg) to rats.

AUCs ratio of valproyl glycine as a metabolite of valproyl glycinamide between liver and plasma and brain to plasma was 1.06 and 0.20, respectively. Thus, valproyl glycine, as a metabolite of valproyl glycinamide, was better partitioned into the brain and the liver than as a preadministered (iv) compound.

These data show that valproyl glycinamide was readily distributed in liver and brain with a similar brain to plasma and liver to plasma partitioning, whereas the distribution of valproyl glycine as a preadministered drug in these organs was quite restrictive. The better partitioning of valproyl glycine as a valproyl glycinamide metabolite in liver and brain may be caused by a biotransformation of valproyl glycinamide to valproyl glycine. A possible different non-specific binding in brain tissue between valproyl glycinamide and valproyl glycine may also explain the different brain distribution of these two analogous compounds. Thus, the metabolism of valproyl glycinamide to valproyl glycine occurs primarily in the liver but may occur in the brain as also reflected by the homogenate studies. In the case of milecamide (12), for example the brain is capable of metabolically converting a glycinamide derivative (N-pentyl glycinamide) to its corresponding glycine analogue. However, unlike milecemide, which is an amine with a pentyl moiety, neither valproyl glycinamide nor valproyl glycine (which are amides with iso-octanoyl moeity) were cleaved of their alkyl moiety to form VPA.

The brain to plasma concentration ratio of valproyl glycinamide was better than that of VPA, although VPA had better partitioning than valproyl glycine (3). Valproyl glycinamide distributes better into the brain than VPA, a fact that may contribute to its better anticonvulsant activity. The primary amide of VPA, VPD, and its isomer valnoctamide (VCD) were evenly distributed among the liver, brain and plasma (3) and thus showed better partitioning into brain and liver than valproyl glycinamide. As valproyl glycinamide was readily distributed among the plasma, brain and liver, similar CL, Vss and $t_{1/2}$ values were calculated from the plasma and tissue data of this

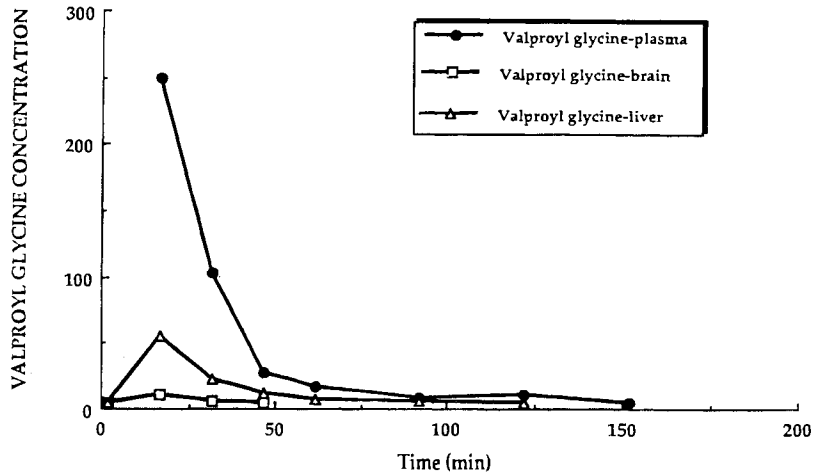

Fig. 3. Plasma (mg/l), brain (mg/kg) and liver (mg/kg) concentrations of valproyl glycine obtained after intravenous administration (103 mg/kg) to rats.

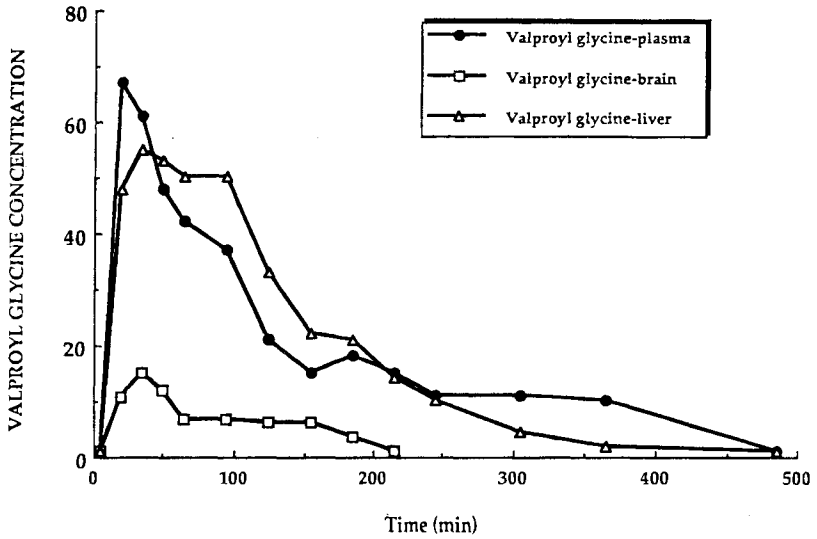

Fig. 4. Plasma (mg/l), of brain (mg/kg) and liver (mg/kg) concentrations of valproyl glycine obtained as a metabolite after intravenous administration (103 mg/kg) of valproyl glycinamide to rats.

compound. This shows that valproyl glycinamide was instantaneously distributed in the brain and liver which are therefore included within the central PK compartment. However, in the case of valproyl glycine, a discrepancy was found between the PK parameters calculated from the plasma, liver or brain data, caused by limited distribution of this compound into brain and liver.

Table I. Pharmacokinetics (PK) of Valproyl Glycinamide and Valproyl Glycine in the Plasma, Liver, Brain, and Urine After Their Intravenous Administration (103 mg/kg) to Rats

	Valproyl glycinamide			Valproyl glycine		
PK Parameters	Plasma	Liver	Brain	Plasma	Liver	Brain
t1/2 (h)	1.1	1.4	1.3	0.37	0.21	
CL (ml/min/kg)	7.1			16		
AUC (mg/l.h)	241	169[a]	158[a]	106[a]	20[a]	1.9
Vss (l/kg)	0.78			0.41		
Vβ (l/kg)	0.69			0.44		
MRT (h)	1.8	2.0	2.1	0.4	0.4	
E (%)	11					
CL_mint (ml/min/kg)	17.7					
Cmax (mg/l)	113	84[b]	65[b]	245	51[b]	5.6[b]
tmax (h)		0.25	0.75		0.25	0.25
fe (%)	13 ± 2			85 ± 25		

Valproyl glycine after Valproyl glycinamide			
t1/2 (h)	1.4	0.83	1.5
AUC (mg/l.hr)	129	137	26
Cmax (mg/l)	66	54	14
tmax (h)	0.25	0.5	0.5
MRT (h)	2.1	1.8	2.3
fm (%)	100		
Mu/D[c](%)	25 ± 5		

[a] AUC is expressed as mg/kg h.
[b] Cmax is expressed as mg/kg.
[c] Fraction of the dose excreted in the urine as valproyl glycine.

In summary, valproyl glycine was barely distributed into the brain a fact which may explain its lack of anticonvulsant activity. The brain was found to be a minor metabolic site, in addition to the liver, of the valproyl glycinamide to valproyl glycine biotransformation. In rats, as in dogs, the biotransformation of valproyl glycinamide or valproyl glycine to VPA is negligible. This paper shows that glycinamide derivatives (1,12,13) have better potential than their corresponding glycine analogues to become new antiepileptics and CNS drugs due to their better brain distribution.

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