Pharmaceutical Research, Vol. 9, No. 8, 1992

Pharmacokinetic Analysis of the Structural Requirements for Forming "Stable" Analogues of Valpromide

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Received December 2, 1991; accepted January 30, 1992

The following valpromide (VPD) analogues were synthesized and their structure–pharmacokinetic relationships explored: 3-ethyl pentanamide (EPD), methylneopentylacetamide (MND), 1-methyl cyclohexanecarboxamide (MCD), cycloheptanecarboxamide (CHD), and t-butylacetamide (TBD). Two aliphatic (EPD and MND) and two cyclic amides (MCD and CHD) underwent complete or partial conversion to their corresponding acids. The only amide found in this study to be "stable" to the amide–acid biotransformation was TBD. It also had the lowest clearance and the longest half-life and mean residence time. Unlike the other investigated amides, TBD contained two substitutions of two methyl moieties at the β position of its chemical structure. A "stable" valpromide analogue must have either two substitutions at the β position, such as in the case of TBD, or a substitution in the α and β positions, such as in the case of the VPD isomer valnoctamide (VCD). This paper discusses the antiepileptic potential of stable VPD analogues which may be more potent and less teratogenic than their biotransformed isomers.

KEY WORDS: valpromide; stable analogues; pharmacokinetics; structural requirements.

INTRODUCTION

Valproic acid (VPA; I) is one of the four major antiepileptic agents. Although the use of VPA in the treatment of epilepsy has grown during recent years, major side effects are still associated with its use. The most serious of these are teratogenicity and hepatotoxicity (1).

Two major problems still exist with current antiepileptic therapy. First, there are only four major drugs (AEDS) in the antiepileptic arsenal: phenobarbital, phenytoin, carbamazepine, and valproic acid (2). Second, about 25% of the epileptic patients are still not seizure-free, and many patients suffer from medication toxicity under the existing AEDS (3). A way of overcoming the above-mentioned problem is by developing improved and better derivatives of the established AEDS.

Keane *et al.* (4), Loscher and Nau (5), and Abbott and Acheampong (6) demonstrated that, within a large series of branched monocarboxylic acids, VPA possessed the optimal chemical structure with regard to the margin between its anticonvulsant activity and its sedative and hypnotic side effects. Loscher and Nau also showed that valpromide (VDP; II) was the most potent compound in a series of 32 analogues and metabolites of VPA (5).

We recently studied the structure–pharmacokinetic relationship in a series of short fatty acid amides with potential antiepileptic activity (7,8), including aliphatic isomers and analogues of VPD, which is the primary amide of VPA (9). The following conclusions were drawn from these studies: (a) all amides were more active as anticonvulsants than their respective acids, and (b) the anticonvulsant activity was affected by the pharmacokinetics in general and by the biotransformation of the amide to the respective acid in particular. An amide which did not undergo hydrolytic metabolism to its acid was more active than its biotransformed isomer or analogue.

Substitution in the β position of the aliphatic amides strongly affected the relationships between the chemical structure and the pharmacokinetic-pharmacodynamic characteristics. An amide with a substituted β position was resistant to hydrolytic metabolism and, therefore, more potent than the labile analogue. Thus, a β-substituted isomer of valpromide (valnoctamide; VCD; III) was found to be stable in dogs (10) and humans (11); VCD was also more potent and possessed a better safety margin than VPD (7,8).

In order to assess the structural requirements for a stable VPD analogue, we investigated the structure pharmacokinetic relationships in a new series of five VPD analogues. The following aliphatic and cyclic amides were synthesized, and their pharmacokinetics was investigated in dogs: 3-ethylpentanamide (EPD; IV), methylneopentylacetamide (in a racemic form, MND; V), 1-methyl-cyclohexanecarboxamide (MCD; VI), cycloheptanecarboxamide (CHD; VII), and t-butylacetamide (TBD; VII). The chemical structures of these amides, and those of VPA, VPD, and VCD, are shown in Fig. 1. In previous studies the corresponding acid of the amide MCD showed anticonvulsant activity similar to that of valproic acid (5,6).

MATERIALS AND METHODS

Materials

The acids 1-methylcyclohexanoic acid (MCA), cycloheptanoic acid (CHA), and t-butyl acetic acid (TBA) were purchased from the Aldrich Chemical Company, Milwaukee, Wisconsin, and their corresponding amides prepared by reacting the acid chloride derivative with aqueous ammonia. The acids, 3-ethylpentanoic acid (EPA) and methylneopentyl acetic acid (MNA), were synthesized by means of the classical method of a condensation between diethylmalonate and the appropriate alkyl halide. The alkylated diethylmalonate was hydrolyzed and the free acid decarboxylated by heating to 150–180°C, until CO_2 production ceased. The acids, MNA and EPA, thus obtained were converted to their primary amides, EPD and MND, respectively, by reacting their acid chloride derivatives with ammonia. The chemical structures of the five investigated amides were confirmed by nuclear magnetic resonance (NMR) and elemental microanalysis.

Animals

The experiments were carried out in six dogs (mongrels), three males and three females, ranging in weight between 18 and 23 kg. The experiment with MND was carried out in four dogs, as two died from causes unrelated to the

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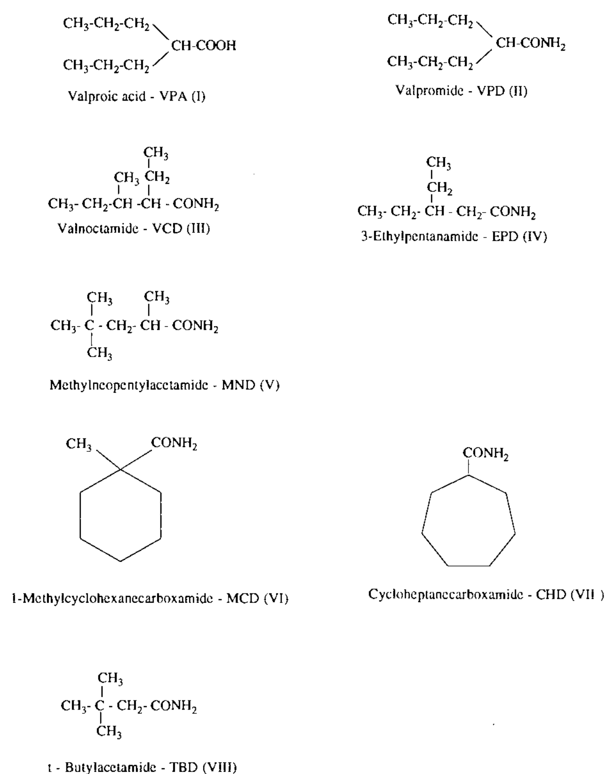

Fig. 1. Chemical structures of valproic acid, valpromide, and the valpromide analogues discussed in the paper.

study. In a randomized crossover design, each dog was injected intravenously with 400 mg (in 1.5 ml 70% alcohol) of the amide (using one of the cephalic veins). In cases where an amide was biotransformed into its homologous acid, the acid itself was also administered (iv, 400 mg). Urine was collected for 12 hr from all dogs by means of an indwelling catheter.

Protocol

Venous blood samples (5 ml) were collected via an indwelling catheter (the other cephalic vein) at specified intervals following injection (0, 5, 10, 15, 20, 30, 40, and 50 min and 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hr). The plasma was then immediately separated by centrifugation at 7000 rpm for 15 min and stored at −20°C. Before each assay, the plasma was allowed to reach room temperature, vortexed, and centrifuged and the residual clot removed. Plasma and urine levels of the amide and its homologous acid were then assayed by gas-liquid chromatography (GLC) using an assay which we have already reported upon for the determination of VPD and VCD (12,13).

As the acids of compounds IV to VIII were considered, a priori, to be potential metabolites of the amides, they were also synthesized. In preliminary studies, we verified the fact that, in our GLC assay, the acids can also be detected and monitored simultaneously with the appropriate amide.

Pharmacokinetic Analysis

The linear terminal slope (β) of log C (amide or acid plasma concentration) versus t (time) was calculated by the method of least squares. The terminal half-life of the compound ($t_{1/2}\beta$) was calculated from the quotient 0.69/terminal slope. The AUC (area under the C versus the t curve) was calculated using the trapezoidal rule with extrapolation to infinity, by dividing the last experimental plasma concentration by the terminal slope (14).

The total-body clearance (CL) of the amides was calculated using the quotient of the i.v. dose (D) and the AUC. The volume of distribution (V_β) was calculated using the quotient of the clearance and the linear terminal slope. The volume of distribution at steady state (V_{ss}) and the mean residence time (MRT) were calculated using Eqs. (1) and (2) (15–17).

$$V_{ss} = \frac{D\ \mathrm{AUMC}}{(\mathrm{AUC})^2} \quad (1)$$

$$\mathrm{MRT} = \frac{\mathrm{AUMC}}{\mathrm{AUC}} \quad (2)$$

The AUMC is the area under the product of time (t) and the plasma drug concentration (C) versus time (t), from time 0 to infinity. The AUMC was calculated by the trapezoidal rule with extrapolation to infinity. The fraction of the amide metabolized (f_m) to its respective homologous acid was calculated using Eq. (3) (18,19), where $(\mathrm{AUC_m})_D$ is the AUC of the acid obtained as a metabolite of the amide following i.v. administration of the amide and $\mathrm{AUC_m}$ is the AUC of the acid obtained following administration of the acid to the same animal which previously received the parent amide. D and D_m are the i.v. doses and CL and CL(m) are the clearances of the amide and acid, respectively.

$$f_m = \frac{(\mathrm{AUC_m})_D}{D} \Big/ \frac{\mathrm{AUC_m}}{D_m} = \frac{(\mathrm{AUC_m})_D}{\mathrm{AUC}} \cdot \frac{\mathrm{CL(m)}}{\mathrm{CL}} \quad (3)$$

Partition, Stability, Water Solubility, and Protein Binding Studies

The blood–plasma concentration ratio (20) of the amides (partition study) was carried out at room temperature by spiking known amounts of the amides in seven samples of fresh blood taken from a dog prior to drug administration. The amides' concentration ranged from 3 to 20 mg/L. Each blood sample was centrifuged immediately after spiking and the separation of the plasma was carried out according to the procedure mentioned above. Plasma levels of the amides were determined by GLC.

A blood stability study of the amides was carried out by incubating 400 μg of each compound in 30 ml of dog blood (placed in heparinized test tubes) at 37°C with continuous shaking. Blood samples (2 ml) were then collected at the following times: 0, 0.5, 1, 2, 3, 4, 5, 6, and 7 hr. Plasma was immediately separated and the amide concentration in the plasma assayed by GLC.

Protein binding of the amides was evaluated using the ultrafiltration method. This was carried out in four amide plasma samples at drug concentrations of 5, 10, 15, and 20 mg/L. The amide levels in the filtrate (plasma water) were assayed by GLC. The free fraction (f_u) of the amides was calculated from the quotient of the drug concentration in the filtrate to the initial drug concentration in the plasma. The

water solubility of each amide was determined by stirring 40 mg of the appropriate amide into 3 ml of distilled water for 2 hr. At the end of the 2-hr period, the sample was centrifuged and 3-μl aliquots were taken for GLC assay.

RESULTS

Figures 2 and 3, respectively, depict the mean plasma levels of the amides MCD (as a representative of the amides that were converted to their corresponding acids) and TBD (a "stable" amide). Table I summarizes their mean pharmacokinetic parameters compared to those of VCD. Of the five investigated valpromide analogues, TBD was the only amide which did not biotransform to its corresponding acid. Table I also shows the mean pharmacokinetic parameters (obtained following i.v. administration to the same dogs) of the acids found to be metabolites of their homologous amides. These acids were 3-ethylpentanoic acid (EPA), methylneopentyl acetic acid (MNA), 1-methylcyclohexanoic acid (MCA), and cycloheptanoic acid (CHA). The fraction of the amide metabolized (f_m) to its corresponding acid showed that EPD and MND were completely biotransformed to their homologous acids, while the cyclic analogues MCD and CHD were partially biotransformed, having a mean f_m value of 66 and 44%, respectively. Analyzing the urine showed that less than 1% of the administered dose of the amides was excreted unchanged.

Stability studies showed that, unlike EPD and TBD, which were stable, MND, MCD, and CHD were not stable in dog blood. The half-life of degradation of MND, MCD, and CHD was 3, 2, and 1.5 hr, respectively.

The data on the partition, plasma protein binding, and water solubility are summarized in Table II. The partition study indicated that MCD and CHD were taken up by the blood cells, while TBD, EPD, and CHD showed a near-even distribution between blood and plasma. The five VPD analogues were not bound extensively to plasma proteins and their free fraction (f_u) was constant throughout the investigated concentration range.

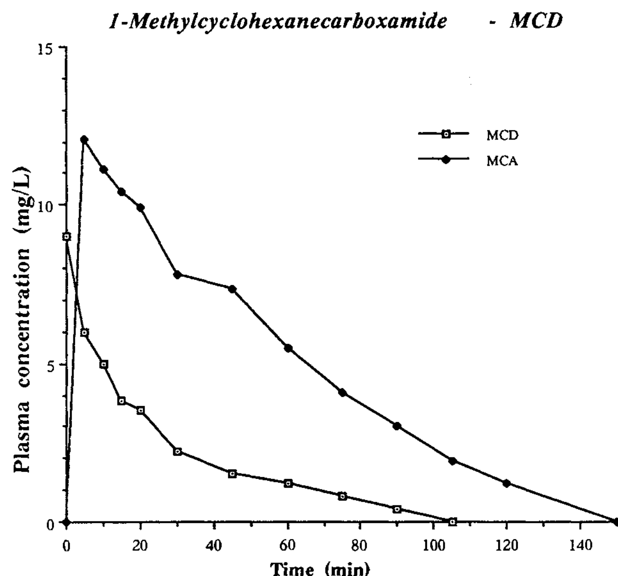

Fig. 2. Mean plasma levels of 1-methylcyclohexanecarboxamide (MCD) and 1-methylcyclohexanoic acid (MCA) following i.v. administration (400 mg) of MCD to six dogs.

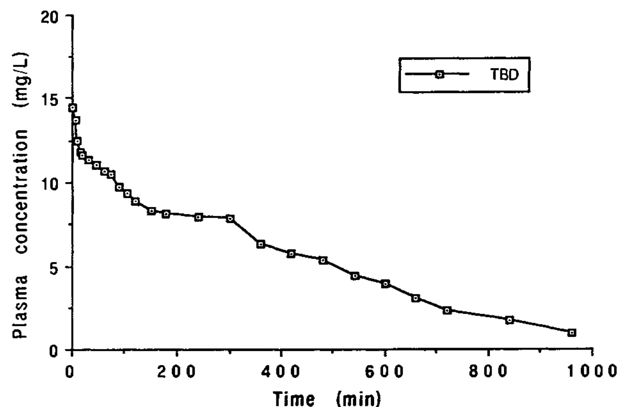

Fig. 3. Mean plasma levels of t-butylacetamide (TBD) following i.v. administration (400 mg) to six dogs.

DISCUSSION

The cyclic VPD analogues MCD and CHD and the aliphatic amide MND had large clearances, which, thus, caused short half-lives of less than 1 hr. The fact that these three amides were unstable in blood and that their biotransformation to their corresponding acids also occurs in blood contributed to their large clearance values. In these circumstances, the cyclic amides were cleared rapidly, similar to some of the aliphatic amides investigated previously (7,8) and, thus, did not show any pharmacokinetic advantage over the aliphatic amides. MCD possessed the largest volume of distribution, while the other four investigated amides possessed a mean volume of distribution ranging from 25 to 33 L. The amides had a larger volume of distribution and clearance than their corresponding acids, and therefore, the half-lives of the amide and its homologous acid were similar. MCD and CHD were rapidly eliminated but underwent only a partial conversion to their corresponding acid. It is plausible that the acid formed was not completely seen in the plasma due to a sequential first-pass effect (21).

Normalizing the plasma clearance (CL_p) by the blood to the plasma ratio of the different amides led to the calculation of the blood clearance (CL_b) (20,22). Dividing the blood (metabolic–hepatic) clearance by the mean dog hepatic blood flow of 560 ml/min (23) gave the following extraction ratios (E): EPD, 40%; and TBD, 11%. For the other three amides investigated calculation of the E values was not feasible, as they were unstable in blood and were therefore susceptible to multisite metabolism. The five amides investigated were not bound extensively to plasma protein, and therefore, protein binding did not play a major role in their disposition. The water solubilities of the investigated amides were all of the same order of magnitude (1.8 to 8.8 mg/ml; Table II).

Two aliphatic (EPD and MND) and two cyclic amides (MCD and CHD) underwent complete or partial conversion to their corresponding acids. The only "stable" analogue of VPD found in this study was TBD, which also had the lowest

Table I. Comparison of Mean Pharmacokinetic Parameters of EPD, EPA, MND, MNA, MCD, MCA, CHD, CHA, TBD, and VCD

	EPD	EPA	MND	MNA	MCD	MCA	CHD	CHA	TBD	VCD
β (hr^{-1})	0.9 ± 0.2	1.3 ± 0.3	1.8 ± 0.2	2.6 ± 0.3	1.0 ± 0.4	1.9 ± 0.4	2.3 ± 0.2	1.6 ± 0.4	0.2 ± 0.02	0.4 ± 0.1
$t_{1/2}\beta$ (hr)	1.0 ± 0.2	0.6 ± 0.2	0.4 ± 0.02	0.3 ± 0.03	0.8 ± 0.3	0.4 ± 0.1	0.3 ± 0.03	0.5 ± 0.1	3.4 ± 0.4	2 ± 0.5
AUC (mg/L · hr)	23 ± 3	36 ± 4	7 ± 2	11 ± 2	4 ± 0.4	21 ± 1	7 ± 1	17 ± 3	91 ± 13	92 ± 21
CL_p										
L/hr	18 ± 3	13 ± 4	63 ± 25	38 ± 7	101 ± 9	19 ± 1	59 ± 8	24 ± 3	4.5 ± 0.7	4.5 ± 0.5
ml/min	301 ± 43	189 ± 21	1046 ± 421	640 ± 112	1689 ± 153	315 ± 18	973 ± 126	393 ± 55	75 ± 11	75 ± 18
CL_b (ml/min)	213 ± 30	—	688 ± 276	—	345 ± 30		207 ± 27		63 ± 9	74 ± 18
V_β (L)	25 ± 5	9 ± 2	35 ± 13	15 ± 4	111 ± 43	10 ± 2	27 ± 5	16 ± 4	22 ± 4	12 ± 3
V_{ss} (L)	25 ± 5	11 ± 1	33 ± 12	16 ± 3	98 ± 32	13 ± 1	32 ± 4	18 ± 3	28 ± 5	13 ± 3
MRT (hr)	1.4 ± 0.2	1 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	1 ± 0.3	0.7 ± 0.1	0.6 ± 0.05	0.8 ± 0.1	6.3 ± 0.5	3 ± 1
f_m (%)	85 ± 12	—	118 ± 26	—	60 ± 8	—	44 ± 10	—	0	0
$t_{1/2}\beta$ acid (hr)	0.7 ± 0.1	—	0.5 ± 0.1	—	0.5 ± 0.1		0.6 ± 0.1			
MRT acid (hr)	1.3 ± 0.2	—	0.6 ± 0.03	—	0.9 ± 0.1		1 ± 0.1			
AUC acid (mg/L · hr)	30 ± 2	—	12 ± 2	—	13 ± 2		7 ± 1			
C_{max} acid (mg/L)	17 ± 2	—	29 ± 4	—	12 ± 2		7 ± 2			
t_{max} acid (hr)	0.3 ± 0.03	—	0.1 ± 0	—	0.1 ± 0.04		0.3 ± 0.1			

Table II. Partition, Protein Binding, and Water Solubility of the Five Amides Investigated

	C_b/C_p[a]	f_u (%)[b]	Water solubility (mg/ml)
EPD (I)	1.4 ± 0.1	42 ± 6	4.1
MND (V)	1.5 ± 0.1	66 ± 3	8.0
MCD (VI)	4.7 ± 0.5	60 ± 5	2.2
CHD (VII)	4.9 ± 0.4	65 ± 4	1.8
TBD (VIII)	1.2 ± 0.1	69 ± 7	8.8

[a] The blood plasma ratio (mean ± SD; $N = 7$).
[b] The free (unbound) fraction in plasma (mean ± SD; $N = 4$).

clearance and E value and the longest half-life and MRT value. Unlike the other investigated amides, TBD contained two substitutions of two methyl moieties at the β position of its chemical structure. The other amides had a free (unsubstituted) β position (MCD, CHD, and MND) or a single substitution at their β position (EPD). The fact that EPD was found not to be a stable amide is quite surprising, because previously (7,8) we postulated that in this series of VPD analogues, a substitution at the β position prevented the amide–acid biotransformation. Reevaluating our previous and current data leads to the conclusion that a β substitution alone is not sufficient to prevent the amide–acid biotransformation. A stable valpromide analogue must have either two substitutions at the β position, such as in the case of TBD, or a substitution in the α and β positions, such as in the case of VCD or other VPD isomers investigated previously, propylisopropylacetamide (PID) and diisopropylacetamide (DID) (7,8).

These stable VPD analogues or isomers have the potential to be more potent as anticonvulsants and less toxic than VPD or VPA. The greater potency will be due to the fact that these amides will act as drugs on their own, and not as prodrugs of their corresponding acid (such as VPD). The amides have a greater intrinsic activity, and as neutral compounds which are not extensively bound to plasma proteins, they can penetrate better into the brain than their corresponding acids. Unlike VPA, VPD is not teratogenic (in rodents). However, as VPD serves as a prodrug of VPA only in humans, because of this interspecies variability, the lack of teratogenecity in rodents does not hold in humans and, therefore, does not have any clinical implications. Thus, a stable VPD isomer such as VCD (11) has the potential of being less teratogenic than VPA. The lower toxicity is due to the fact that "stable" amides are less teratogenic than VPA (24).

Using the approach implied in this paper of structure–pharmacokinetic relationships, the second major side effect of VPA—the hepatotoxicity (25)—also may be minimized. VPA hepatotoxicity has been associated with a VPA metabolite (4-ene-VPA or delta-4-VPA) which has a terminal double-bond in its chemical structure (26,27). However, in the case of TBD, such a terminal olefin cannot be formed, as the ω-1 position is already substituted and the ω-1 carbon is a quaternary one.

This study shows that in this series of VPD analogues, minor changes in the chemical structure affect the pharmacokinetics in general and, in particular, the "stability" of the amide to amide–acid conversion. In order to become stable, aliphatic and cyclic analogues of VPD require substitutions at their α and β positions or two substitutions in their β position. A "stable" amide has the potential of having a higher anticonvulsant potency and fewer side effects than its biotransformed isomer.

ACKNOWLEDGMENTS

This work is abstracted in part from the doctoral dissertations of Dr. Abdulla Haj-Yehia and Mr. Salim Hadad, in partial fulfillment of the Ph.D. degree requirements of the Hebrew University of Jerusalem.

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