Pharmaceutical Research, Vol. 6, No. 8, 1989

Research Article

Structure–Pharmacokinetic Relationships in a Series of Valpromide Derivatives with Antiepileptic Activity

Abdulla Haj-Yehia[1] and Meir Bialer[1,2]

Received November 10, 1988; accepted March 8, 1989

The following valpromide (VPD) derivatives were synthesized and their structure–pharmacokinetic relationships explored: ethylbutylacetamide (EBD), methylpentylacetamide (MPD), propylisopropylacetamide (PID), and propylallylacetamide (PAD). In addition, the anticonvulsant activity of these compounds was evaluated and compared to that of VPD, valnoctamide (VCD), and valproic acid (VPA). MPD, the least-branched compound had the largest clearance and shortest half-life of all the amides investigated and was the least active. All other amides had similar pharmacokinetic parameters. Unlike the other amides, PID and VCD did not metabolize to their respective homologous acids and were the most active compounds. Our study showed that these amides need an unsubstituted β position in their aliphatic side chain in order to biotransform to their homologous acids. An amide which is not metabolized is more potent as an anticonvulsant than its biotransformed isomer. All amides were more active than their respective homologous acids. In this particular series of aliphatic amides, which were derived from short-branched fatty acids, the anticonvulsant activity was affected by the pharmacokinetics in general and by the biotransformation of the amide to its homologous acid in particular. This amide–acid biotransformation appeared to be dependent upon the chemical structure, especially upon the substitution at position β of the molecule.

KEY WORDS: valpromide; valproic acid; antiepileptic activity; SAR; pharmacokinetics.

Introduction

Valpromide or dipropylacetamide (VPD-I; Fig. 1), a primary amide of valproic acid, is widely used in several European countries, both as an antiepileptic and as an antipsychotic drug (1–3).

Previous reports (3–5) have shown that, upon oral administration to humans, valpromide was biotransformed to valproic acid (VPA-II; Fig. 1), a well-known antiepileptic agent (6), before reaching the systemic circulation. Pharmacokinetic analysis demonstrated that VPD is a prodrug of VPA (2–5,7,8) and that this may account for its antiepileptic activity.

Loscher and Nau (9) reported that among a series of VPA analogues tested in mice for anticonvulsant activity, VPD was found to be the most potent, being two to five times more potent than VPA. However, VPD also exerted a more significant sedative side effect.

Recent articles have reported that VPD also possesses specific properties of its own (unrelated to VPA), i.e., the induction of an elevation in the plasma levels of carbamazepine-10,11-epoxide, the active metabolite of carbamazepine (10–14).

Following i.v. administration, VPD was shown to be rapidly and almost completely metabolized to VPA in humans, with an f_m value of 80% (f_m = the metabolized fraction of VPD to VPA) (8). In dogs, VPD's biotransformation to VPA was only partial and was independent of the route of administration, the f_m being in the range of 30–40% (15,16).

Valnoctamide (VCD-III; Fig. 1; valmethamide or 2-ethyl-3-methylpentamide), an isomer of VPD, has also proven useful as a tranquilizer in the treatment of anxiety and tension (17–19). In a recent study in dogs, it was reported that VCD's major pharmacokinetic parameters were similar to those of VPD (20), the main difference being that VCD was not a prodrug of its homologous acid (valnoctic acid; VCA-IV; Fig. 1). This pharmacokinetic (or metabolic) difference may explain the different pharmacological properties of the two isomers. The extent of biotransformation of an aliphatic amide (such as VPD or VCD) to its homologous acid, therefore, appears to be a key issue in these compounds' pharmacological activity.

Another compound, similar to both VPD and VCD, is allylisopropylacetamide (AIA-V; Fig. 1). In contrast to VPD and VCD, which are used as drugs, AIA is defined as a "suicide substrate" (21,22). Despite the fact that VPD, VCD, and AIA are chemically similar, there are marked differences in their pharmacological properties.

Keane *et al.* (23) and Loscher and Nau (9) have demonstrated that within a large series of branched monocarboxylic acids, VPA had the optimal chemical structure with regard to margins between its anticonvulsant effect and its sedative/hypnotic side effects. Since pharmacokinetics plays a major role in the pharmacological activity of these ali-

[1] Department of Pharmacy, School of Pharmacy, Hebrew University of Jerusalem, POB 12065, Jerusalem 91120, Israel.
[2] To whom correspondence should be addressed.

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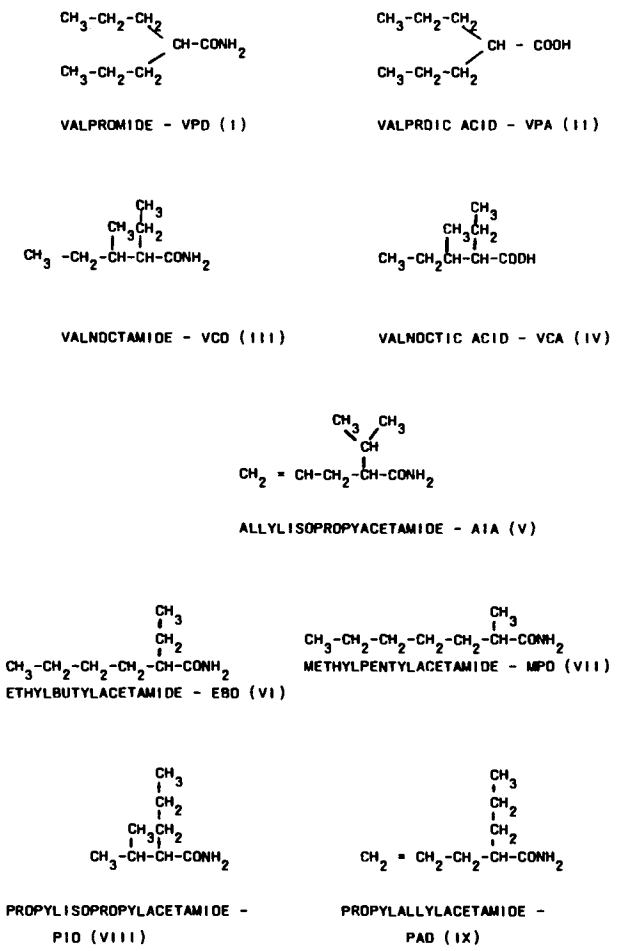

Fig. 1. Chemical structures of the different aliphatic amides and acids discussed in the paper.

phatic amides, we decided to explore the structure–pharmacokinetic relationships that may exist within a series of VPD (or VCD) isomers or derivatives which contain eight carbon atoms per molecule.

The following aliphatic branched-chain amides were synthesized and their pharmacokinetics investigated in dogs (following i.v. administration): ethylbutylacetamide (EBD-VI), methylpentylacetamide (MPD-VII), propylisopropylacetamide (PID-VIII), and propylallylacetamide (PAD-IX). The chemical structures of these amides are depicted in Fig. 1.

In order to evaluate whether any relationships exist among chemical structure, pharmacokinetics, and anticonvulsant activity in the above-mentioned compounds, we tested and evaluated the antiepileptic data of our compounds by using the anticonvulsant screening project of the NIH Epilepsy Branch (24).

MATERIALS AND METHODS

Materials

The amides (compounds VI to IX) and their homologous acids were synthesized by means of the classical method of a condensation between the diethylmalonate ester and the appropriate alkyl halide (all chemicals were purchased from Aldrich, Milwaukee, Wis.). The acids were then obtained by decarboxylation (heating to 150–180°C until the elaboration of all of the CO_2 stopped) of the condensation product and the amides (compounds VI to IX) by amidation of the acyl chloride with ammonia. The chemical structures were confirmed by nuclear magnetic resonance (NMR) and elementary microanalysis.

Animals

The experiments were carried out in six dogs (mongrels), three males and three females, ranging in weight between 18 and 23 kg. Although mice and rats are usually used for anticonvulsant screening (24), these animals are too small to be used in pharmacokinetic studies with a crossover design. In addition, the disposition of drugs in dogs has the potential of being more similar to that in humans than the disposition of the same drugs in rodents. In a randomized crossover design, each dog was injected intravenously with 400 mg (in 1.5 ml 70% alcohol) of the amide (into one of the cephalic veins). In cases where an amide was biotransformed into its homologous acid, the acid was also administered (i.v., 400 mg). Urine was collected systematically for 16 hr from all dogs by means of an indwelling catheter. A washout period of 3 weeks was allowed between any two consecutive studies.

Protocol

Venous blood samples (6 ml) were collected via an indwelling catheter (the other cephalic vein) at specified intervals following injection (0, 2, 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, and 10 hr, respectively). 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 levels of the amide and its homologous acid were assayed by gas–liquid chromatography (GLC), an assay which we have reported on for the determination of VPD and VCD (25,26).

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

Anticonvulsant Activity

The amides VPD (I), VCD (III), EBD (VI), MPD (VII), PID (VIII), and PAD (IX) and their respective homologous acids were screened in mice for their anticonvulsant activity by the NIH Epilepsy Branch (24). The screening procedure involved the following: (i) the maximal electroshock (MES) test, which measures seizure spread; (ii) the subcutaneous pentylenetetrazol test (s.c. Met. test), which measures seizure threshold; and (iii) the rotorod ataxia test, which assesses neurotoxicity.

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 by using the trapezoidal rule with extrapolation to infinity—by dividing the last experimental plasma concentration by the terminal slope (27).

The total body clearance (CL) of the amides was calculated by 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) (28–30).

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

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

The AUMC is the area under the product of time (t) and the plasma drug concentration (C) versus (t), from time zero to infinity. The AUMC was calculated by the trapezoidal rule with extrapolation to infinity. The fraction metabolized of the amide to its respective homologous acid (f_m) was calculated using Eq. (3) (31,32), where $(\text{AUC}_m)_D$ is the AUC of the acid obtained as a metabolite of the amide after i.v. administration of the amide and $(\text{AUC})_m$ is the AUC of the acid obtained after i.v. 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.

$$f_m = \frac{(\text{AUC}_m)_D}{D} : \frac{\text{AUC}_m}{D_m} = \frac{(\text{AUC}_m)_D}{\text{AUC}} \cdot \frac{\text{CL (m)}}{\text{CL}} \quad (3)$$

All of the pharmacokinetic parameters were calculated in a noncompartmental manner based on the statistical moment theory (30,33).

Partition, Stability, Water Solubility, and Protein Binding Studies

The blood–plasma concentration ratio (34,35) 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/liter. 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 20 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, 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 by using the ultrafiltration method. This was carried out in four amide plasma samples at drug concentrations of 5, 10, 15, and 20 mg/liter. The amides' levels in the filtrate (plasma water) were assayed by GLC. The free fraction (f_u) of the amides was calculated form 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 in 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

The mean plasma levels of the amides EBD, MPD, PID, and PAD are presented in Figs. 2–5, respectively and Table I summarizes their mean pharmacokinetic parameters as compared to those of VCD. Unlike EBD, MPD, and PAD, PID was not biotransformed to its homologous acid. Table I also shows the mean pharmacokinetic parameters (obtained after i.v. administration to the same dogs) of the acids found to be metabolites of their homologous amide. These acids were ethylbutyl acetic acid (EBA), methylpentyl acetic acid (MPA), and propylallyl acetic acid (PAA). The f_m (the fraction metabolized of the amide to its respective homologous acid) calculations showed that MPD and PAD were completely biotransformed to their homologous acids, while EBD was only partially biotransformed, having an f_m value

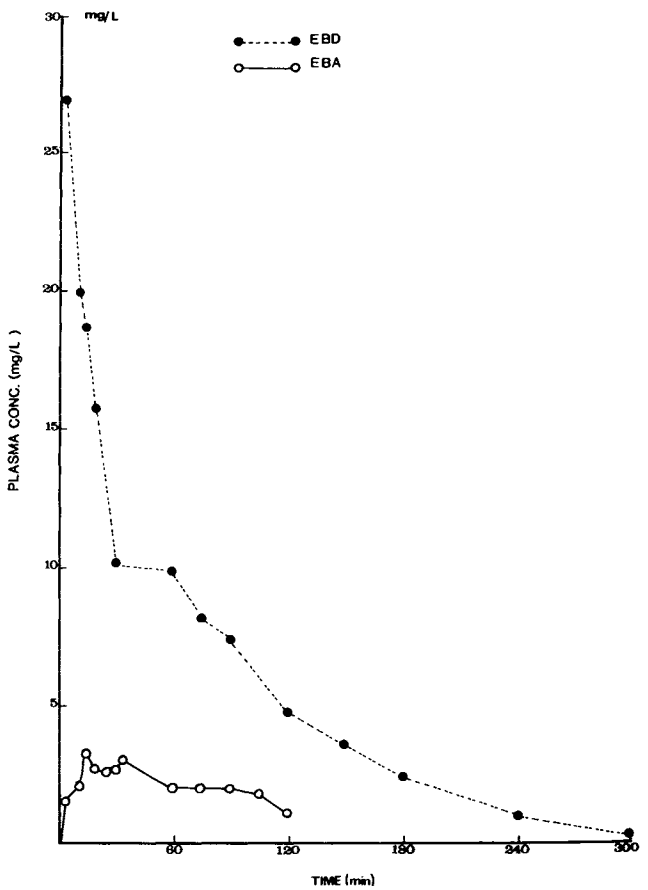

Fig. 2. Mean plasma levels of ethylbutylacetamide (EBD) and ethylbutyl acetic acid (EBA) following i.v. administration (400 mg) of EBD to six dogs. The coefficient of variation of these mean data ranged between 20 and 40%.

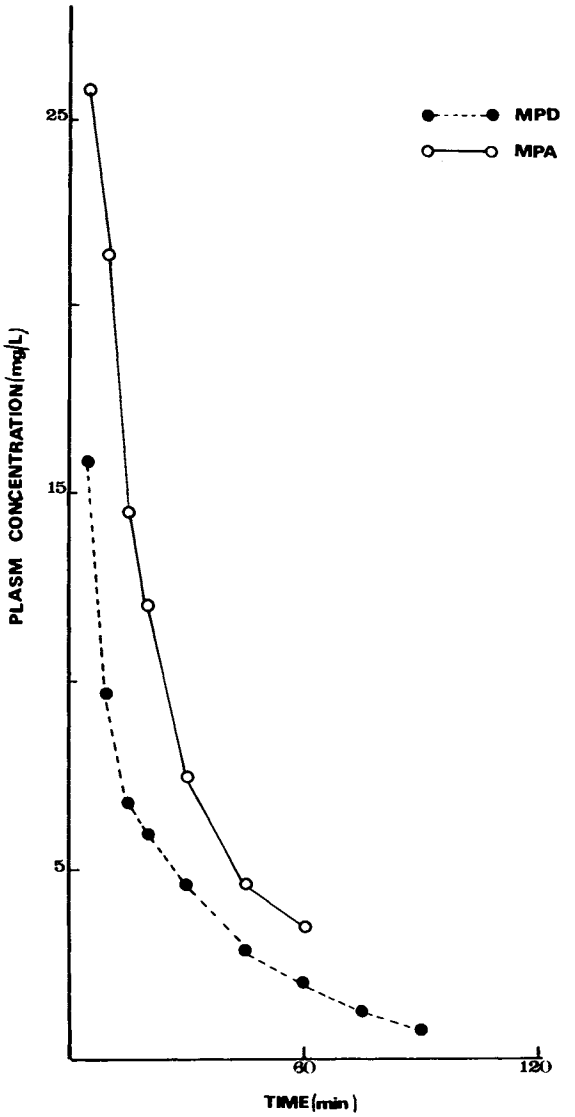

Fig. 3. Mean plasma levels of methylpentylacetamide (MPD) and methylpentyl acetic acid (MPA) following i.v. administration (400 mg) of MPD to six dogs. The coefficient of variation of these mean data ranged between 20 and 50%.

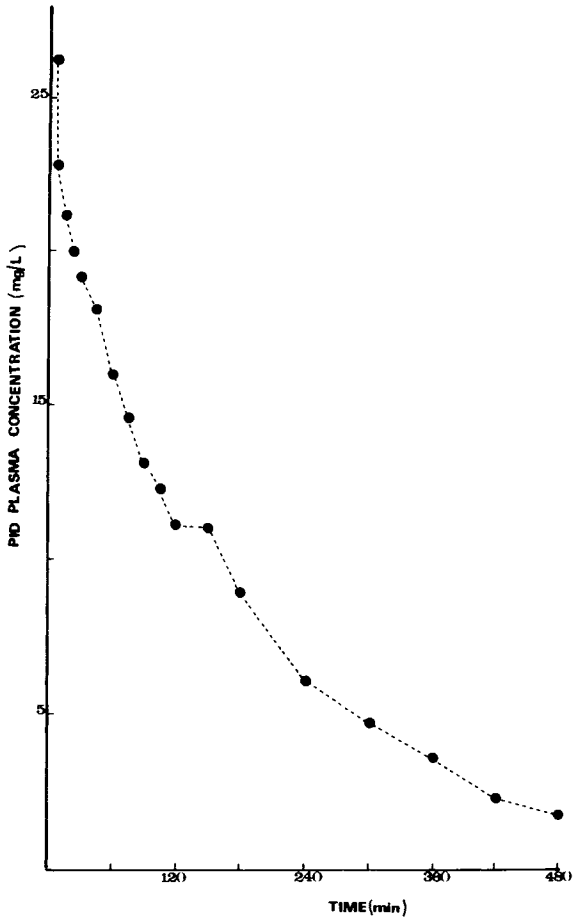

Fig. 4. Mean plasma levels of propylisopropylacetamide (PID) following i.v. administration (400 mg) to six dogs. The coefficient of variation of these mean data ranged between 20 and 35%.

of only 16%. The individual f_m calculations for MPD in two dogs gave a value greater than one. This may be due to (i) a deviation from the assumptions (such as linear kinetics) inherent in Eq. (3) and/or (ii) a rapid and multisite metabolism, accounting for the quick conversion of MPD to MPA. MPD was rapidly metabolized to MPA, its peak plasma concentration being obtained 5 min after the i.v. administration of MPD. Analyzing the urine showed that less than 1% of the administered dose of the amides was excreted unchanged.

Stability studies showed that, unlike MPD, which is unstable, EBD, PID, and PAD are stable in dog blood. Assuming first-order kinetics, the half-life of degradation of MPD was 4.8 hr. After 7 hr, there was a 40% decrease in the MPD concentration and a proportional increase in the concentration of its homologous acid (MPA). MPD was stable in plasma.

The data of the partition, plasma protein binding, stability, and water solubility studies are summarized in Table II. The partition study indicated that EBD and MPD are taken up by blood cells, while PID and PAD showed little and balanced uptakes, respectively.

In Phase 1 of the anticonvulsant screening project of the NIH Epilepsy Branch, all of the amides were found to be more active than their respective homologous acids. Therefore, for Phase 2 the amides were subsequently tested in order to determine their ED_{50}'s, TD_{50}'s, and protective indices (PI; the ratio between the TD_{50} and the ED_{50} values). The results are shown in Table III. Even though the PI values of VCD and PID were not dramatically higher than those of VPA, VPD, PAD, and EBD (in the PI MES values), VCD and PID were the most active of all of the compounds tested. MPD was found to be the least active of all of the compounds tested.

DISCUSSION

Following i.v. administration the plasma levels of the investigated amides declined in a biphasic fashion. MPD (VII) had the shortest mean terminal half-life (0.4 hr), while both PID and PAD had a half-life of 2.5 hr, a value which was similar to that obtained previously for VPD and VCD (15,20). The mean volume of distribution of MPD was the

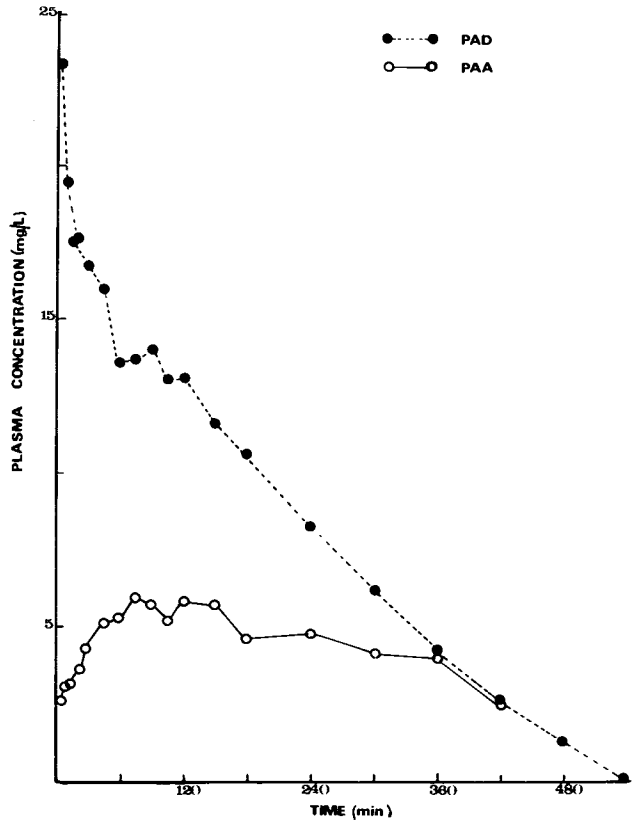

Fig. 5. Mean plasma levels of propylallylacetamide (PAD) and propylallyl acetic acid (PAA) following i.v. administration (400 mg) of PAD to six dogs. The coefficient of variation of these mean data ranged between 20 and 35%.

largest (V_{ss} = 38 ± 20 liters) and it also produced the largest interdog variability. The other three amides had similar volumes of distribution (20 liters), a value which was of the same order of magnitude as that of VPD and twice that of VCD.

As reflected by its short half-life, the total-body (plasma) clearance of MPD (VII) was 10 times that of VPD, VCD, PID, and PAD and 3 times that of EBD. Since this value is greater than the blood flow through any individual organ in the dog (36), it indicates that the metabolism of MPD, including its biotransformation to its homologous acid, may occur at several metabolic sites. In addition, the facts that MPD is unstable in blood and that its biotransformation to MPA occurs in blood also contribute to this large clearance value.

The other three amides (EBD, PID, and PAD) were taken up by blood cells and were found to be stable in blood. This indicates that blood cells and/or plasma are not among their metabolic sites. As very low amounts of the three amides were found in the urine and as they were stable in blood, it appears that these three, like VPD and VCD, are eliminated from the body by metabolic processes.

In order to compare plasma clearance with hepatic blood flow, the amides' blood clearance had to be calculated. This was carried out by using Eq. (4) (34):

$$\frac{\text{plasma clearance}}{\text{blood clearance}} = \frac{\text{blood concentration}}{\text{plasma concentration}} \quad (4)$$

Using Eq. (4), the following mean values of blood clearance (ml/min) were obtained: EBD, 244; MPD, 753; PID, 251; and PAD, 89. Dividing the blood clearance by the mean dog hepatic blood flow of 560 ml/min (36) gave the following extraction ratios (E): EBD, 43%; PID, 45%; and PAD, 16% (Table I). This means that the metabolic clearance of PAD, like that of VCD, was low and restrictive (37). Thus, PAD has a low extraction ratio (E) by the liver, which indicates that it is not susceptible to a first-pass effect upon oral administration. PAD's low extraction ratio indicates that although its clearance will not be affected by changes in blood flow, it may be affected by changes in plasma protein binding (37). EBD and PID have intermediate metabolic clearances and, thus, they may be susceptible to a partial first-pass effect upon oral administration.

The mean f_u values of the amides ranged between 0.46 and 0.86. This indicated that these amides were not highly bound to plasma proteins. Therefore, plasma protein binding does not appear to be a major factor in the disposition and pharmacokinetics of these amides. The water solubility of the investigated amides were all of the same order of magnitude (3.5 to 9.5 mg/ml; Table II).

Table I compares the major pharmacokinetic parameters of the four amides investigated in this study to those of VCD. The values for CL, V, and $t_{1/2}$ were similar for VPD, VCD, PID, and PAD. However, MPD had a large clearance value and, therefore, the shortest half-life. Unlike VPD, EBD, MPD, and PAD, PID and VCD did not biotransform to their homologous acids. They were eliminated from the body by metabolic pathways which did not include hydrolysis of their amide moiety. This may be due to the fact that, in these two compounds, one of the β positions in the molecule has an alkyl substituent. Thus, an amide in this series of compounds investigated needs a free (unsubstituted) β position in its aliphatic side chain in order to undergo metabolism to its homologous acid. The differences in the extent of biotransformation to the respective homologous acid may therefore account for the differences in the pharmacological activities of the investigated compounds. In addition, the two amides which did not biotransform to their homologous acids were the most potent in the anticonvulsant screen. Therefore, in this series of aliphatic amides derived from short-branched fatty acids, the biotransformation of the amide to its homologous acid depends upon the chemical structure of the compound, especially upon whether the β position of the molecule is substituted or not.

Results from other laboratories (9,23) also showed that, in a series of VPA derivatives evaluated, a branched chain was essential for anticonvulsant activity. This may explain why MPD (the least-branched compound) showed the least activity. Keane *et al.* reported that when a series of VPA homologoues and analogues was tested for their anticonvulsant activity, a significant correlation existed between side-chain length and anticonvulsant potency (23). In the Keane study, no sedative or toxic effects were observed with VPA homologues containing seven or fewer carbon atoms or with VPA (which possesses eight carbon atoms). However, 2-ethylhexanoic acid (which is an isomer of VPA) and three VPA analogues with 9 or 10 carbons all exhibited sedative and/or toxic properties. Thus, VPA appears to have the optimal chemical structure in this series, as it possesses a very

Table I. Comparison of the Mean Pharmacokinetic Parameters of EBD, EBA, MPD, MPA, PID, PAD, PAA, and VCD

	EBD	EBA	MPD	MPA	PID	PAD	PAA	VCD[d]
β (hr^{-1})	1.1 ± 0.4	1.1 ± 0.6	2.1 ± 0.8	2.4 ± 1	0.31 ± 0.05	0.33 ± 0.20	1 ± 0.3	0.4 ± 0.1
$t_{1/2}\beta$ (hr)	0.7 ± 0.2	0.7 ± 0.3	0.4 ± 0.1	0.4 ± 0.2	2.4 ± 0.6	2.6 ± 1.1	0.7 ± 0.3	1.9 ± 0.5
AUC (mg/L · hr)	21 ± 9	40 ± 7	6.5 ± 2.4	10 ± 7	73 ± 20	85 ± 30	54 ± 19	92 ± 21
CL_p[a] L/hr	28 ± 18	10 ± 2	70 ± 31	63 ± 45	6 ± 2	6 ± 3	9 ± 3	4.5 ± 0.5
ml/min	467 ± 300	171 ± 30	1167 ± 516	1056 ± 757	100 ± 30	100 ± 50	148 ± 54	75 ± 18
CL_b[b] (ml/min)	244 ± 157		753 ± 332		251 ± 154	89 ± 45		74 ± 18
E[c] (%)	43		>100		45	16		13
V_β (L)	25 ± 8	11 ± 7	35 ± 17	24 ± 10	20 ± 7	18 ± 2	9 ± 2.5	12 ± 3
V_{ss} (L)	25 ± 7	9 ± 3	38 ± 20	25 ± 7	19 ± 5	22 ± 6	10 ± 2.5	13 ± 3
MRT (hr)	1.1 ± 0.4	49 ± 11	0.6 ± 0.1	34 ± 22	3 ± 1	4 ± 1	1 ± 0.4	3 ± 0.8
f_m (%)[e]	16 ± 5		124 ± 42		0	100 ± 30		0
$t_{1/2}\beta$ acid (hr)[f]	0.9 ± 0.5		0.4 ± 0.2			5 ± 3		
MRT acid (hr)[f]	1.5 ± 0.7		0.6 ± 0.2			8 ± 5		
AUC (mg/L · hr)[f]	6 ± 3		12 ± 5			56 ± 26		
C_{max} (mg/L)[f]	3 ± 1.2		26 ± 9			7 ± 2		
t_{max} (hr)[f]	0.4 ± 0.2		0.8 ± 0			1.3 ± 0.5		

[a] Plasma clearance.
[b] Blood clearance.
[c] Extraction ratio (MPD had a large E value due to its multisite metabolism).
[d] VCD data are taken from Ref. 20.
[e] The fraction metabolized of the amide to its respective homologous acid.
[f] A parameter of the acid as a metabolite of its respective homologous amide.

good anticonvulsant activity without producing sedative side effects. Therefore, in our study, we also focused on amides and acids that were isomers or analogues of VPD or VPA which contained eight carbon atoms in their molecule.

Our study showed that the amides were more active as anticonvulsants than their respective homologous acids. However, amides may possess other biological activities, such as antipsychotic (VPD) or anxiolytic (VCD). In the case of VCD and PID, the anticonvulsant activity appears to be due in the parent compounds. However, with amides which undergo biotransformation to their homologous acids, the acid also appears to contribute to the anticonvulsant activity. Taking this into consideration, there may be a species difference in the extent of this biotransformation. This may then influence the anticonvulsant activity—a fact which should be taken into consideration when anticonvulsant activity studies in animals are being extrapolated to humans.

ACKNOWLEDGMENTS

The authors thank Dr. Harvey J. Kupferberg and Mr. James Stables of the NIH Epilepsy Branch for screening the compounds in their anticonvulsant screening project. Our thanks to Mr. Abu Salach Omar and Mr. Abed Lutef for their skillful technical assistance.

Table II. Results of the Partition, Protein Binding, and Water Solubility Studies of the Four Investigated Amides

	C_b/C_p[a]	f_u (%)[b]	Water solubility (mg/ml)
EBD (VI)	1.91 ± 0.33	46 ± 3	4.4
MPD (VII)	1.55 ± 0.18	63 ± 2	7.1
PID (VIII)	0.39 ± 0.03	86 ± 4	3.5
PAD (IX)	1.12 ± 0.05	81 ± 3	9.5
VCD (III)[c]	1.01 ± 0.05	68 ± 2	8.7

[a] The blood/plasma ratio (mean ± SD; $N = 7$).
[b] The free (unbound) fraction in plasma (mean ± SD; $N = 4$).
[c] The data on VCD are taken from Ref. 20.

Table III. Results of Phase 2 of the NIH Anticonvulsant Screening Project: ED_{50}, TD_{50} (mg/kg), and PI (Mice i.p.)

	VPA	EBD	MPD	PID	PAD	VPD	VCD
MES test ED_{50}	200	78	167	58	67	56	58
s.c. Met test ED_{50}	146	103	268	49	67	55	32
Neurotoxicity TD_{50}	283	116	205	99	96	81	81
PI, MES	1.4	1.5	1.2	1.7	1.4	1.4	1.4
PI, s.c. Met	1.9	1.1	0.8	2.0	1.4	1.5	2.5

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

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