Pharmacokinetics and Anticonvulsant Activity of Three Monoesteric Prodrugs of Valproic Acid

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The pharmacokinetics of valproic acid (VPA) were compared in dogs with those of the prodrugs ethyl valproate (E-VPA), trichloroethyl valproate (T-VPA), and valproyl valproate (V-VPA). Valproic acid, E-VPA, T-VPA, and V-VPA were administered intravenously and orally to six dogs at equimolar doses. The three VPA prodrugs were rapidly converted to VPA. The biotransformation was complete in the case of E-VPA and T-VPA but was only partial in the case of V-VPA. Because of the rapid conversion to the parent drug, after administration of the prodrugs, VPA plasma levels did not yield a sustained-release profile. Further, the anticonvulsant activity of prodrugs was compared in mice to that of VPA and valpromide (VPD). The anticonvulsant activity of E-VPA, T-VPA, and V-VPA was less than that of VPA.

KEY WORDS: valproic acid; esteric prodrugs; pharmacokinetics.

INTRODUCTION

Valproic acid (VPA) is one of the major antiepileptic drugs, with a broad spectrum of activity against different kinds of epilepsy (1,2). Valproic acid has the shortest half-life of all existing antiepileptics. As a result, there are fluctuations in VPA plasma levels during chronic treatment, and therefore the drug has to be administered several times a day. A sustained-release formulation of VPA has been developed to overcome this problem (3). Alternatively, the slow biotransformation of a prodrug to the parent drug can be used to obtain sustained plasma levels of the drug. In the case of valproic acid, prodrugs can minimize side effects such as gastric irritation and assist with different pharmaceutical problems associated with valproic acid (which is a liquid) or sodium valproate (a hygroscopic material).

The primary amide of valproic acid—valpromide (VPD)—was found to be a prodrug of valproic acid after oral and intravenous administration to humans (4–7). Further, there are two reports on esteric prodrugs of valproic acid: glyceryl trivalproate (8) and valproyl valproate (9). However, no pharmacokinetic analysis of these prodrugs has been reported.

This paper describes a pharmacokinetic analysis in dogs of three monoesteric prodrugs of valproic acid: ethyl valproate (E-VPA), trichloroethyl valproate (T-VPA), and valproyl valproate (V-VPA). T-VPA is an ester between valproic acid and trichloroethanol, which is the active metabolite of chloral hydrate, which possesses sedative and hypnotic properties similar to those of the barbiturates (10). The combination between valproic acid and trichloroethanol may lead to synergistic activity. Valproyl valproate is an ester formed by the esterification of valproic acid with its alcoholic congener, 2-propyl pentanol. After metabolic hydrolysis by the esterases, V-VPA will be cleaved to valproic acid and its alcoholic congener 2-propyl pentanol, which in turn can be further oxidized by dehydrogenases to valproic acid. Thus, each prodrug molecule could yield two molecules of the parent drug.

Taillandier *et al.* investigated a series of analogues of valproic acid, including esteric derivatives (11). However, in these ester derivatives 2-propyl pentanol was esterified by acetic, isobutyric, and benzylic acid.

In order to evaluate the pharmacokinetics of E-VPA, T-VPA, and V-VPA in comparison to valproic acid, the three prodrugs and the parent drug were administered intravenously and orally to dogs. In addition, we tested the antiepileptic activity of the three prodrugs in comparison to VPA and VPD by using the anticonvulsant screening project of the NIH Epilepsy Branch (12).

MATERIALS AND METHODS

Materials

E-VPA and V-VPA were supplied by Chemische Fabriek Katwijk (Katwijk, The Netherlands). T-VPA was synthesized by esterification of valproyl (acyl) chloride (a product of VPA and $SOCl_2$) with chilled trichloroethanol containing dimethylamino pyridine as a catalyst. VPA was obtained from Teva (Jerusalem, Israel), sodium valproate was obtained from Sanofi-Labaz (Paris, France), and trichloroethanol was purchased from Aldrich (Milwaukee, WI). The chemical structures of the three esteric prodrugs and their purity were confirmed by NMR and elemental microanalysis.

Animals

The experiments were carried out in six dogs (mongrels), three males and three females, ranging in weight between 16 and 20 kg. Although mice and rats are usually used for anticonvulsant screening (12), these animals are too small to be used in pharmacokinetic studies with a crossover design. In a randomized crossover design, each dog was administered intravenously (iv bolus into one of the chphalic veins) with a dose equimolar to 400 mg VPA (in 1.5 ml 70% alcohol) of the compounds E-VPA, T-VPA, and V-VPA and a parenteral preparation of 50 mg/ml sodium valproate in saline. After completing the four-crossover design iv dosings, the same dog also received oral doses (raw material in a gelatin capsule) of E-VPA, T-VPA, V-VPA, and valproic acid in a similar design.

The equimolar iv and oral doses to 400 mg of VPA were 478 mg of E-VPA, 767 mg of T-VPA, and 350 mg of V-VPA. The equimolar dose for V-VPA was calculated by assuming

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the valproyl valproate undergoes metabolism to VPA and 2-propyl pentanol, which is further oxidized to VPA. 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 (from the other cephalic vein) at specified intervals following injection (0, 5, 10, 15, 20, 30, 40, and 50 min, 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, and 10 hr). After oral dosing, the sampling times were the same except for the first hour, during which blood was withdrawn every 15 min. The plasma was immediately separated by centrifugation at 3000 rpm for 15 min and stored at −20°C. Before each assay, the plasma was allowed to reach room temperature, vortexed, then centrifuged, and the residual clot removed. Plasma levels of valproic acid and its prodrug were assayed by GLC (13). In preliminary studies we established that the prodrugs can also be detected and monitored, simultaneously with valproic acid, in our GLC assay.

Anticonvulsant Activity

The compound VPA, VPD, E-VPA, T-VPA, and V-VPA were screened in mice (after ip administration) for their anticonvulsant activity by the NIH Epilepsy Branch using a procedure previously described in detail (12).

Pharmacokinetic Analysis

The linear terminal slope (β) of log C (VPA plasma concentration) versus t (time) was calculated by the method of least squares. The terminal half-life of VPA ($t_{1/2}\beta$) was calculated from the quotient 0.69/terminal slope. The peak plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were determined by inspection. The AUC (area under the C-versus-t curve) was calculated by the classical method (14). The total-body clearance (CL), volume of distribution (V_β), volume of distribution at steady state (V_{ss}), and mean residence time (MRT) were calculated by using noncompartmental classical methods which are based on the statistical moment theory (14–17).

The relative bioavailability of VPA after administration of the prodrug was determined from the ratio of the AUCs after the administration of the prodrug and the parent drug by the same route (F_1). The relative oral bioavailabilities (F_2) of E-VPA, T-VPA, and V-VPA were calculated from the ratio of the AUC obtained after their oral and intravenous administration. Statistical analysis of the AUC values obtained after the different administrations was performed by using an analysis of variance.

Stability Studies

A blood stability study of E-VPA, T-VPA, and V-VPA 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, 7, and 8 hr. Plasma was immediately separated, and the compound concentration in the plasma was then assayed by GLC.

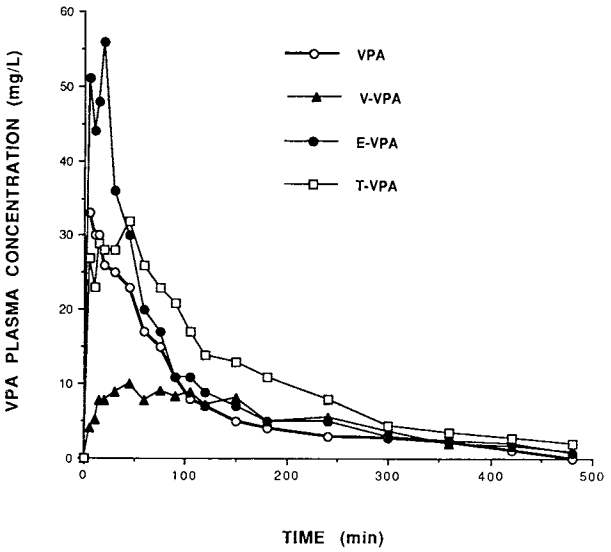

Fig. 1. Mean plasma levels of valproic acid (VPA) obtained after iv administration of VPA, ethyl valproate (E-VPA), trichloroethyl valproate (T-VPA), and valproyl valproate (V-VPA) to six dogs.

RESULTS

After iv or oral administration, no plasma levels of the prodrugs were detectable. E-VPA, T-VPA, and V-VPA were rapidly biotransformed to VPA. Figures 1 and 2 show the mean plasma levels of VPA obtained after intravenous (Fig. 1) and oral (Fig. 2) administration of VPA, E-VPA, T-VPA, and V-VPA. Tables I and II summarize the mean pharmacokinetic parameters of the four investigated compounds after intravenous and oral administration, respectively.

Stability studies showed that compounds E-VPA, T-VPA, and V-VPA were stable in dog blood for 8 hr at physiological conditions. In phase I of the anticonvulsant screening project of the NIH Epilepsy Branch, the three esteric prodrugs of VPA demonstrated (qualitatively) some anticon-

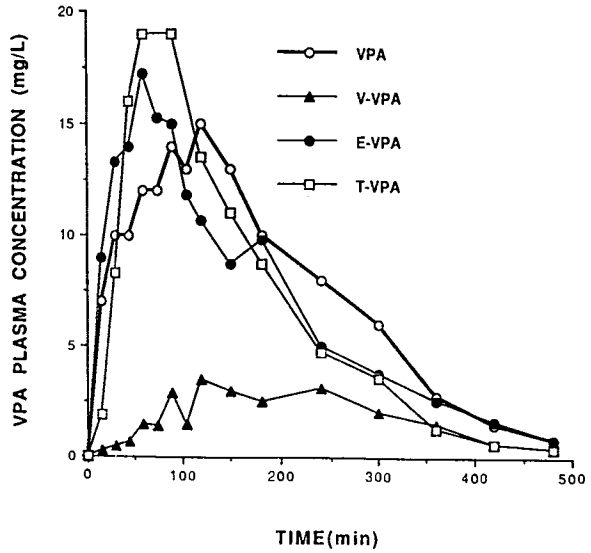

Fig. 2. Mean plasma levels of valproic acid (VPA) obtained after oral administration of VPA, ethyl valproate (E-VPA), trichloroethyl valproate (T-VPA), and valproyl valproate (V-VPA) to six dogs.

Table I. Comparison of the Mean Pharmacokinetic Parameters of Valproic Acid (VPA) Obtained After Intravenous Administration (400 mg) of VPA, E-VPA, T-VPA, and V-VPA to Six Dogs

Pharmacokinetic parameter	VPA	E-VPA	T-VPA	V-VPA
$t_{1/2}\beta$ (hr)	1.6 ± 0.6	2.8 ± 1.9	2.3 ± 0.9	2.7 ± 1.9
AUC (mg/L × hr)	58 ± 11	88 ± 42	93 ± 21	46 ± 15
C_{max} (mg/L)	—	64 ± 52	38 ± 11	12 ± 9
t_{max} (min)	—	8 ± 6	31 ± 17	105 ± 41
MRT (hr)	2.0 ± 0.6	2.8 ± 1.4	3.0 ± 0.7	4.5 ± 2.4
F_1[a]	—	1.52 ± 0.5	1.65 ± 0.5	0.82 ± 0.4
CL (ml/min)	130 ± 30	—	—	—
V_β (L)	18 ± 9	—	—	—
V_{ss}	16 ± 5	—	—	—

[a] Bioavailability relative to intravenous administration of VPA.

vulsant activity. Therefore, it was decided subsequently to test compounds E-VPA, T-VPA, and V-VPA in phase II of the NIH anticonvulsant screening project in order to determine their ED_{50} and TD_{50} values, as well as their protective indices (PI; the ratio between the TD_{50} and the ED_{50} values). The results in comparison to VPA and VPD are shown in Table III.

DISCUSSION

Unlike valpromide, which in dogs underwent a relatively slow and partial biotransformation to valproic acid (18), the three monoesteric prodrugs of VPA investigated in this study underwent a rapid conversion to VPA (after iv administration). The fastest conversion was noted in the case of E-VPA and the slowest in the case of V-VPA. The bioavailability of VPA (F_1) after iv administration of E-VPA, T-VPA, and V-VPA (relative to iv administration of VPA) was complete. A higher interdog variability was observed with the prodrug administration than after the administration of VPA. The AUC of VPA obtained after iv administration of E-VPA and T-VPA was larger than that obtained after iv administration of V-VPA.

After oral administration, the relative bioavailability of E-VPA and T-VPA was complete, but was only partial for V-VPA. A comparison of the AUCs (of VPA) obtained after oral administration of E-VPA and T-VPA in relation to the AUCs (of VPA) obtained after their iv administration gave F_2 values that were lower than the F_1 values. The absorption rate of VPA after oral administration of E-VPA and T-VPA was faster than that obtained after oral administration of VPA, as can be seen from the different t_{max} values (Table II, Fig. 2).

V-VPA was administered in a dose of 375 mg, assuming that the enzymes, esterases, and alcohol dehydrogenase will form two equivalents of VPA from each equivalent of V-VPA. The partial oral bioavailability and the relatively low levels of VPA obtained after iv and oral administration of V-VPA suggest that the metabolic conversion of V-VPA to VPA is incomplete.

Peak plasma concentration of VPA, obtained after oral administration of compounds E-VPA, T-VPA, and V-VPA, was reached at a later time than after iv administration of the esteric prodrugs. However, plasma profiles of VPA obtained after oral administration of the three esteric prodrugs did not resemble that of a VPA sustained release dosage form (3).

Table III shows that E-VPA, T-VPA, and V-VPA were less toxic, but also less active, than VPA or VPD in mice. The anticonvulsant activity occurred at much higher doses than with VPA or VPD, and therefore their ED_{50} values could not be determined.

Generally, it may be better to investigate the pharmacokinetics and anticonvulsant activity of drugs in the same animal species due to different rate and/or metabolic pathways, a possibility which cannot be ruled out in this study as well. However, dogs and mice are the most used animal models for pharmacokinetic evaluation and anticonvulsant

Table II. Comparison of the Mean Pharmacokinetic Parameters of Valproic Acid (VPA) After Oral Administration of (400 mg) VPA, E-VPA, T-VPA, and V-VPA to Six Dogs

Pharmacokinetic parameter	VPA	E-VPA	T-VPA	V-VPA
$t_{1/2}\beta$ (hr)	1.6 ± 0.6	2.0 ± 1.4	1.6 ± 1.2	1.0 ± 0.33
AUC (mg/L × hr)	61 ± 20	59 ± 13	58 ± 32	16 ± 25
C_{max} (mg/L)	19 ± 5	19 ± 3	22 ± 15	4 ± 4
t_{max} (min)	103 ± 41	50 ± 46	93 ± 33	120 ± 39
MRT (hr)	3.1 ± 0.5	3.4 ± 1.8	3.1 ± 1.8	2.8 ± 0.9
F_1[a]	—	1.0 ± 0.3	0.99 ± 0.60	0.21 ± 0.26
F_2[b]	1.05 ± 0.25	0.67 ± 0.12	0.64 ± 0.42	0.49 ± 0.89

[a] Bioavailability relative to oral administration of VPA.
[b] Bioavailability relative to intravenous administration of each compound.

Table III. Results of Phase II of the NIH Anticonvulsant Screening Project: ED_{50} and TD_{50} (mg/kg) Obtained in Mice

Test	VPA	VPD	E-VPA	T-VPA	V-VPA
MES, ED_{50}	200	56	>500	>500	>500
sc MET, ED_{50}	146	55	>500	>500	>500
Neurotoxicity, TD_{50}	283	81	>500	>500	>500

screening, respectively. Dogs provide a good animal for crossover pharmacokinetic studies which can be extrapolated to humans. Mice are the common animal model for anticonvulsant screening of compounds with potential antiepileptic activity (12).

The anticonvulsant testing was done in mice after ip administration. It is possible that the rate of delivery of VPA was slower than its rate of elimination. VPA has a short half-life of about 50 min in mice (19). It is plausible that the rate of absorption of the VPA prodrugs (after ip administration to mice) or their rate of biotransformation was slower than VPA's rate of elimination, leading to low VPA plasma levels similar to the situation of a formation rate-limited metabolite.

The above, tentative explanation, in addition to the different species used in the study, may explain the lack of pharmacokinetic pharmacodynamic correlation that was found in this study in the case of E-VPA and T-VPA.

From the pharmacokinetic profile of E-VPA, T-VPA, and V-VPA in dogs and the lack of anticonvulsant activity in mice (at doses equivalent to VPA) in phase II of the NIH Anticonvulsant Screening Project, it can be concluded that the prodrugs of VPA investigated in this study were inferior to the parent compound VPA.

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