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Stereoselective Pharmacokinetic Analysis and Antiepileptic Activity of N-2-Hydroxypropyl Valpromide, a Central Nervous System–Active Chiral Valproylamide

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Summary: The purpose of this study was to evaluate the anticonvulsant activity and pharmacokinetics (PK) of a novel chiral CNS-active 2-hydroxypropyl valpromide (HP-VPD), a derivative of valproic acid (VPA). The individual enantiomers, R, S, and racemic (R,S)-HP-VPD were synthesized and evaluated for their pharmacokinetics and pharmacodynamics in a stereoselective manner. A stereoselective gas chromatography (GC) assay for simultaneous quantification of HP-VPD enantiomers in plasma and urine was developed and used to investigate the pharmacokinetics of HP-VPD in dogs. Pharmacodynamic analysis in rats showed that (S)-HP-VPD was 2.5 times more potent as an anticonvulsant in the maximal electroshock seizure (MES) test than its enantiomer and approximately 10 times more potent than VPA. No significant differences were observed in major PK parameters (clearance, volume of distribution, and half-life) between S and (R)-HP-VPD, and this suggested that pharmacodynamic differences could be attributed to the intrinsic pharmacodynamics of each enantiomer rather than to a preferable pharmacokinetic profile. The pharmacokinetic (metabolic) analysis showed that the fraction metabolized to HP-VPD-glucuronide ranged from 5% to 7% and no biotransformation of HP-VPD to VPA and 2-ketopropyl valpromide was observed. This is the first report of significant stereoselectivity in the anticonvulsant activity of a valproylamide with a chiral carbon situated on the alkyl chain of the amine moiety. **Keywords:** Stereoselective anticonvulsant activity—Pharmacokinetics—Biotransformation—Chiral valproylamides.

Valproic acid (VPA) I (Fig. 1) is one of the major antiepileptic drugs. Whereas it has a wide spectrum of antiepileptic activity, its anticonvulsant potency is less than that of the three other major antiepileptic drugs, phenobarbital, phenytoin, and carbamazepine (1). In addition, VPA causes two rare but severe and sometimes fatal side effects: teratogenicity and hepatotoxicity (2,3).

Valpromide (VPD) II, the primary amide of VPA, is a nonteratogenic central nervous system (CNS)-active entity that is more potent than VPA as an anticonvulsant. However, its better anticonvulsant profile and lack of teratogenicity have no clinical implications because VPD serves as a prodrug of VPA in humans (4). Consequently, several isomers and analogues of VPD were synthesized and evaluated pharmacokinetically and pharmacodynamically to explore the structural requirements for metabolically stable VPD analogues that do not undergo hydrolytic metabolism to their corresponding inactive acids (5). The following three valproylamides (Fig. 1) have emerged as the optimal compounds: valnoctamide III, N-methyl-tetramethylcyclopropyl carboxamide IV, and propylisopropyl acetamide (PID) V. These compounds possessed better anticonvulsant activity than VPA and were nonteratogenic (5). The pharmacokinetics of both valnoctamide and PID were

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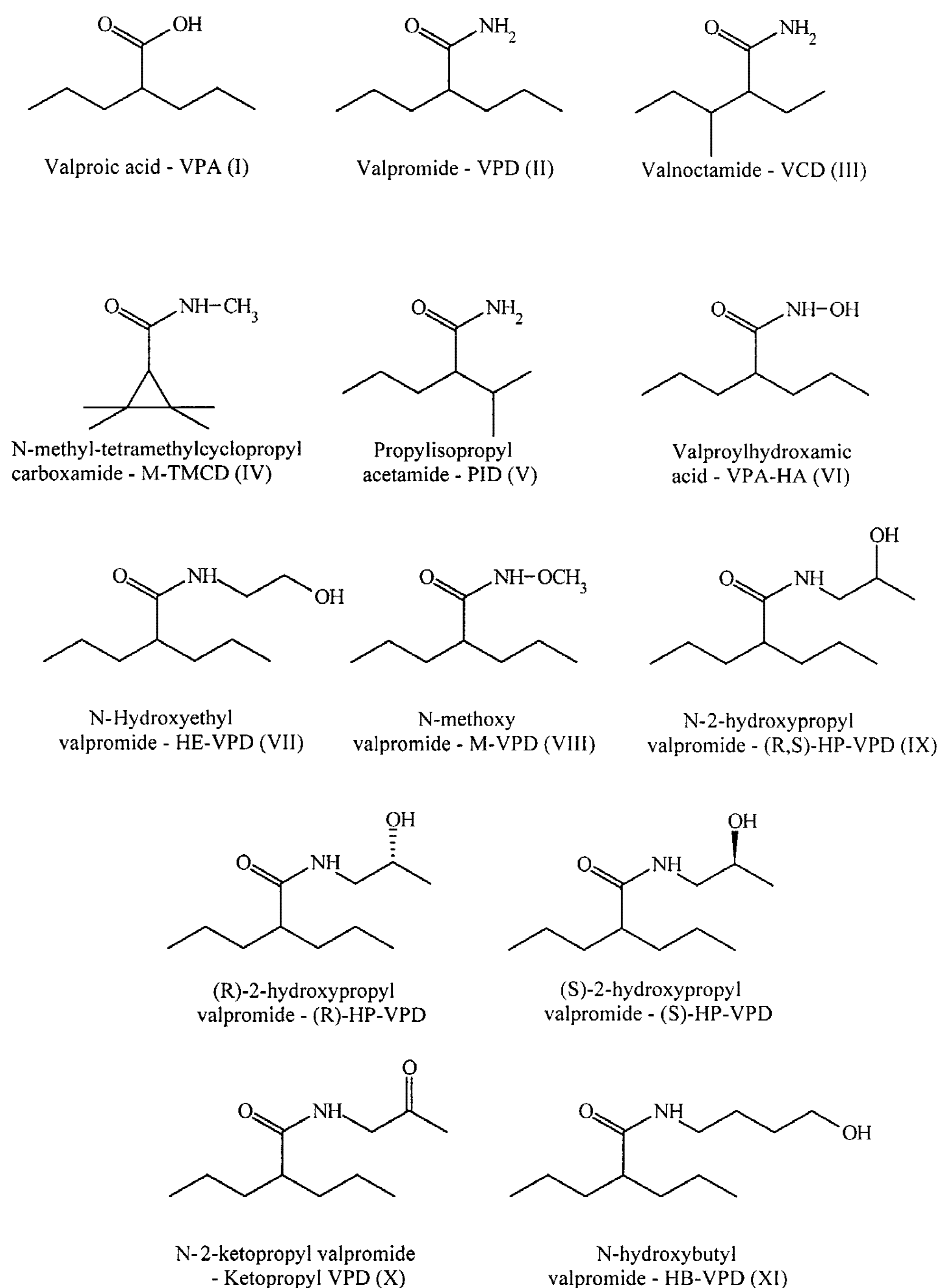

FIG. 1. Chemical structures of valproic acid and valpromide analogues and derivatives.

stereoselective (6,7). Propylisopropyl acetamide had a stereoselective anticonvulsant activity with a stereoselective index of 1.6. Subsequently, the VPD derivatives valproyl hydroxamic (VPA-HA) acid VI and hydroxyethyl valpromide (HE-VPD) VII were substituted on the amine moiety of the molecule, (Fig. 1) and synthesized, and their anticonvulsant activity and pharmacokinetics were evaluated. Both VPA-HA and HE-VPD exerted a greater anticonvulsant activity than VPA, and unlike VPD they did not undergo biotransformation to VPA (8). An additional VPA-HA derivative, N-methoxy valpromide (M-VPD) VIII, was more active as an anticonvulsant than VPA; however, after intravenous administration to dogs it was rapidly and extensively biotransformed to VPA

(9). The importance of the chirality of the active entity in drug development and evaluation has been generally accepted (10).

Following up on our structure–pharmacokinetic–pharmacodynamic relationship (SPPR) study of chiral valproylamides and using the above data as a base, the current study focused on 2-hydroxypropyl valpromide (HP-VPD) IX, a chiral derivative of valpromide that possesses a chiral carbon on an amine moiety of the molecule (Fig. 1). The two enantiomers of the chiral valproylamide, (S)-HP-VPD and (R)-HP-VPD, and a racemate of HP-VPD were synthesized and analyzed pharmacokinetically and pharmacodynamically in a stereoselective manner.

The current study was designed to evaluate the influence of the absolute configuration of the HP-VPD enantiomers on stereoselective anticonvulsant activity and pharmacokinetics.

MATERIALS AND METHODS

Chemicals

All chemicals and solvents used were purchased from Aldrich (Milwaukee, WI) and were of analytical grade.

Chemical Synthesis

Racemic-HP-VPD and the R- and S- enantiomers of HP-VPD were synthesized according to the methods given below, their purity was confirmed by elemental analysis, and their structures were confirmed by proton nuclear magnetic resonance (NMR) using $CDCl_3$ as a solvent.

(R,S)-N-2-Hydroxypropyl Valpromide (IX)

Valproyl chloride (5 g, 30 mmol) in 20 mL dichloromethane was slowly added to a cooled (0°C) stirred solution of (R,S)-1-amino-2-propanol (5 g, 67 mmol) in 30 mL dichloromethane at 0°C. After the addition the reaction was stirred for 1 hour. Dichloromethane was evaporated and 50 mL ethyl acetate was added. The organic phase was washed three times with water, 0.25 mol HCl, and 0.5 mol $NaHCO_3$, dried over Na_2SO_4 and evaporated. The resulting crude material was recrystallized from ethyl acetate : petroleum ether (2:3) solution. The yield was 70% analytically pure (R,S)-HP-VPD. M.P.: 72°C.

Elemental Analysis: ($C_{11}H_{23}NO_2$): found (calculated) C, 65.59% (65.67%); O, 11.40% (11.44%); N, 7.10% (6.97%).

^1H NMR (300 MHz): δ (ppm) 5.90 (s, br., 1H, NH), 3.95 (s, br., 1H), 3.43 (m, 1H), 3.12 (m, 1H), 2.52 (s, br., 1H, OH), 2.04 (m, 1H) 1.2–1.65 (m, 8H), 1.18 (d, 3H), 0.88 (t, 6H).

(R)-Hydroxypropyl Valpromide

(R)-Hydroxypropyl valpromide was prepared from (R)-1-amino-2-propanol and valproyl chloride following the same procedure described above in (R,S)-HP-VPD preparation. The yield was 75%. M.P.: 72°C.

Elemental Analysis: ($C_{11}H_{23}NO_2$): found (calculated) C, 65.63% (65.67%); O, 11.32% (11.44%); N, 7.13% (6.97%).

^1H NMR (300 MHz): δ (ppm) 5.90 (s, br.,1H, NH), 3.95 (s, br.,1H), 3.43 (m, 1H), 3.12 (m, 1H), 2.52 (s, br., 1H, OH), 2.04 (m, 1H), 1.2–1.65 (m, 8H), 1.18 (d, 3H), 0.88 (t, 6H).

(S)-Hydroxypropyl Valpromide

(S)-Hydroxypropyl valpromide was prepared from (S)-1-amino-2-propanol and valproyl chloride following the same procedure described above in (R,S)-HP-VPD preparation. The yield was 68%.

M.P.: 72°C

Elemental Analysis: ($C_{11}H_{23}NO_2$): found (calculated) C, 65.67% (65.67%); O, 11.16% (11.44%); N, 6.73% (6.97%).

^1H NMR (300 MHz): δ (ppm) 5.90 (s, br.,1H, NH), 3.95 (s, br.,1H), 3.43 (m, 1H), 3.12 (m, 1H), 2.52 (s, br., 1H, OH), 2.04 (m, 1H), 1.2–1.65 (m, 8H), 1.18 (d, 3H), 0.88 (t, 6H).

N-Hydroxybutyl Valpromide (XI)

A solution of valproyl chloride (2.55 g, 15.7 mmol) in 10 mL dry dichloromethane was added dropwise to a cooled (0°C) stirred solution of 4-hydroxybutyl amine (2 g, 22.5 mmol) and triethylamine (5 g, 49.4 mmol) in 20 mL dry dichloromethane. The reaction mixture was removed from the ice bath and stirred for 1 hour at room temperature. The work-up of the reaction and product isolation was identical to that used in HP-VPD synthesis. The crude material was recrystallized from ethyl acetate : petroleum ether (2:3) yielding 1.84 g (55%) HB-VPD. M.P.: 75°C.

Elemental analysis: ($C_{12}H_{25}NO_2$): found (calculated) C, 66.95% (66.97%); O, 11.43% (11.63%); N, 6.47% (6.51%).

^{1}H NMR (300 MHz): δ (ppm) 5.77 (s, br.,1H, NH), 3.65 (m, 2H), 3.27 (m, 2H), 2.18 (s, br., 1H, OH), 1.98 (m, 1H), 1.12–1.6 (m, 12H), 0.88 (t, 6H).

N-2-Ketopropyl Valpromide (X)

Jones reagent was prepared by dissolving 3.5 g chromium trioxide in 4 mL sulfuric acid. The obtained vivid orange solution was diluted to 10 mL with water. (R,S)-HP-VPD (2 g, 10 mmol) in 20 mL acetone was titrated with freshly prepared Jones reagent until the reaction mixture turned orange. The reaction progress was followed by thin layer chromatography (2% methanol in chloroform) until the starting material disappeared completely. The acetone was evaporated and 30 mL ethyl acetate and 30 mL water were added. The green jelly material readily dissolved in water. The organic phase was washed with water (×3), 0.5 mol $NaHCO_3$ (×3), 0.25 mol HCl (×3) and saturated brine solution, dried over Na_2SO_4, and evaporated. The crude material was recrystallized from ethyl acetate : petroleum ether (2:3). The yield was 0.8 g (40%). M.P.: 55°C.

Elemental analysis: ($C_{11}H_{21}NO_2$): found (calculated) C, 66.14% (66.33%); O, 11.74% (11.55%); N, 7.11% (7.04%).

^{1}H NMR (300 MHz) : δ (ppm) 6.17 (s, br.,1H, NH), 4.18 (d, 2H), 2.2 (s, 3H), 2.12 (m, 1H), 1.2–1.65 (m, 8H), 0.88 (t, 6H).

IR (cm^{-1}): 3286, 3087, 2956, 2873, 1728, 1639, 1558, 1467, 1077.

Pharmacodynamic Studies

Anticonvulsant activity and neurotoxicity of HP-VPD enantiomers were tested at the NIH-Epilepsy Branch in mice after intraperitoneal injection and in Sprague-Dawley rats after oral administration. The testing was performed by standard procedures (11,12) including the following models: maximal electroshock seizure (MES) test, which measures seizure spread, subcutaneous metrazol (sc Met) test, which measures seizure threshold, and rotorod ataxia test which assesses minimal neurotoxicity.

The stereoselective index (SI) was calculated from the ratio of the median effective dose (ED_{50}) values of the enantiomers (SI = High ED_{50}/ Low ED_{50}) (12).

Pharmacokinetic Studies

The studies were approved by the Institutional Animal Care and Use Committee at the Faculty of Medicine, Hebrew University of Jerusalem. Hydroxypropyl valpromide pharmacokinetic (PK) experiments were carried out in six beagle dogs weighing an average of 15 kg. Dogs were housed at the University animal farm and were brought to the laboratory every 2 to 3 weeks for the crossover experiments after an overnight fast. Venous catheters (20 gauge × 32 mm [Venflon; Helsingborg, Sweden]) were inserted into two different legs of each dog, and one urinary catheter (2.7 × 530 mm, Pennine Healthcare; Derby, UK) was also inserted. Each dog received 300 mg (20 mg/kg) of racemic HP-VPD and 150 mg (10 mg/kg) each of (R)- and (S)-HP-VPD. Venous blood samples (5–6 mL) were withdrawn at 5, 10, 20, 30, and 45 minutes; and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 hours after the injection from the indwelling catheter (not the one through which the drug was injected) and were transferred to heparinized test tubes. The tubes were immediately centrifuged at 3000*g* for 10 minutes, after which plasma was separated and stored at −20°C prior to the analysis. Using an indwelling urinary catheter, urine samples were collected every 2 hours for as many as 12 hours.

Enantioselective Gas Chromatograph Assay for HP-VPD, HP-VPD-Glucuronide and 2-Ketopropyl VPD Quantification in Dog Plasma and Urine HP-VPD

Plasma Samples

To dry test tubes, 4 μL internal standard solution (hydroxybutyl valpromide 1mg/mL in chloroform) were added after the addition of 0.5 mL plasma and 4 mL chloroform. The mixture was vigorously vortex-mixed for 1 minute and centrifuged at 3000*g* for 15 minutes. The organic phase was separated and evaporated under reduced pressure. Derivatization procedures were performed in the following manner: 100 μL acetic anhydride and 100 μL pyridine were added to each test tube. The test tubes were sealed and lightly vortex-mixed for 45 minutes. Acetic anhydride and pyridine were then evaporated under reduced pressure. The dry residue was reconstituted in 30 μL chloroform and a 2-μL aliquot was injected into the GC apparatus.

Urine Samples

The extraction procedure for urine was similar to the one used for plasma. The urine volume (0.2 mL), and internal standard solution volume (5 μL) were used. The derivatization procedure was identical to the one described above.

HP-VPD-Glucuronide

Urine samples (0.2 mL) were diluted with 0.2 mL phtalate buffer (pH 5.0) and incubated for 24 hours at 37°C with β-glucuronidase (type H-2, 110350 units/mL (Sigma; St. Louis, MO). The HP-VPD quantification was carried out using the procedure described above. The only difference between the procedures was the addition of the internal standard (HB-VPD) dissolved in methanol, which took place after the treatment of the samples with β-glucuronidase. The amount of HP-VPD glucuronide in the urine was determined by subtractimg free HP-VPD, obtained from assaying the samples without β-glucuronidase treatment, from the total amount of HP-VPD, obtained after treating the samples with β-glucuronidase.

2-Ketopropyl Valpromide

Plasma Samples

To dry test tubes, 5 μL internal standard solution ((R,S)-HP-VPD 1 mg/mL in chloroform) was added. The chloroform was allowed to evaporate and then 0.5 mL plasma and 4 mL chloroform were added. The test tubes were vigorously vortex-mixed for 1 minute and centrifuged at 3000*g*. The lower phase (chloroform) was separated and chloroform was evaporated under reduced pressure. The dry residue was reconstituted in 30 μL chloroform and 2 μL aliquot was injected into the GC apparatus.

Urine Samples

The procedure used for the extraction of urine was similar to the one used for the extraction of plasma. Two milliliters of urine was used in the procedure.

The GC apparatus consisted of a HP 5890 series II gas chromatograph equipped with a capillary split inlet, HP 7673 automatic injector, flame ionization detector and HP 3396-A integrator (Hewlett-Packard; Avondale, PA). Stereoselective separation was achieved on a capillary column (10 m × 0.25 mm × 0.25 μm) coated with Heptakis (2,3-di-O-methyl-6-O-tert-butyldimethylsilyl)-β-cyclodextrine as the stationary phase. The carrier gas was nitrogen; the column head pressure was set at 50 kPa. The split ratio was 1:19. The initial oven temperature was 95°C for 2 minutes, the gradient was 30°C/min, and the final temperature was 150°C until the end of the run. The injector was set at 250°C and the detector was set at 300°C. Under these conditions the retention times were 12.7 minutes for acetylated (S)-HP-VPD, 13.5 minutes for acetylated (R)-HP-VPD, 33.2 minutes for acetylated HB-VPD (internal standard), 8.5 minutes for 2-ketopropyl VPD, and 10.9 minutes for unacetylated HP-VPD (both racemate and individual enantiomers).

Limit of quantitation for HP-VPD enantiomers, HB-VPD, and 2-ketopropyl VPD was less than 1 μg/mL. Concentrations of HP-VPD enantiomers in plasma and urine ranged from 0 to 250 μg/mL. Plasma and urine samples were examined for the presence of valproic acid and valproic acid glucuronide using a published assay (13).

Pharmacokinetic Analysis

The PK parameters were calculated by noncompartmental analysis according to statistical moment theory (14) using the PK software package WinNonlin version 1.1 (SCI Software; Lexington, KY). The terminal half-life ($t_{1/2}$) was calculated as 0.693/β, where β is the linear terminal slope of the log concentration–time curve. The area under the concentration–time curve (AUC) was calculated numerically using the trapezoidal rule with extrapolation to infinity by dividing the last experimental plasma concentration by the terminal slope. The total body plasma clearance (CL), volumes of distribution at steady state (V_{ss}), and mean residence time (MRT) were calculated using a classic method and moment analysis (14). The fraction of HP-VPD excreted unchanged (fe) in the urine was calculated from the ratio of the cumulative amount excreted intact in the urine and the dose. The fraction metabolized (fm) of HP-VPD to its urinary metabolite HP-VPD-glucuronide was calculated from the quotient of the cumulative amount of the metabolite excreted in the urine and the dose. Results are reported as mean values ± SD. Statistical comparisons of kinetic parameters of the two enantiomers were made using the Wilcoxon paired-sample test.

RESULTS

Anticonvulsant screening of the enantiomers of HP-VPD was performed at the Epilepsy Branch of the NIH. After oral administration to rats the S enantiomer had an ED_{50} of 48 mg/kg and was 2.5 times more potent than (R)-HP-VPD in the MES test. The data of anticonvulsant activity and neurotoxicity of (R,S)-HP-VPD, (R)-HP-VPD, and (S)-HP-VPD are presented in Table 1. Table 2 summarizes the mean PK parameters of (R,S)-HP-VPD, (R)-HP-VPD, and (S)-HP-VPD after intravenous administration to six dogs. Figure 2 depicts the mean plasma levels of HP-VPD enantiomers after intravenous administration of (R,S)-HP-VPD to six dogs. Figure 3 depicts

TABLE 1. *Anticonvulsant activity of HP-VPD*†*

Compound	VPA	(R)-HP-VPD	(S)-HP-VPD	(R,S)-HP-VPD	SI§
Test					
MES	490	117	48	92	2.5
scMet	180	—	—	—	
Neurotoxicity	280	>500	>650	>500	
PI‡-MES	0.6	>4.2	>13.5	>5.4	
PI‡-scMet	1.6				

* ED_{50} and TD_{50} values, rats, oral.
† ED_{50} and TD_{50} in mg/Kg.
‡ SI: High ED_{50}/Low ED_{50}.
§ PI: TD_{50}/ED_{50}.
ED_{50}, median effective dose; HP-VPD, 2-hydroxypropal valpromide; MES, maximal electroshock seizure; PI, protective index; scMet, subcutaneous metrazol; SI, stereoselective index; TD_{50}, median toxic dose; VPA, valproic acid.

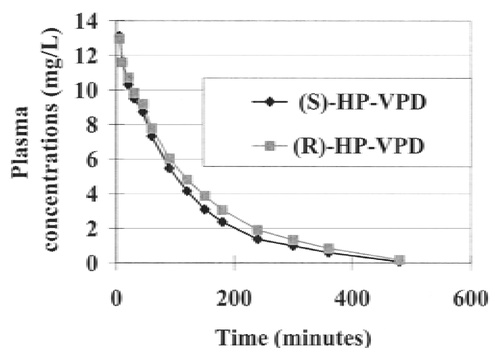

FIG. 2. Mean plasma concentrations of 2-hydroxypropyl valpromide after IV administration of (R,S)-HP-VPD to six dogs.

the mean cumulative amounts of the enantiomers of HP-VPD-glucuronide excreted in the urine. Similar graphs were obtained after intravenous administration (in a crossover design) of the individual enantiomers, (R)-HP-VPD and (S)-HP-VPD, to the same dogs. No significant stereoselectivity was found in the pharmacokinetics of HP-VPD enantiomers or in the formation of HP-VPD-glucuronides (fm = 5–7%). No biotransformation of HP-VPD to VPA or to 2-ketopropyl VPD was observed. Less than 1% of HP-VPD was excreted unchanged in the urine, suggesting predominance of metabolic elimination.

DISCUSSION

In the current study chiral VPD derivatives, with the chiral center situated on a 2-hydroxypropyl substituent attached to the nitrogen, were synthesized and their pharmacokinetics and pharmacodynamics were evaluated stereoselectively. In the MES test (in rats) the anticonvulsant activity of the R and S enantiomers of HP-VPD and of the (R,S)-HP-VPD was greater than that of VPA. Marked stereoselectivity was observed in the anticonvulsant activity of HP-VPD enantiomers with (S)-HP-VPD being 2.5 times more potent than (R)-HP-VPD. Consequently, a stereoselective PK analysis of HP-VPD was performed to verify whether the superior anticonvulsant activity of (S)-HP-VPD over its enantiomer could be attributed to a preferable PK profile or to different pharmacodynamic intrinsic properties. Enantioselective analysis should be done in a crossover design, and this was only feasible using dogs. Previous studies showed that valproylamides exhibit similar pharmacokinetics in rats and dogs (15,16). Pharmacokinetic analysis of HP-VPD enantiomers showed no significant difference in the PK parameters between the enantiomers, and this suggested that the greater potency of (S)-HP-VPD compared with that of (R)-HP-VPD is the result of different intrinsic pharmacodynamics. Less than 1% of HP-VPD dose was excreted unchanged in the urine. Metabolic studies showed that less than 10% of a dose of HP-VPD was excreted in the urine as a glucuronide conjugate. No biotransformation of HP-VPD to VPA or to 2-ketopropyl VPD was observed.

TABLE 2. *Main pharmacokinetic parameters obtained following IV administration of (R,S)-HP-VPD, (R)-HP-VPD and (S)-HP-VPD to dogs*

Parameter	(S)-HP-VPD in racemate	(S)-HP-VPD alone	(R)-HP-VPD in racemate	(R)-HP-VPD alone
Cl (mL/min)	116 ± 45	133 ± 61	108 ± 38	126 ± 62
V_{ss} (L)	11 ± 1.4	9.3 ± 1.8	11 ± 1.3	10 ± 1.3
$t_{1/2}$ (min)	71 ± 14	55 ± 26	76 ± 16	66 ± 30
MRT (min)	102 ± 20	69 ± 12	114 ± 26	97 ± 40
fe (%)	0.11* ± 0.09		0.37* ± 0.12	
fm to HP-VPD glucuronide (%)	5.4 ± 2.8		7.2 ± 2.3	

* $p < 0.05$ (S versus R). All other pharmacokinetic parameters (S versus R) were not significantly different ($p > 0.05$).
Cl, clearance; fe, fraction of HP-VPD excreted unchanged; fm, fraction of HP-VPD metabolized to HP-VPD glucuronide; HP-VPD, 2-hydroxypropyl valpromide; MRT, mean residence time; $t_{1/2}$, half-life; V_{ss}, volume of distribution at steady state.

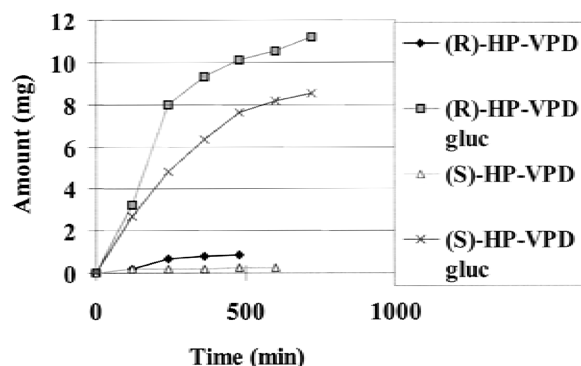

FIG. 3. Mean cumulative amount of (R) and (S) enantiomers of 2-hydroxypropyl valpromide excreted unchanged and as glucuronide conjugates after administration of racemic 2-hydroxypropyl valpromide to six dogs.

Previous reports demonstrated stereoselectivity in the teratogenicity of VPA analogues with (S)-4-yn-VPA being eight times more teratogenic than (R)-4-yn-VPA, whereas no difference was observed in their pharmacokinetics (17). In a recent study it was shown that the CNS-active valproylamide PID, having the chiral carbon on the alkylcarboxylic moiety, possessed stereoselective pharmacokinetics and pharmacodynamics (anticonvulsant activity) with an SI of 1.6 in favor of (R)-PID (7). In conclusion, the present results have shown stereoselectivity in the anticonvulsant activity of a CNS-active valproylamide. This is the first report of significant stereoselectivity in anticonvulsant activity of a valproylamide with a chiral carbon located in the amine moiety of the molecule. Thus, it might be assumed that the superior anticonvulsant activity of (S)-HP-VPD relative to that of (R)-HP-VPD, (SI = 2.5) is caused by different intrinsic pharmacodynamics rather than by a favorable pharmacokinetic profile. This fact should provide new leads for the future rational design of novel and safer anticonvulsant drugs derived from the alkanoid acid amides. In this context it is likely that individual enantiomers of HP-VPD may be used as model compounds for the study of the metabolic processes involving short-chain fatty acid amides and for explanations of the anticonvulsant activity exerted by other valproylamides.

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