Stereoselective Pharmacokinetics and Pharmacodynamics of Propylisopropyl Acetamide, a CNS-Active Chiral Amide Analog of Valproic Acid

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Purpose. The purpose of this study was to evaluate there existed stereoselective effects in the pharmacokinetics, anticonvulsant activity, microsomal epoxide hydrolase (mEH) inhibition, and teratogenicity of the two enantiomers of propylisopropyl acetamide (PID), a CNS-active chiral amide analogue of valproic acid.

Methods. Racemic PID, as well as the individual enantiomers, were intravenously administered to six dogs in order to investigate the stereoselectivity in their pharmacokinetics. Anticonvulsant activity was evaluated in mice (ip) and rats (oral), mEH inhibition studies were performed in human liver microsomes, and teratogenicity was evaluated in an inbred susceptible mice strain.

Results. Following intravenous administration to dogs of the individual enantiomers, (R)-PID had significantly lower clearance and longer half-life than (S)-PID, however, the volumes of distribution were similar. In contrast, following intravenous administration of racemic PID, both enantiomers had similar pharmacokinetic parameters. In rats (oral), (R)-PID had a significantly lower ED_{50} in the maximal electroshock seizure test than (S)-PID; 16 and 25 mg/kg, respectively. PID enantiomers were non-teratogenic and did not demonstrate stereoselective mEH inhibition.

Conclusions. (R)-PID demonstrated better anticonvulsant activity, lower clearance and a longer half-life compared to (S)-PID. When racemic PID was administered, the clearance of (S)-PID was significantly reduced, reflecting an enantiomer-enantiomer interaction.

KEY WORDS: anticonvulsant activity; enantiomer-enantiomer interaction; enantiomers of propylisopropyl acetamide; microsomal epoxide hydrolase; pharmacokinetics, teratogenicity.

INTRODUCTION

In spite the introduction of several new antiepileptic drugs (AEDs), valproic acid (VPA, Fig. 1) remains as one of the four major antiepileptic drugs. Furthermore, VPA has found new indications in other central nervous system (CNS) disorders (1). VPA therapy has been associated with two rare, but severe adverse effects: hepatotoxicity and teratogenicity (2,3). As a result, the use of VPA has been restricted in two sub-populations: children, especially under the age of two years, and women of childbearing age. The most common congenital malformation in infants that were exposed *in utero* to VPA, during the first trimester of pregnancy, is spina bifida (4). The incidence of this form of neural tube defect (NTD) is approximately 20 times greater in infants that have been exposed *in utero* to VPA than infants of untreated epileptic pregnant women or the general population (5).

Valpromide (VPD, Fig. 1), the amide derivative of VPA, is 3–15 times more potent than VPA in animal models for anticonvulsant activity (6). VPD was found to be non-teratogenic in mice (7), therefore, Nau and co-workers empirically suggested a mandatory structural requirement for a teratogenic-VPA analogue was to possess a carboxylic acid moiety (7,8). However, in humans, unlike rats or dogs, VPD is a prodrug of VPA (9), therefore, its greater anticonvulsant efficacy and lack of teratogenicity in rodents have no clinical implications.

Propylisopropyl acetamide (PID) and the anxyolytic drug valnoctamide (VCD, Nirvanil®), are two VPD isomers possessing one and two chiral carbons, respectively (Fig. 1). Both racemic VCD and racemic PID showed anticonvulsant potencies in mice and rats similar to that of VPD (6,10). Unlike VPD, PID and VCD did not undergo metabolic hydrolysis in dogs to their corresponding inactive acids, propylisopropyl acetic acid and valnoctic acid, respectively (10,11). Similar to VPD, both racemic PID and racemic VCD were found to be potent inhibitors of the detoxifying enzyme (12), human microsomal epoxide hydrolase (mEH). The median inhibitory concentration (IC_{50}) value of PID (13) was within the *in vivo* concentration range obtained following iv administration to dogs (10). The IC_{50} value of racemic VCD (13,14) was within the *in vivo* concentration ranges obtained following oral administration in humans (14) and iv administration in dogs (11). All previous pharmacokinetic and pharmacodynamic data related to PID were obtained with the racemic mixture and non-enantioselective methods.

Stereoselectivity in pharmacodynamics and pharmacokinetics of VPA analogues was reported in several studies. Nau and co-workers demonstrated the (S)-enantiomer of the VPA metabolite 4-ene-VPA, was four times more teratogenic and

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ABBREVIATIONS: AED, antiepileptic drug; CL, total body plasma clearance; CL_{blood}, blood clearance; CL_{int}, intrinsic clearance; CMC, carboxymethyl cellulose; CNS, central nervous system; DID, diisopropyl acetamide; E, liver extraction ratio; ED_{50}, median effective concentration; fe, fraction of parent compound excreted unchanged in the urine; fu, fraction unbound to plasma proteins; GC, gas chromatography; IC_{50}, median inhibitory concentration; ip, intraperitoneal; iv, intravenous; LOQ, limit of quantification; mEH, microsomal epoxide hydrolase; MES, maximal electroshock seizure test; NTD, neural tube deffect; PED, S-(+)-1-phenyl-1,2-ethanediol; PID, Propylisopropyl acetamide; QC, quality control; sc Met, subcutaneous metrazol test; SD, standard deviation; SI, stereoselective index; SO, S-(+)-styrene oxide; $t^1/_2$, terminal half-life; TBME, *tert*-butyl methyl ether; TD_{50}, median neurotoxic dose; V_β, volume of distribution; VCD, valnoctamide; VPA, valproic acid; VPD, valpromide; Vss, volume of distribution at steady state.

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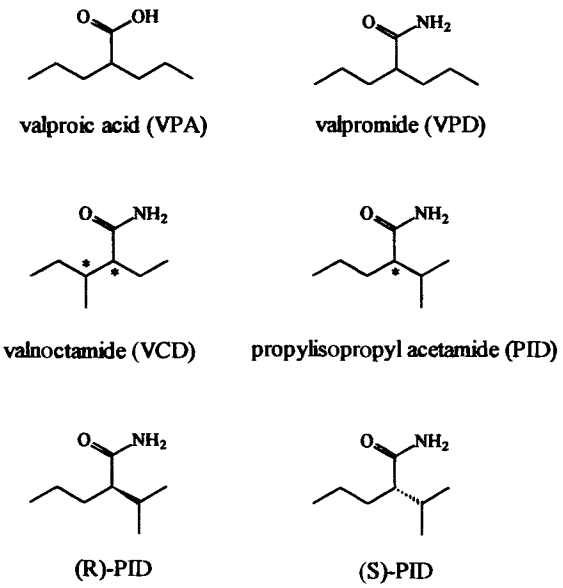

Fig. 1. Chemical structures of valproic acid (VPA), valpromide (VPD), valnoctamide (VCD) and propylisopropyl acetamide (PID).

embryotoxic in mice than its enantiomer, (R)-4-ene-VPA (15). Thus, the stereoselective index (SI), which is calculated as the ratio between the high to the low value of a pharmacokinetic or pharmacodynamic parameter, was equal to four (SI = 4). A similar trend in enantioselective teratogenicity was observed with the unsaturated VPA analogue, 4-yn-VPA (16). In this study the (S)-enantiomer was 7.5 times more teratogenic than the (R)-enantiomer (SI = 7.5).

VCD was recently found to possess stereoselective pharmacokinetics following oral administration of the racemate, in both healthy human subjects and epileptic patients (17). In that study, (2S,3R)-valnoctamide (18) had a significantly larger clearance and a shorter half-life than its enantiomer and two diastereomers. Consequently, the aim of the present study was to evaluate the stereoselective pharmacokinetics and pharmacodynamics of PID enantiomers. Stereoselective pharmacodynamic analysis was conducted in regard to the anticonvulsant activity and the untoward effects: neurotoxicity, teratogenicity, and mEH inhibition.

MATERIALS AND METHODS

Chemicals

Enantiomerically pure (S)-PID and (R)-PID (enantiomeric excess > 99.4%) were obtained by asymmetric synthesis (19). All solvents were of analytical or chromatographic grades.

Pharmacokinetic Studies

The Institutional Animal Care and Use Committee at the Hebrew University of Jerusalem approved this study. Pharmacokinetic experiments were carried out on six mongrel dogs weighing 18–25 kg. Dogs were brought to the lab repeatedly every two-three weeks for crossover experiments after an overnight fast. Each dog was inserted with a urinary (Levin's tube, Pennine Healthcare, Derby, UK) and two venous (20G/32 mm Venflon 2, Ohmeda, Helsingborg, Sweden) catheters located on different legs.

Dogs were randomly assigned to treatments and received an intravenous bolus dose of each individual PID enantiomer (10 mg/kg) or the racemic mixture (20 mg/kg), dissolved prior to administration in 2 ml of 96% ethyl alcohol for injection. Venous blood samples (6 ml) were withdrawn via an indwelling catheter (from the catheter other than the one used for drug administration) and transferred into heparinized tubes. The blood samples were centrifuged at 3000 g for 10 minutes, plasma was separated and stored at −20°C until analyzed. Blood samples were collected at 5, 10, 20, 30, 45 minutes and 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, and 12 hours after injection. Urine samples were collected at 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 hours after injection. The urine volume was recorded and an aliquot stored at −20°C until analyzed.

Enantioselective Gas Chromatographic (GC) Assay

Plasma Sample

The internal standard (2 μg diisopropyl acetamide-DID) and 5 ml of *tert*-butyl methyl ether (TBME) was added to plasma (0.5 ml). After vortexing (30 sec) the tubes were centrifuged at 3000 g for 10 minutes. The organic phase was separated and dried under reduced pressure using a vortex evaporator (Buchler Instruments, Lenexa, KS, U.S.A.). The dry residue was reconstituted in 150 μl of chloroform, which was evaporated without vortexing. The dry residue was reconstituted in 35 μl of chloroform and 2 μl were injected into the GC apparatus.

Urine Samples

The internal standard (2 μg DID), 2M potassium hydroxide solution (0.1 ml) and 5 ml of TBME was added to urine (0.5 ml). After vortexing (30 sec) the tubes were centrifuged at 3000 g for 10 minutes. To the separated organic phase bi-distilled water (0.2 ml) was added and the tubes were vortexed (30 sec) and centrifuged at 3000 g for 10 min. The organic phase was separated and evaporated under reduced pressure. The dry residue was reconstituted with 70 μl of chloroform and 2 μl were injected into the GC apparatus.

The GC apparatus consisted of HP 5890 series II gas chromatograph equipped with a capillary split inlet, HP 7673 automatic injector, flame ionization detector and HP 3396-A integrator. Enantioselective 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-cyclodextrin (20) as the stationary phase and nitrogen as carrier gas. Column head pressure was set at 50 KPa, split ratio 1:8 (plasma samples) and 1:17 (urine samples), oven temperature 120°C, injector at 250°C and detector at 300° C. At these conditions the two enantiomers had baseline separation (19) with retention times of 4.6 min for (S)-PID and 5.1 min for (R)-PID.

Plasma Protein Binding and Partitioning

Protein binding of PID enantiomers to plasma proteins was assessed both *in vivo* and *in vitro* using ultrafiltration kits (Centrisart-C30, Sartorius, Gottingen, Germany) with a 20,000 molecular weight cut-off at a total PID plasma concentration

range of 2–20 μg/ml. Plasma for the *in vivo* binding assay was taken from samples obtained during the pharmacokinetic experiments whereas blank dog plasma spiked with individual PID enantiomers or the racemate was used for *in vitro* binding. Assay procedure: duplicate plasma samples (3 ml at 37°C) were transferred into the ultrafiltration tubes (the membranes pre-rinsed with bi-distilled water to remove glycerol, which interferes with the GC analysis) and centrifuged 45 min at 4000 g to obtain 0.35–0.4 ml of the ultrafiltrate. To the ultrafiltrate (0.3 ml) was added the internal standard (1.2 μg DID), 5 ml TBME and processed further as the plasma samples. The free (unbound) fraction (fu) was calculated from the quotient of PID concentrationin the ultrafiltrate by the total PID plasma concentration.

Blood to plasma partitioning was determined as previously published (10) with spiked (R)-PID and (S)-PID at 1, 4, and 16 μg/ml (individually and concomitantly).

Pharmacokinetic Calculations

The pharmacokinetic parameters of PID enantiomers were determined by non-compartmental analysis using the pharmacokinetic software package WinNonlin version 1.1 (SCI Software, Lexington KY, U.S.A.). Blood clearance (CL_{blood}) was calculated from the quotient of CL to the blood to plasma partitioning coefficient. The fraction of PID excreted unchanged in urine (fe) was calculated from the cumulative amount of drug excreted intact in the urine (U_∞)/Dose. The liver extraction ratio (E) was calculated from the quotient of CL_{blood} and liver blood flow (21), assuming the liver was the major metabolic site of PID and utilizing dog liver blood flow data (1.85 L/h/kg) as published by Davies and Morris (22). Intrinsic Clearance (CL_{int}) was calculated from the quotient: CL/fu(1-E). Comparative statistical analysis of the pharmacokinetic parameters was done using two-tailed paired t-tests.

Anticonvulsant Evaluation

Anticonvulsant activity and neurotoxicity were tested at the NIH-Epilepsy Branch in Carworth Farm No. 1 mice following ip injection and in Sprague-Dawley rats following oral administration (23). The testing procedure included the following models: (a) maximal electroshock seizure (MES) test, which measures seizure spread, (b) subcutaneous metrazol (sc Met) test, which measures seizure threshold; and (c) rotorod ataxia test, which assesses minimal neurotoxicity (TD_{50}). Each ED_{50} and TD_{50} value was calculated from dose-response curves that were obtained from 25–50 animals each. Analysis for statistical significance between the dose-response curves was done by means of probits. ED_{50} values were compared using the 95% confidence intervals of the log transforms provided by the probitanalysis.

Microsomal Epoxide Hydrolase (mEH) Inhibition Assay

The *in vitro* mEH inhibition potency of PID enantiomers was tested in human liver microsomes, prepared from liver #135 (24), with S-(+)-styrene oxide (SO) as substrate at 25 μM (equals the Km). The formation rate of the product, (S)-(+)-1-phenyl-1,2-ethanediol (PED), was measured in microsomal incubations, as previously described (25). Inhibitors were added as 15 μl methanolic solutions (final organic solvents concentration was 1%). Inhibition reactions were investigated in triplicates at five concentrations ranging from 4 to 30 μM. VPD at a concentration of 5 μM (equal to its IC_{50}) was used as a positive control.

PED was extracted from the incubation mixtures and assayed using a published HPLC procedure (25). Internal standard was felbamate 1.6 μg. Extracting solvent was 7 ml TBME. Mobile phase composition was 20% acetonitrile and 10% methanol in water.

Teratogenicity Study

Teratogenicity was evaluated in the highly inbred SWV mice strain on the basis of its known susceptibility to VPA-induced NTDs (26) and according to a published procedure (27). Dams were randomly assigned to the following treatments: racemic PID, (S)-PID, (R)-PID, VPA as an active control or the vehicle (1% carboxymethyl cellulose- CMC). At day 8.5 of gestation, each dam was exposed to a single intraperitoneal (ip) injection of the tested compound (500–600 mg/kg) or the vehicle. At day 18.5 of gestation the dams were sacrificed by cervical dislocation, the location of all-viable fetuses and resorption sites were recorded, and the fetuses were examined for the presence of exencephaly (NTD).

RESULTS

Pharmacokinetics

The plasma concentrations vs. time plots of (R)-PID and (S)-PID, following iv administration to six dogs of the racemic mixture and the individual enantiomers are presented in Figs. 2 and 3, respectively. The pharmacokinetic parameters of (R)-PID and (S)-PID are presented in Table I. The assay was linear up to 30 mg/L and the limit of quantification (LOQ) was 0.2 mg/L (plasma) and 1 mg/L (urine) for both enantiomers. Quality control (QC) samples from plasma had an accuracy of 0.5–2.6% (11.7% at LOQ) for (R)-PID and 0.3–5.0% (9. 1% at LOQ) for (S)-PID. Reproducibility of the QC samples was 3.6–6.8% (15.9% at LOQ) for (R)-PID and 5.2–7.2% (26.5% at LOQ) for (S)-PID. QC samples from urine had an accuracy of 3.1–10.4% with reproducibility of 7.8–13.9% for the two enantiomers (including LOQ).

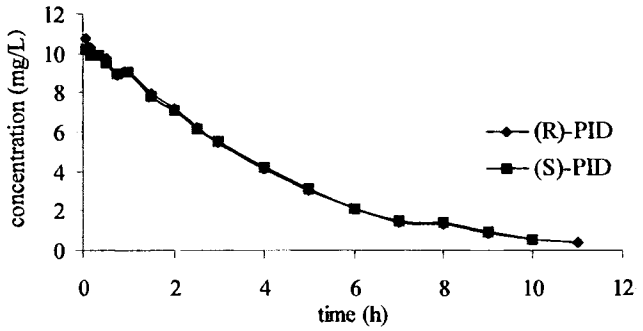

Fig. 2. Mean plasma concentrations vs. time plots of PID enantiomers following iv administration of the racemic mixture (20 mg/kg) to six dogs.

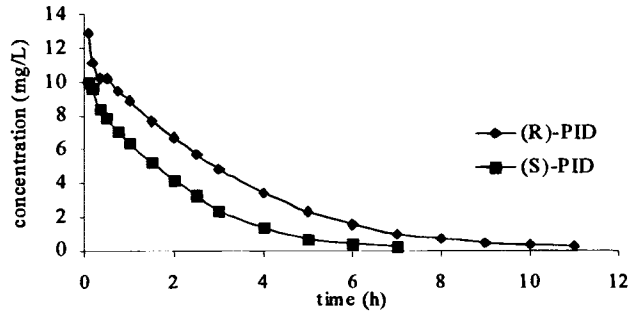

Fig. 3. Mean plasma concentrations vs. time plots of PID enantiomers following iv administration of the individual enantiomers (10 mg/kg) to six dogs.

Partitioning of the individual PID enantiomers between blood and plasma was independent of their concentration in blood, nor was it dependent upon the presence of the antipode. Mean partitioning (± SD) was: (R)-PID 1.14 ± 0.12 (individually) and 1.18 ± 0.16 (racemate); (S)-PID 1.23 ± 0.10 (individually) and 1.22 ± 0.16 (racemate). Binding of PID enantiomers to plasma proteins at concentrations of 2–20 μg/ml was found to be enantioselective (Table I): The free fraction of (R)-PID in plasma was 66.2 ± 5.0% (individually) and 68.6 ± 7.5% (racemate). The protein binding of (R)-PID was significantly lower ($p < 0.05$) than that of (S)-PID 72.0 ± 5.5% (individually) and 75.5 ± 8.5% (racemate). The enantioselective binding to plasma proteins was independent of the total plasma concentration and unaffected by presence of the antipode.

All pharmacokinetic parameters of PID enantiomers were almost identical following administration of racemic PID, with SI values of about unity (Table I). Only CL_{int} was found to be significantly different ($p < 0.05$) between enantiomers: 7.7 L/h for (R)-PID and 7.1 L/h for (S)-PID. Contrary to administration of racemic PID, administration of the individual enantiomers resulted in statistically significant ($p < 0.05$) enantioselectivity in almost all pharmacokinetic parameters (Table I). Parameter values for (R) PID vs. (S)-PID are: CL (6.8 vs. 11.3 L/h, respectively), CL_{int} (8.7 vs. 12.2 L/h), t1/2 (1.37 vs. 1.04 h), MRT (2.57 vs. 1.83 h), E (15.0 vs. 23.7%) and fe (0.50 vs. 0.39%). No chiral inversion was observed following administration of the two individual enantiomers.

Anticonvulsant Activity and Neurotoxicity

The anticonvulsant activity and neurotoxicity data of PID enantiomers are presented in Table II. Anticonvulsant activity: In mice following ip administration, (R)-PID had an ED_{50} of 110 mg/kg in the MES model, (S)-PID had an ED_{50} of 145 mg/kg (SI = 1.32) and racemic PID an ED_{50} of 122 mg/kg. In the sc Met model in mice, (R)-PID had an ED_{50} of 67 mg/kg, (S)-PID had an ED_{50} of 80 mg/kg (SI = 1.19) and racemic PID an ED_{50} of 77 mg/kg. In rats following oral administration, (R)-PID was significantly more potent ($p < 0.05$) in the MES test model than (S)-PID: ED_{50} values were 16 and 25 mg/kg, respectively (SI = 1.58). In addition, in the MES test, (R)-PID was significantly more potent ($p < 0.05$) in rats than racemic PID with an ED_{50} of 31 mg/kg.In the sc Met model in rats racemic PID had an ED_{50} of 37 mg/kg.

In mice and rats (R)-PID had a TD_{50} below 145 and below 100 mg/kg, respectively. In mice and rats (S)-PID had a TD_{50} of 118 and below 100 mg/kg, respectively. In mice and rats racemic PID had a TD_{50} of 112 and 63 mg/kg, respectively.

mEH Inhibition

Inhibition of mEH mediated SO hydrolysis by racemic PID, (S)-PID, and (R)-PID was examined in microsomal suspensions prepared from a single human liver. Measures of mEH mediated formation of PED expressed as remaining activity of the enzyme are presented in Figure 4. Racemic PID was found to have an IC_{50} of 9.3 μM, which was in between the IC_{50} values of the individual enantiomers: 8.5 μM for (S)-PID and 11.8 μM for (R)-PID (SI = 1.39). Average non-enzymatic (background) SO hydrolysis rates were 6.7 ± 0.5% of control hydrolysis rates. VPD (5 μM) inhibited mEH by 51.5 ± 2.4% of the control rates. QC samples had an accuracy of 0.6–2.7% and reproducibility of 1.5–5.6%.

Teratogenicity

Teratogenicity in the SWV mice strain was evaluated following a single ip injection to ten pregnant dams at day 8.5 of

Table I. Mean (±SD) Pharmacokinetic Parameters of PID Enantiomers Obtained Following iv Administration of the Individual Enantiomers (10 mg/kg) and the Racemic Mixture (20 mg/kg) to Six Dogs

Administration:	Individual enantiomers			Racemic mixture			I/R[b]	I/R[b]
Parameter	(R)-PID	(S)-PID	SI[a]	(R)-PID	(S)-PID	SI[a]	(R)-PID	(S)-PID
CL (L/h)	6.8 ± 1.6[c]	11.3 ± 1.5[c]	1.66 ± 0.32	6.0 ± 1.7	6.0 ± 1.8	1.0 ± 0.05	1.13 ± 0.15[d]	1.88 ± 0.51[d]
CLint (L/h)	8.7 ± 1.6[c]	12.2 ± 1.4[c]	1.42 ± 0.22	7.7 ± 1.9[c]	7.1 ± 1.9[c]	1.1 ± 0.05	1.14 ± 0.16[d]	1.86 ± 0.40[d]
Vss (L)	16.0 ± 1.2	19.1 ± 2.7	1.19 ± 0.17	15.7 ± 1.5	15.7 ± 0.9	1.0 ± 0.05	1.02 ± 0.10	1.22 ± 0.14[d]
V_β (L)	13.1 ± 2.2	17.0 ± 2.9	1.29 ± 0.31	10.2 ± 0.9	10.4 ± 1.4	1.0 ± 0.16	1.28 ± 0.22[d]	1.63 ± 0.45[d]
$t^1/_2$ (h)	1.37 ± 0.2[c]	1.04 ± 0.1[c]	1.34 ± 0.15	1.25 ± 0.3	1.27 ± 0.3	1.0 ± 0.15	1.10 ± 0.26	0.82 ± 0.20
MRT (h)	2.57 ± 0.5[c]	1.83 ± 0.3[c]	1.42 ± 0.17	2.83 ± 0.6	2.87 ± 0.7	1.0 ± 0.04	0.91 ± 0.11	0.64 ± 0.12[d]
E (%)	15.0 ± 4.6[c]	23.7 ± 3.5[c]	1.58 ± 0.33	12.5 ± 4.0	12.1 ± 4.1	1.0 ± 0.05	1.19 ± 0.15	1.90 ± 0.49[d]
fe (%)	0.50 ± 0.35[c]	0.39 ± 0.36[c]	1.70 ± 0.49	0.63 ± 0.45	0.66 ± 0.46	1.0 ± 0.06	1.00 ± 0.37	0.50 ± 0.33[d]
fu(%)	66.2 ± 5.0[c]	72.0 ± 5.5[c]	1.13 ± 0.03	68.6 ± 7.5[c]	75.5 ± 8.5[c]	1.1 ± 0.05	0.98 ± 0.19	1.01 ± 0.23

[a] Stereoselective index (SI): the ratio between the high and the low value.
[b] Parameter ratio between the two modes of administration (I/R): Individual/Racemic.
[c] Statistical significant difference was observed between the enantiomers (paired t-test, $p < 0.05$).
[d] Statistical significant difference was observed between the two administrations, i.e. Individual vs. Racemic mixture (paired t-test, $p < 0.05$).

Table II. Anticonvulsant Activity and Neurotoxicity Obtained Following ip Administration to Mice and Oral Administration to Rats

Compound	MES-ED_{50} mg/kg (CI[a])	sc Met-ED_{50} mg/kg (CI[a])	Neurotoxicity-TD_{50} mg/kg (CI[a])	PI, MES[b]	PI, sc Met[b]
Mice-ip					
Racemic PID	122 (106–140)	77 (66–85)	112 (106–118)	0.92	1.46
(R)-PID	110 (78–133)	67 (58–80)	<145	<1.3	<2.2
(S)-PID	145 (111–169)	80 (62–115)	118 (89–137)	0.81	1.46
SI[c]	1.32	1.19	<1.23		
Rats-oral					
Racemic-PID	31 (21–39)[d]	37 (23–58)	63 (48–76)	2.05	1.72
(R)-PID	16 (10–23)	<100	<100	<6.3	
(S)-PID	25 (19–30)[d]				
SI[c]	1.58				

[a] 95% confidence interval (CI).
[b] Protective index (PI, safety margin): TD_{50}/ED_{50}.
[c] Stereoselective index (SI): (S)-PID value/(R)-PID value.
[d] Significantly higher than (R)-PID ($p < 0.05$) after oral administration to rats.

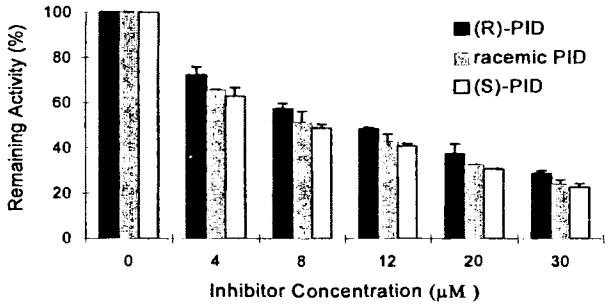

Fig. 4. *In vitro* inhibition of microsomal epoxide hydrolase (mEH) by racemic propylisopropyl acetamide (PID) and its individual enantiomers: (S)-PID and (R)-PID. The data were obtained by measuring formation rates of (S)-(+)-1-phenyl-1,2-ethanediol (PED) from a single human liver with (S)-(+)-styrene oxide (SO) as substrate at 25 μM.

gestation. Teratogenicity data of racemic PID and the individual enantiomers are presented in Table III. Racemic PID and (R)-PID were administered at 600 mg/kg, whereas (S)-PID was injected at 500 mg/kg, due to a few incidences of maternal lethality observed at 600 mg/kg. Both racemic PID and (R)-PID failed to induce exencephaly in the SWV embryos, whereas a single case of exencephaly (0.8%) was observed in the (S)-PID group (not significantly different from control values, $p > 0.05$). Resorption rates induced by racemic PID, (S)-PID and (R)-PID were 6.3%, 6.1% and 10.4%, respectively (not significantly different from control values). In contrast to PID, administration of VPA (600 mg/kg) to pregnant SWV dams induced 73.3% exencephaly and 9.3% resorptions.

Discussion

In vivo inhibition of mEH constitutes a possible drawback to the development of PID enantiomers as a new stereospecific AED since it may have toxicological implications (12,28). In this study, the *in vitro* mEH inhibition by PID enantiomers was shown to be relatively insensitive to the stereochemical configuration, as IC_{50} values of 8.5 and 11.8 μM (1.2 and 1.7 mg/L, respectively) were obtained (SI = 1.39). This degree of stereoselectivity is insignificant, since IC_{50} values for both enantiomers are within their in vivo concentration range obtained following iv administration to dogs (Fig. 2 and 3). Assuming that these unsubstituted amides inhibit mEH competitively (29), under our experimental conditions (where the substrate concentration equals the Km of the enzyme), the Ki of each PID enantiomer would be approximately half of its IC_{50}, i.e., 4–6 μM (30). As this degree of enantioselectivity is nil and insignificant, we found it unnecessary to further pursue Ki values for the individual PID enantiomers.

Neither racemic PID, nor the individual enantiomers (that were administered at twice the dosage compared to when the

Table III. Teratogenic Effects of Racemic PID, (R)-PID and (S)-PID in SWV Mice Compared to Controls and VPA

Compound	Dose mg/kg	Litters n	Implants n	Resorptions[a] n (%)	Live Fetuses n	Exencephaly[b] n (%)
Racemic PID	600	10	126	8 (6.3)	118	0
(R)-PID	600	10	134	14 (10.4)	120	0
(S)-PID	500	10	131	8 (6.1)	123	1 (0.8)
Control[c]	0	12	152	13 (8.6)	139	0
VPA[d]	600	13	148	13 (9.3)	135	99 (73.3)

[a] Percent of implants.
[b] Percent of live fetuses.
[c] Controls were administered with the vehicle: 1% aqueous carboxymethyl cellulose (CMC).
[d] VPA was administered in: 1% CMC and 1% cyclodextrin aqueous solution.

racemic mixture was administered) induced a significant increase in the incidence of NTDs. These observations support our findings in NMRI mice (19) and are in accordance to the empirical hypothesis postulated by Hendrickx and Nau, who suggested that a teratogenic VPA analogue must possess a carboxylic acid moiety (7). This hypothesis was also supported by a recent study demonstrating that racemic VCD is non-teratogenic as well (31). Nevertheless, rigorous substantiation of this hypothesis requires additional studies of valproyl amide analogues and their corresponding teratogenic valproyl acid analogues.

The anticonvulsant activity data suggests that (R)-PID is a more potent anticonvulsant than (S)-PID (Table II). Following ip injection to mice, the ED_{50} of (R)-PID was 19–32% lower than (S)-PID, however the differences were not statistically significant ($p > 0.05$). In contrast to mice, statistically significant stereoselectivity ($p > 0.05$) was observed after oral administration to rats, where the MES-ED_{50} of (R)-PID was 58% lower than that of (S)-PID (SI = 1.58).

When PID enantiomers were administered individually, stereoselective pharmacokinetics was observed in all parameters except Vss and V_β (Table I). Compared to (R)-PID, (S)-PID had a higher CL (SI = 1.66), a higher CL_{int} (SI = 1.42), a shorter half-life (SI = 1.34) and a larger liver extraction ratio (SI = 1.58). It is important to note that in this study stereoselectivity was demonstrated in highly hybridized pharmacokinetic parameters such as t1/2, clearance, and extraction ratio (32). Consequently, a greater degree of stereoselectivity would be expected in low-hybridized pharmacokinetic parameters (reflecting specific drug- macromolecule interactions) such as intrinsic formation clearances of specific metabolites.

Contrary to administration of the individual PID enantiomers, administration of racemic PID resulted in similar plasma concentrations and pharmacokinetic parameters for both enantiomers which reflects a pharmacokinetic enantiomer-enantiomer interaction. Comparing the pharmacokinetic parameters of each enantiomer when administered individually vs. in the racemic mixture (I/R, Table I) shows that the largest changes occurred with (S)-PID. Most importantly, (S)-PID had an 86–88% reduction in CL and CL_{int} ($p < 0.05$) when administered with its enantiomer, (R)-PID. On the other hand, the t1/2 of (S)-PID was not significantly prolonged after administration of the racemic mixture, due to concomitant changes in the volume of distribution. Nevertheless, the MRT of (S)-PID was significantly longer and the E significantly smaller when administered in the racemic mixture. Contrary to (S)-PID only modest changes were observed with (R)-PID when it was administered concomitantly with (S)-PID. The most probable explanation for the decrease in clearances of (S)-PID and (R)-PID is mutual inhibition of metabolism. The different degrees of inhibition observed for PID enantiomers in this study may probably be explained by different affinities of each enantiomer to the metabolizing enzyme(s).

The observed stereoselectivity in the anticonvulsant activity in favor of (R)-PID in rats may arise from stereoselectivity in pharmacodynamic (a more potent interaction with an endogenous chiral receptor or ion channel) and/or pharmacokinetic processes. Our present data in dogs show pharmacokinetic superiority of (R)-PID when administered individually. Regarding the latter mechanism, it is interesting to note that valproyl amide analogues exhibit similar pharmacokinetics in rats and dogs (33). If the same phenomenon occurs in rats, stereoselectivity in pharmacokinetics may contribute to or explain the more favorable anticonvulsant profile of (R)-PID relative to (S)-PID.

From the present study, we can conclude that the enantiomers of the CNS-active valproyl amide analogue-PID were non-teratogenic and demonstrated no significant stereoselectivity with respect to mEH inhibition. On the other hand, enantioselectivity in favor of (R)-PID was demonstrated in the anticonvulsant activity and pharmacokinetics. In addition, an enantiomer-enantiomer interaction between PID enantiomers was observed, probably due to metabolic inhibition. The present study serves also to underline the importance of investigating the pharmacokinetics of chiral drugs following administration of the individual enantiomers as well as the racemic mixture, as enantiomer-enantiomer interactions might occur, resulting in potential misinterpretation of related pharmacokinetic and pharmacodynamic data.

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