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Pharmacokinetic Interaction in Rabbits between a New Anticonvulsant, DL-3-Hydroxy-3-ethyl-3-phenylpropionamide, and Phenytoin

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Abstract

The effect of phenytoin on the disposition of DL-3-hydroxy-3-ethyl-3-phenylpropionamide (HEPP) has been studied in New Zealand white rabbits.

Plasma HEPP levels decreased when the drug was administered with phenytoin. The area under the plasma concentration–time curve was reduced by 56·49% (from 43·23 ± 7·0 to 18·81 ± 2·03 μg h mL^{-1}), the elimination half-life was also significantly ($P < 0·01$) reduced (from 2·68 ± 0·35 to 1·04 ± 0·07 h) and the clearance was increased (from 0·35 to 0·81 L h^{-1} kg^{-1}). In-vitro protein binding to bovine serum albumin (BSA) and plasma was evaluated by equilibrium dialysis. Plasma protein binding was low (between 33·69 and 37·43% at concentrations ranging from 6·25 to 100 μg mL^{-1}). The compound binds preferentially to albumin with an association constant (Ka) of 3·81 × 10^3 M^{-1} at 37°C.

The results suggest a pharmacokinetic interaction between phenytoin and HEPP, probably on the drug-metabolizing enzyme system in the liver.

The pharmacological activity of a new series of anticonvulsant drugs, DL-4-hydroxy-4-ethyl-4-phenylbutyramide (HEPB) and its inferior homologues DL-3-hydroxy-3-ethyl-3-phenylpropion-amide (HEPP) and DL-2-hydroxy-2-ethyl-2-phenyl-acetamide (HEPA) has been studied in animals (Meza et al 1990). The results indicated that the compounds might be of value in the treatment of generalized absence seizures and because HEPP was found to be least neurotoxic it was selected for toxicological and neurochemical studies (Chávez & Martínez de Muñóz 1996; Mendoza et al 1996).

HEPP has a high potency against pentylenetetrazole-induced seizures in rats and there is a direct correlation between the anticonvulsant effect and the time-course of the drug in the brain (Javier & Martínez de Muñóz 1996). HEPP has also been shown to be highly effective against the GABA withdrawal syndrome, a new model of epileptogenesis. This effect is remarkable because the common drugs of choice for status epilepticus are ineffective against that syndrome (Brailowsky & Montiel 1996). Because these results indicated that HEPP might be a promising anticonvulsant, its safety, efficacy and pharmacokinetics were tested in animals (Gómez & Lehmann 1995; Gómez et al 1995). The drug is now under clinical phase I study.

In Mexico, phenytoin is the drug of choice for the treatment of generalized tonic–clonic seizures and status epilepticus (Brailowsky & Montiel 1996). Despite the trend towards monotherapy, phenytoin is often used with other anticonvulsant drugs. Because phenytoin has been shown to induce liver enzyme systems (Johannessen 1990; Leppik & Wolff 1993), the objective of this study was to investigate the influence of phenytoin on the disposition of HEPP, and vice versa.

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Materials and Methods

Chemicals

HEPP and HEPA were kindly provided by Armstrong Laboratories (Mexico). Phenytoin (Hidantoina; Rudefsa Laboratories, lot 600743) was supplied by the National Institute of Neurology and Neurosurgery of Mexico. HPLC-grade acetonitrile was purchased from Mallinckrodt. Bovine serum albumin (crystallized, essentially fatty acid-free) was obtained from Sigma. Fresh plasma was

obtained from the hospital blood bank. The dialysis cell and the Spectra/Por membranes (molecular weight cut-off, 12 000–14 000) were obtained from Spectrum Medical Industries. All chemicals and solvents used were of analytical grade.

The extent of binding of HEPP to human plasma and to bovine serum albumin

Plasma or bovine serum albumin (1 mL) was dialysed against buffer (1 mL) in the dialysis cell. HEPP was added to the plasma compartment at concentrations of 6·25, 12·5, 25, 50 or 100 μg mL^{-1}. After 3 h incubation at 37°C and stirring at 30 rev min^{-1}, samples (0·5 mL) were removed from both compartments and analysed the same day by an HPLC method reported elsewhere (Gómez & Lehmann 1995). No corrections were made for drug binding to the dialysis membrane (< 1·59%), for the Donnan effect, or for changes in albumin content before and after dialysis. Total protein concentration was measured by the Biuret method.

Pharmacokinetic study

The study was performed on nine male New Zealand white rabbits which were randomly assigned to three groups (n = 3 in each group) according to a balanced incomplete block design of three treatments and two periods with a wash-out period of one month between the two phases of the study. Group I received intraperitoneal phenytoin (30 mg kg^{-1} every 12 hours) for five days (treatment 1). Group II received a single intravenous dose of HEPP (15 mg kg^{-1}; treatment 2). Group III received intraperitoneal phenytoin (30 mg kg^{-1} every 12 h) for five days followed by a single intravenous dose of HEPP (15 mg kg^{-1}; treatment 3). One month later, group I received treatment 3, group II received treatment 1, and group III received treatment 2.

Blood samples were drawn from the marginal ear vein at time 0 (just after the eleventh dose of phenytoin and before dosing with HEPP) and after 1·0, 2·0, 3·0, 5·0, 8·0, 12·0 and 24·0 h, for the treatment 1; at time 0, 0·083, 0·25, 0·5, 0·75, 1·0, 2·0, 3·0, 5·0, 8·0 and 10·0 h for treatment 2 and after 0·083, 0·25, 0·5, 0·75, 1·0, 2·0, 3·0, 4·0, 5·0, 8·0, 10·0, 12·0 and 24·0 h for the treatment 3.

HEPP assay

Analysis of HEPP was performed by HPLC (Gómez & Lehmann 1995). Briefly, a methanolic solution of HEPA (100 μg mL^{-1}, internal standard; 50 μL) was added to the sample (500 μL) then sodium hydroxide solution (2 M; 500 μL) was added, the sample was mixed, and then phosphate buffer (0·1 M, pH 8; 500 μL) was added and the sample mixed again. The sample was then extracted with dichloromethane (5 mL) by mixing for 15 min in a horizontal shaker. After centrifugation for 15 min at 1650 *g*, the organic layer was separated and evaporated to dryness. The residue was dissolved in mobile phase (acetonitrile–water, 25 : 75; 200 μL) and 100 μL of this were injected into a Gold-System liquid chromatograph fitted with a Spherisorb ODS C_{18} column (200 mm × 4·6 mm; 5-μm particles). The flow-rate was 0·8 mL min^{-1} and ultraviolet detection was performed at 219 nm. The relationship between response and concentration was linear in the range 0·39 to 100 μg mL^{-1}. Mean intra-day and inter-day reproducibilities were 4·3 and 4·2%, respectively. The recovery ranged between 91·7 and 100·5%.

Phenytoin assay

Plasma concentrations of phenytoin were determined by an enzyme immunoassay method (EMIT; Syva Company, lot 6B009 UL-H1A).

Pharmacokinetic data analysis

The area under the plasma concentration–time curve (AUC) was determined by the trapezoidal method. The area from the last concentration point (C_{last}) to infinity was calculated as C_{last}/λ_n, where λ_n was the terminal elimination rate constant. The mean residence time (MRT) was calculated for each rabbit by use of the equation MRT = AUMC/AUC, where AUMC is the area under the first moment curve. The volume of distribution at steady state was calculated by multiplying CL and MRT, where CL, the total body clearance, was determined from the equation CL = D/AUC, where D is the dose kg^{-1}.

Statistical analysis

Pharmacokinetic data were analysed by one-way analysis of variance and by Student's unpaired *t*-test. Differences were considered significant when $P < 0.01$. Results are expressed as means ± s.d.

Results and Discussion

The extent of binding of HEPP to human plasma and to bovine serum albumin

The binding of HEPP to plasma and to BSA was between 32·57 and 38·88% and between 30·88 and 37·43%, respectively, indicating that HEPP is bound exclusively to BSA. The association constant K_a was 3·81 × 10^3 M^{-1}.

Influence of HEPP on the pharmacokinetics of phenytoin

The dependence of average concentrations of phenytoin in plasma on the time after administration of 2×30 mg kg^{-1} day^{-1} were highly variable at each sampling time. This inter-individual variability has been reported by numerous workers and arises mainly because the pharmacokinetics of the drug are dose-dependent. In our study it was not possible to observe concentration-dependence in the rate of elimination of phenytoin and we could not, therefore, calculate K_m and V_{max}. For this reason, the parameters AUC_{0-t}, $AUC_{0-\infty}$, half-life ($t_{1/2}$) and MRT were used to evaluate the interaction.

Curves for phenytoin alone and for phenytoin combined with HEPP were almost superimposable and there were no significant differences between the pharmacokinetic parameters found for the two treatments.

Influence of phenytoin on the pharmacokinetics of HEPP

A particularly important feature of phenytoin is that it has enzyme-inducing properties which can stimulate the metabolism of other drugs. It was, therefore, considered of value to establish whether phenytoin influences the pharmacokinetics of HEPP.

Mean HEPP plasma concentrations with and without administration of phenytoin are shown in Figure 1. It is apparent that plasma disappearance of HEPP was significantly accelerated by administration of phenytoin and that, as a result, HEPP levels were significantly reduced. The pharmacokinetics of HEPP were described by a two-compartment open model. Table 1 summarizes the effect of phenytoin on the pharmacokinetics of HEPP. $AUC_{0-\infty}$ for HEPP was reduced by 56·49% when the compound was

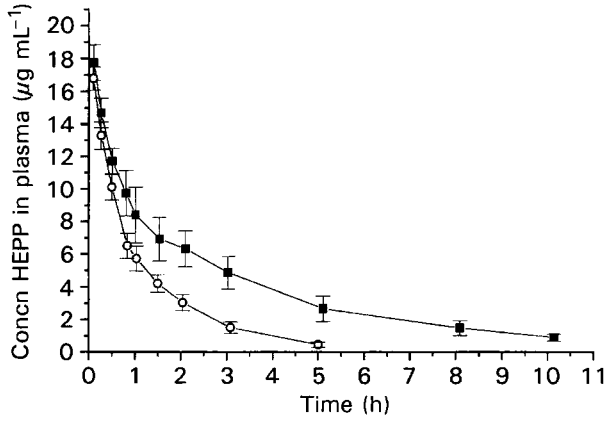

Figure 1. Plots of average plasma concentration against time after administration of HEPP to rabbits in the presence (■) and absence (○) of phenytoin. Values are means ± s.d. of results from six rabbits.

Table 1. Mean values of pharmacokinetic parameters of HEPP with and without administration of phenytoin in rabbits.

Parameter	Monotherapy (HEPP)	Combined therapy (HEPP + phenytoin)
AUC_{0-t} (μg h mL^{-1})	40·36 ± 6·22	17·56 ± 2·37*
$AUC_{0-\infty}$ (μg h mL^{-1})	43·23 ± 7·01	18·81 ± 2·03*
$t_{1/2}$ (h)	2·68 ± 0·35	1·05 ± 0·07*
CL (L h^{-1} kg^{-1})	0·35 ± 0·05	0·81 ± 0·09*
MRT (h)	3·53 ± 0·49	1·35 ± 0·10*
Vdss (L kg^{-1})	1·24 ± 0·18	1·09 ± 0·04

Results are means ± s.d. *$P < 0·01$, significantly different from result obtained in the absence of phenytoin. HEPP = DL-3-hydroxy-3-ethyl-3-phenylpropionamide; AUC = area under the plasma concentration = time curve; $t_{1/2}$ = half-life; CL = clearance; MRT = mean residence time; Vdss = volume of distribution at steady state.

administered with phenytoin ($P < 0·01$) and the parameters $t_{1/2}\beta$ (elimination half-life), MRT and total body clearance showed similar behaviour–elimination half-life dropped from 2·68 ± 0·35 h for rabbits on monotherapy to 1·05 ± 0·07 h for rabbits on combined therapy; MRT decreased from 3·53 ± 0·49 h to 1·35 ± 0·10 h and total body clearance was increased from 0·35 ± 0·05 to 0·81 ± 0·09 L h^{-1} kg^{-1}.

Interactions between phenytoin and other anticonvulsants have frequently been reported—clonazepam, valproate, methsuximide, felbamate, carbamazepine, primidone and phenobarbital are some examples (Levy 1995; Levy & Meldrum 1995). The majority of these are pharmacokinetic interactions and involve induction or inhibition of biotransformation or alteration of plasma-protein binding. Because in this work the possibility of a binding interaction was discounted (because of the low extent of binding to plasma proteins shown for HEPP), our results suggest strong induction by phenytoin of the metabolism of HEPP. When phenytoin acts as an enzyme inducer of microsomal P450, lower plasma concentrations, reduced half-life and, therefore, reduced therapeutic efficacy are observed (Conney 1967; Leppik & Wolff 1993; Levy 1995; Levy & Meldrum 1995). The current study was performed with rabbits taking into account the considerable trans-species homology in P450 isozymes between animal and man. In man, as in rabbit, phenytoin is eliminated primarily by hepatic metabolism via the cytochrome P450 monooxygenase system with the major metabolite being the 4-hydroxylation product 5-(4-hydroxyphenyl)-5-phenylhydantoin (Doecke et al 1990). There is no information available about the metabolism of HEPP, but our results imply that HEPP might be metabolized by cytochrome P450, perhaps of the same genetic subfamily on which

phenytoin acts as enzyme inductor. It will be necessary to perform other in-vitro studies and HEPP metabolism studies to determine the mechanisms involved in the biotransformation of this drug.

References

Brailowsky, S., Montiel, T. (1996) Efectos de la DL-3-hidroxi, 3-etil, 3-fenil propionamida (HEPP) sobre un nuevo modelo de status epilepticus focal: el síndrome de abtinencia al GABA (SAG). Arch. Neurocien (Mex). 1: 104–107

Chávez, J. L., Martínez de Muñóz, D. (1996) Mecanismo de acción del anticonvulsivante DL-3-hidroxi, 3-etil, 3-fenil propionamida (HEPP). Arch. Neurocien (Mex). 1: 173–177

Conney, A. H. (1967) Pharmacological implications of microsomal enzyme induction. Pharmacol Rev. 19: 317–366

Doecke, C., Sansom, L., McManus, M. (1990) Phenytoin 4-hydroxylation by rabbit liver P450IIC3 and identification of orthologs in human liver microsomes. Biochem. Biophys. Res. Commun. 166: 860–866

Gómez, L., Lehmann, F. (1995) Pharmacokinetics of the novel anticonvulsant HEPP after single intravenous administration of three different doses in dogs. Biopharm. Drug Disp. 16: 105–112

Gómez, L., Cuevas-Rolón, R., Lehmann, F. (1995) Disposition kinetics of HEPP in rats after intravenous, oral and intraperitoneal administration. Correlation of plasma and brain levels with the anticonvulsant effect. Biopharm. Drug Disp. 16: 77–89

Javier, A. M., Martínez de Muñóz, D. (1996) Espectro de acción antiepiléptica de la DL-4-hidroxi, 4-etil, 4-fenil butiramida y sus homólogos inferiores. Arch. Neurocien (Mex). 1: 76–80

Johannessen, S. (1990) Pharmacokinetics of Anti-epileptic Drugs and their Clinical Significance. Behav. Neurol. 3(Suppl. 1): 1–13

Leppik, Y. E., Wolff, D. L. (1993) Antiepileptic medication interactions. Neurol. Clin. 14: 905–921

Levy, R. (1995) Cytochrome P450 isozymes and antiepileptic drug interactions. Epilepsia 36 (Suppl. 5): S8–S13

Levy, M., Meldrum, M. (1995) Antiepileptic Drugs. Raven Press, USA, pp 283–338

Mendoza, F., López, M. L., Hernández, A. (1996) Estudio preeliminar de la hepatotoxicidad del anticonvulsivante DL-4-hidroxi, 4-etil, 4-fenil butiramida (HEPB) y sus homólogos inferiores (HEPP y HEPA). Arch. Neurocien (Mex). 1: 169–172

Meza, S., Zenteno, M., Carbajal, G. (1990) A new homologous series of anticonvulsants: phenyl–alcohol–amides. Arzneim. Forsch. 40: 1289–1291