Comparative Pharmacokinetic and Pharmacodynamic Analysis of Phthaloyl Glycine Derivatives with Potential Antiepileptic Activity

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Received January 19, 1994; accepted May 10, 1994

Glycine, in addition to GABA, is one of the most important neurotransmitter amino acids. The described structure pharmacokinetic pharmacodynamic relationships (SPPR) study explored the possibility of utilizing phthaloyl derivates of glycine as new antiepileptics. This was carried out by investigating the pharmacokinetics and pharmacodynamics (anticonvulsant activity and neurotoxicity) of the following four phthalimide derivatives: phthaloyl glycine, phthaloyl glycaminde, N,N-diethyl phthaloyl glycaminde and N,N-disopropyl phthaloyl glycaminde. Phthaloyl glycine did not demonstrate anticonvulsant activity, possibly because of its poor pharmacokinetics, high clearances, low volume of distribution and short half life. The three glycaminde derivatives showed anticonvulsant activity and had better pharmacokinetic profiles, longer half life and mean residence time, than phthaloyl glycine. Phthaloyl glycaminde was more potent than one of the major antiepileptic agents—valproic acid and showed a better margin between activity and neurotoxicity. The four investigated phthaloyl glycine derivates did not operate as chemical drug delivery systems (CDDS) of glycine, but acted rather as drugs on their own. Phthaloyl glycine was excreted unchanged in the urine while the urinary metabolites of the glycaminde derivatives were phthaloyl glycine and phthaloyl glycaminde. In this analogous series of phthalimide derivatives, minor chemical changes affected dramatically the compounds' pharmacokinetics. The current study demonstrates the benefit of the SSPR approach in developing and selecting a potent antiepileptic compound in intact animals based not only on its intrinsic pharmacodynamic activity, but also on its better pharmacokinetic profile.

KEY WORDS: phthaloyl glycine; phthaloyl glycaminde and its N,N-dialkyl analogues; anticonvulsant activity; structure pharmacokinetic pharmacodynamic relationships (SPPR).

INTRODUCTION

GABA is an inhibitory neurotransmitter, which plays an important role in the control of neuronal activity in the mammalian central nervous system (CNS). A deficiency in brain GABA levels has been found to cause convulsions or epilepsy (1,2). Therefore, drugs which increase the amount of GABA available in the brain for neurotransmission have the potential of becoming antiepileptic agents. The first GABA derivative to become an antiepileptic was progabide which was approved in France in the mid 1980’s (3). Two of the five newest antiepileptics recently approved; vigabatrin and gabapentin, contain GABA in their molecule (4). Vigabatrin, γ-vinyl GABA, was designed to increase GABA brain levels by inhibiting GABA transaminase (5). Gabapentin contains a GABA molecule symmetrically integrated into a lipophilic cyclohexane system, which unlike GABA has the ability of crossing the blood brain barrier (4,6). However, at present it appears that gabapentin has a novel mechanism of action which is unrelated to its original design as a GABA analogue (6).

Next to GABA, glycine is one of the most important inhibitory neurotransmitter amino acids. Glycine has also been incorporated into a new antiepileptic agent—milacemide-I (7). Recent reports have shown that in several rats models, co-administration of glycine together with other antiepileptics, such as phenytoin, phenobarbital and vigabatrin, potentiate their anticonvulsant activity due to synergism (8–11).

In spite of their intrinsic potency neither GABA nor glycine are effective upon oral or systemic administration, due to their inability to cross the blood brain barrier (BBB) and their liver metabolic deactivation which minimizes their availability to the brain (12). The delivery of these two inhibitory neurotransmitters into the brain can be accomplished by designing active lipophyllic derivatives which will act as drugs on their own, or as chemical drug delivery systems (CDDS) which will serve as BBB penetrative carriers (13,14).

Recently, it was reported that the new compound taltramide—(2-phthalimidoethanesulphon-N-isopropylamide-II) and its dealkylated metabolite showed promising anticonvulsant activity in animal models (15). Taltramide, which is currently undergoing clinical trials, is a phthalimide derivative of taurine, which like glycine is a neuroinhibitory transmitter (15,16). A second phthalimide derivative, phthaloyl GABA (III) has also been reported to possess antiepileptic activity in different animal models (17,18).

A comparative analysis of the structures of milacemide, taltramide and phthaloyl GABA led us to the idea of designing and evaluating several glycine derivatives of phthalimide with potential antiepileptic activity. These phthalimide derivatives could serve as CDDS for glycine and glycaminde or may become potent glycine derivatives on their own. Consequently, glycine brain penetrability will be enhanced leading to glycine derivatives with anticonvulsant activity in intact animals. The purpose of the current study was to comparatively evaluate the pharmacokinetics and pharmacodynamics (anticonvulsant activity and neurotoxicity) of the following four phthalimide derivatives (Fig 1): phthaloyl glycine (IV), phthaloyl glycaminide (V), N,N-diethyl phthaloyl glycaminide (VI) and N,N-disopropyl phthaloyl glycaminide (VII). The analysis was carried out utilizing a structure pharmacokinetic pharmacodynamic relationship (SPPR) study. This approach enabled pharmacokineti pharmacodynamic correlation as well as investigation of how the in vivo performance (pharmacokinetics in intact animals) affected the anticonvulsant activity in a series of analogous phthaloyl glycine derivatives.
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, and 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hr). The plasma was immediately separated by centrifugation at 3000 g for 15 min and stored at −20°C. Before each assay, the plasma was allowed to reach room temperature, was vortexed, centrifuged, and the residual clot removed. Plasma concentrations of the investigated phthaloyl derivatives were monitored by an HPLC assay whose details are described below. Urine was collected systematically at 1 hour intervals for 12 hours after dosing, by means of an indwelling catheter. Urine levels of the phthaloyl derivatives were assayed by HPLC.

Rats

Pharmacokinetic studies were carried out on Sabra rats weighing 250–260 g placed in metabolic cages. In three parallel studies the rats were intravenously (20 mg) injected with phthaloyl glycine (IV), phthaloyl glycaminde (V) and N,N-diethyl phthaloyl glycaminde (IV).

Protocol. Following each administration, four rats were sacrificed and blood collected at each of the following time intervals; 5, 15, 30, 45, 60 min 1, 1.25, 1.5, 1.75, 2, 3, 4, 5, 6, 7, 8, 9, and 10 hours following dosing. Urine was collected systematically at one hour intervals for 12 hours after dosing, utilizing the metabolic cages. The remainder of the protocol was as described above for the dog studies.

Assay Methodology

A new HPLC assay was developed, which allowed simultaneous monitoring and quantification of phthaloyl glycine, phthaloyl glycaminde, N,N-diethyl phthaloyl glycaminde and N,N-diisopropyl phthaloyl glycaminde.

The assay procedure was as follows: To 0.25 ml plasma (containing the above mentioned phthalimide derivatives), internal standard solution (10 ul of phthaloyl GABA solution, 1 mg/ml in acetonitrile), and methanol (1 ml) were added. The mixture was vortexed for 30 minutes and 1 ml of acetonitrile was added followed by a 30 min vortex. The mixture was then centrifuged for 10 minutes at 3000 g and tert. butyl methyl ether (3 ml) was added, followed by a 30 second vigorous vortex. The mixture was centrifuged for 10 minutes at 3000 g and the organic phase was separated, and evaporated (using a vortex evaporator) to dryness. To the dry residue, mobile phase (120 ul) was added, the mixture was vortexed and 20 ul were injected into the HPLC apparatus (LDC Milton Roy, USA).

HPLC Conditions: Column — RP-18 reverse phase column (Lichrosphere — RP-18 5 μ—Merck, Germany) equipped with a precolumn. Mobile phase: acetonitrile 35%, bidistilled water 65%, and trifluoroacetic acid (TFA) 0.1%. UV wave length — 220 nm. The minimum quantifiable concentration of the assay was 0.1 mg/L. The interday coefficient of variation among replicates ranged between 5 and 9.5%.

Materials and Methods

Materials

Phthaloyl glycine, phthalimide and tert-butyl methyl ether were purchased from Aldrich, Wisconsin, USA. Phthaloyl glycaminde (V) was synthesized by a classical method, reacting N-carboethoxy phthalimide with glycaminde. The N,N-diethyl and N,N-diisopropyl phthaloyl glycaminde were prepared by reacting phthaloyl glycine with diethylamine and diisopropylamine, respectively. Phthaloyl GABA (III) (17,18) was also synthesized (for comparative means and for utilization as an internal standard) by reacting N-carboethoxy phthalimide with GABA. All reagents used were of analytical grade or HPLC pure. The chemical structures of the synthesized phthaloyl derivatives were confirmed by nuclear magnetic resonance (NMR) and elemental microanalysis.

Pharmacokinetic Studies

Dogs

The pharmacokinetic studies were carried out on four mongrel dogs ranging in weight between 18 and 21 kg. In a randomized cross over design, each dog was intravenously injected (into one of the cephalic veins) with the following phthaloyl derivatives: phthaloyl glycine (400 mg), phthaloyl glycaminde (400 mg), N,N-diethyl phthaloyl glycaminde (510 mg) and N,N-diisopropyl phthaloyl glycaminde (562 mg). The doses of the latter two compounds were equivalent to 400 mg of phthaloyl glycaminde.