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Pharmacokinetic analysis and anticonvulsant activity of glycine and glycinamide derivatives

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Abstract

The objective of this study was to investigate the pharmacokinetics and pharmacodynamics (anticonvulsant activity and neurotoxicity) of a series of amide derivatives of glycinamide in order to explore their structure pharmacokinetic–pharmacodynamic relationship and to discover a glycinamide derivative which might have the potential to become a new antiepileptic agent. The following compounds were investigated: glycylglycine, glycylglycinamide, gaboylglycinamide, *N*-acetylglycine, *N*-acetylglycinamide, *N*-acetylglycylglycinamide, *N*-acetyl, *N′*-benzylglycinamide, *N*-benzyloxycarbonylglycine or *Z*-glycine, *Z*-glycinamide, *Z*-glycylglycine and *Z*-glycylglycinamide. The anticonvulsant activity and neurotoxicity study was carried out in classical animal models for anticonvulsant screening. The pharmacokinetics of the active compounds was studied in dogs, which is a common animal model for a comparative crossover pharmacokinetic studies. Of the compounds investigated in this study, all the dipeptides of glycinamide and the glycine derivatives were found to be inactive. The only two active compounds were: *N*-acetyl,*N′*-benzylglycinamide (VII) and *Z*-glycinamide (IX). These compounds demonstrated similar pharmacokinetic profiles. Unlike glycine or glycinamide, compounds VII and IX, being lipophilic derivatives of glycinamide, showed anticonvulsant activity in animal models due to their better pharmacodynamic and pharmacokinetic properties. The pharmacodynamics and pharmacokinetics of compounds VII and IX were similar to that of the potential new antiepileptics; *N*-valproylglycinamide and phthaloylglycinamide. This study provides certain clues concerning the structural requirements for the design of anticonvulsant-active glycine derivatives.

Keywords: Glycinamide derivatives; Glycine; Anticonvulsant activity; Pharmacokinetics; New antiepileptics

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1. Introduction

GABA is an inhibitory neurotransmitter which plays an important role in the control of neuronal activity in the mammalian central nervous system (CNS) (Porter, 1986). A deficiency in brain GABA levels, has been shown to cause convulsions or epilepsy (Roberts et al., 1976; Dreifuss, 1987). Therefore, drugs which increase the amount of GABA available in the brain for neurotransmission have the potential to become a new antiepileptic agents. GABA derivatives, such as γ-vinyl-GABA (GVG) (Mumford and Canon, 1994) and gabapentin (Chadwick, 1993) are two of the new antiepileptics which have been approved in recent years. Next to GABA, glycine is one of the most important inhibitory neurotransmitter amino acids. Glycine itself does not readily cross the blood–brain barrier due to its zwitterionic character and the absence of an active transport. Similar to GABA, glycine has also been incorporated into the new antiepileptic agents—milacemide (Roba et al., 1986) and remacemide (Clark et al., 1995). Several reports have shown that coadministration of glycine and other antiepileptics, such as carbamazepine, phenobarbital and GVG, potentiate the anticonvulsant activity in several rats models, due to synergism (Seiler and Sarhan, 1984; Toth and Lajtha, 1984; Wood et al., 1988; Liu et al., 1990; Peterson et al., 1990). However, neither GABA nor glycine are effective upon oral or systemic administration due to their inability to cross readily the blood–brain barrier (BBB) and their liver metabolic deactivation which minimizes their availability to the brain (Krogsgaard-Larsen et al., 1988).

Lambert et al. (1994) reported that a glycine derivative *N*-benzyloxy-carbonylglycine (*Z*-glycine) was found to be far more active than glycine in rats following chemically and electrically induced seizures. Subsequently, the anticonvulsant activity of ester and amide-type lipid conjugates of glycine and *N*-benzyloxycarbonylglycine (*Z*-glycine) was evaluated utilizing the maximal electroshock (MES) and the strychnine tests (Lambert et al., 1996). In all cases the *Z*-glycine derivatives were always more potent than the corresponding glycine derivatives, with the amide lipid being more active than the ester derivatives (Lambert et al., 1996).

We recently explored the pharmacokinetics and pharmacodynamics (anticonvulsant activity and neurotoxicity) of *N*-phthaloyl and *N*-valproyl derivatives of GABA and glycine (Abu Salach et al., 1994; Hadad and Bialer, 1995). Of the valproyl derivatives, only valproylglycinamide showed a good anticonvulsant activity in both mice and rats due to its better pharmacokinetic profile (Hadad and Bialer, 1995). *N*-Valproylglycinamide (current commercial name, TV 1901) is currently undergoing phase II clinical trials (Bialer et al., 1996a). Subsequently, we developed and evaluated analogous and isomers of TV 1901 which showed good anticonvulsant activity in rodents such as tetramethylcyclopropylcarbonylglycinamide (Bialer et al., 1996c), 2-ene valproylglycinamide (Bialer et al., 1996b), valnoctylglycinamide and diisopropylacetylglycinamide (Hadad and Bialer, 1997). All these four derivatives of TV 1901 showed similar anticonvulsant activity and safety margin to that of the parent compound in mice and rats.

As a consequence of the anticonvulsant activity described in the literature for *Z*-glycine (Lambert et al., 1994, 1996) and the above *N*-valproyl derivatives of glycinamide (Hadad and Bialer, 1995; Bialer et al., 1996a,b,c; Hadad and Bialer, 1997), the current study was designed in order to investigate the anticonvulsant activity, neurotoxicity and safety margin of a series of amide derivatives of glycinamide (compounds I–XI) and to assess the pharmacokinetics of the active compounds. This pharmacokinetic–pharmacodynamic structure relationship study provides certain clues regarding the structural requirements for the design of antiepileptic-active glycine derivatives. This may also lead to the discovery of a glycinamide derivative that might be a potential new antiepileptic agent.

2. Materials and methods

2.1. Materials

N-Benzyloxycarbonylglycine-*Z*-glycine (VIII),

N-benzyloxycarbonyl glycylglycine (X), *N*-acetylglycine (IV), *N*-acetylglycinamide (V) and Boc-glycine were purchased from Aldrich (Milwaukee, WI). *Z*-Glycinamide (IX), glycylglycine, *Z*-GABA, dicyclohexyl-carbodiimide (DCC) and 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Sigma (St. Louis, MO). The chemical structure of the glycine and glycinamide derivatives investigated in this study are presented in Fig. 1. Compounds II, III, VI, VII, and XI were prepared according to the following respective methods.

2.1.1. Glycylglycinamide (II)

To a solution of 2 g (11.4 mmol) of *N*-(*tert*-butoxycarbonyl)- glycine (Boc-glycine) and 1.26 g (22.8 mmol) of glycinamide hydrochloride in 100 ml of DMF at neutral pH, 3.54 g (17.1 mmol) of dicyclohexylcarbodiimide (DCC) was added and 0.28 g (2.3 mmol) of 4-dimethylaminopyridine (DMAP) was added as a catalyst. The reaction mixture was stirred for 24 h at room temperature, the insoluble materials were filtered and the filtrate was evaporated to dryness in vacuum.

The residue was dissolved in 100 ml of a mixture of dichloromethane–TFA (8:2). The mixture was stirred for 2 h to remove the Boc group and the mixture was concentrated in vacuum leaving an oil product. The oil product was dissolved in 100 ml of hydrochloric acid solution (0.1 N), washed four times with 100 ml of dichloromethane, and the water fraction was evaporated to dryness in vacuum. The product was recrystallized from methanol–ethylacetate (2:8) to get 0.95 g (5.7 mmol) of the dipeptide as a hydrochloride salt, yield of 50% (m.p., 177–180°C).

Anal. calculated for $C_4H_9N_3O_2$. HCl: Calculated: C, 28.65%; H, 6%; N, 25%. Found: C, 28.65%; H, 5.7%; N, 23.8% ^{1}H NMR (D_2O): 3.747 (s, 2H, CH_2), 3.844 (s, 2H, CH_2) ppm.

2.1.2. Gaboylglycinamide (III)

The same procedure as for glycylglycinamide (II) was used to prepare gaboylglycinamide by using Boc-GABA, yield of 55% (m.p., 155–157°C).

Anal. calculated for $C_6H_{13}N_3O_2$. HCl: Calculated: C, 36.82%; H, 7.08%; N, 21.26%. Found: C, 36.52%; H, 6.99%; N, 21.19%. ^{1}H NMR (D_2O): 3.85 (s, 2H, CH_2), 3.62 (T, 2H, CH_2); 2.42 (T, 2H, CH_2), 2.12 (Q, 2H, CH_2) ppm.

2.1.3. N-Acetylglycylglycinamide (VI)

N-Acetylglycine (5 g, 43 mmol) and 9.5 g (86 mmol) of glycinamide hydrochloride were dissolved in 150 ml of a mixture of water–acetonitrile (50:50), 8.65 g (86 mmol) of triethylamine (TEA) and 12.6 g (64 mmol) of 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (EDC) as a coupling reagent were added to the mixture and the mixture was refluxed for 12 h. The mixture was left to cool at room temperature, the insoluble materials were filtered and the filtrate was evaporated to dryness in vacuum. The residue was recrystallized from a big fraction of acetonitrile to get a mixture of the product with the salt TEA:HCl, the solid crystals were dissolved in distilled water and purified from the salt by a strong cation-exchange chromatography (120 plus). The water fractions were collected and the water was removed by lyophilization to get a pure product 4.5 g (26 mmol), yield of 60%.

Anal. calculated for $C_6H_{11}N_3O_3$: Calculated: C, 41.62%; H, 6.36%; N, 24.27%. Found: C, 41.90%; H, 6.34%; N, 23.9%. ^{1}H NMR (D_2O): 3.919 (s, 2H, CH_2), 3.896 (s, 2H, CH_2), 2.037 (s, 3H, CH_3) ppm.

2.1.4. N-Acetyl,N′-benzylglycinamide (VII)

N-Acetylglycine (5 g, 43 mmol) and 8.4 g (86 mmol) of benzylamine were dissolved in 150 ml of a mixture of water–acetonitrile and two equivalent of EDC (1-ethyl-3(3-dimethyl aminopropyl)carbodiimide) were added as a coupling reagent and the mixture was refluxed for 6 h. The mixture was left to cool at room temperature, the insoluble material was filtered and the filtrate was evaporated to dryness in vacuum. The dry residue was dissolved in 200 ml of hydrochloric acid solution (0.1 N) and extracted four times with 100 ml of dichloromethane and the fractions of dichloromethane were collected, dried and evaporated to dryness. The product

was recrystallized from ethylacetate to get a pure product 6.2 g (30 mmol), yield of 70%.

Anal. calculated for $C_{11}H_{14}N_2O_2$: Calculated: C, 64.07%; H, 6.79%; N, 13.59%. Found: C, 64.15%; H, 6.75%; N, 13.41%. ^{1}H NMR ($CDCl_3$): 7.3 (M, 5H, Ar), 6.58 (br,s, 1H, NH), 6.78 (br,s, 1H, NH), 4.41 (D, 2H, CH_2), 3.93 (D, 2H, CH_2), 2.05 (s, 3H, CH_3) ppm.

2.1.5. *N*-Benzyloxycarbonyl glycylglycinamide (XI)

N-Benzyloxycarbonylglycine (3 g, 14.4 mmol) was dissolved in 200 ml of dichloromethane and 4.5 g (21.6 mmol) of DCC were added. The mixture was stirred at room temperature for 2 h, the insoluble materials were removed and 3.2 g (28.8 mmol) of glycinamide hydrochloride and 2.93 g (28.8 mmol) of TEA (triethylamine) were added to the filtrate. The reaction was stirred for 24 h at room temperature, the insoluble materials were filtered and the filtrate was evaporated to dryness in vacuum. The residue was recrystallized from distilled water to get a pure product 1.9 g (7.2 mmol), yield of 50%.

Anal. calculated for $C_{12}H_{15}N_3O_4$: Calculated: C, 54.34%; H, 5.66%; N, 15.85%. Found: C, 54.54%; H, 5.66%; N, 15.95%. ^{1}H NMR (CD_3OD): 6.1 (M, 5H, Ar), 3.75 (s, 2H, CH_2), 2.5 (s, 2H, CH_2), 2.42 (s, 2H, CH_2) ppm.

2.2. Animals

The pharmacokinetic experiments were carried out on six male dogs (mongrels), ranging in age and weight between 2 and 4 years and 18 and 22 kg, respectively. In a randomized crossover design, each dog was injected intravenously (in 1.5 ml of DMSO) with a dose of 400 mg of *N*-acetyl,*N*′-benzylglycinamide (VII), 404 mg (on dose equivalent to 400 mg of compound VII) of *Z*-glycinamide (IX) and 406 mg of *Z*-glycine (VIII). Urine was collected systematically for 12 h after dosing, by means of an indwelling catheter. No adverse effects were observed following i.v. administration of compounds VII, VIII and IX to dogs.

Compounds I–XI were screened in adult male Carworth Farm No. 1 albino mice, ranging in weight between 20 and 25 g (i.p., in a volume of 0.01 ml/g of body weight) and adult male Sprague–Dawley albino rats ranging in weight between 105 and 145 g (p.o., in a volume of 0.004 ml/g of body weight) for their anticonvulsant activity and neurotoxicity by the NIH Epilepsy Branch (Porter et al., 1984). The screening procedure involved the following: (1) the maximal electroshock (MES) test, which measures seizure spread; (2) the subcutaneous pentylenetetrazol test (s.c. Met test), which measures seizure threshold; and (3) the rotorod ataxia test, which assesses neurotoxicity.

2.3. Protocol

Venous blood samples (6 ml) were collected via an indwelling catheter (from the cephalic vein) at specified intervals following injection (0, 5, 10, 20, 30, 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10 and 12 h, respectively). The plasma was then immediately separated by centrifugation at $3000 \times g$ for 15 min and stored at -20°C. Before each assay, the plasma was allowed to reach room temperature, vortexed, centrifuged and the residual clot removed. Urine samples were collected via a urine catheter at specified intervals following injection (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 h, respectively), the sample volumes were measured and the urine samples were stored at -20°C. Plasma and urine levels of compounds VII, VIII and IX were then assayed by a new HPLC assay described below.

2.3.1. HPLC assay for monitoring *N*-acetyl,*N*′-benzylglycinamide (VII) and *N*-benzyloxycarbonylglycinamide (IX) in dog plasma and urine

Plasma samples are allowed to reach room temperature, vortexed and centrifuged. The plasma was then assayed as follows:

To 0.5 ml of plasma, 10 μg of an internal standard (*N*-benzyloxycarbonylglycinamide (IX)) and 4 ml of *tert*-butylmethylether were added. The mixture was vigorously vortexed for 30 s, centrifuged for 10 min at $3000 \times g$, the organic

phase was separated and evaporated to dryness (using a vortex evaporator). To the residue, 80 μl of methanol were added, the mixture was vortexed and 30 μl were injected to the HPLC.

Urine samples were allowed to reach room temperature, vortexed and centrifuged. The urine was then assayed as follows:

To 0.25 ml of urine, 10 μg of internal standard (*N*-benzyloxycarbonylglycinamide (IX)), 100 μl of hydrochloric acid solution (1 N) and 4 ml of *tert*-butylmethylether were added. The mixture was vigorously vortexed for 30 s and centrifuged for 10 min at $3000 \times g$, the organic phase was separated and transferred to another test tube containing 0.5 ml of sodium hydroxide solution (1 N), the mixture was vigorously vortexed for 30 s, centrifuged for 10 min at $3000 \times g$, the organic phase was separated and evaporated to dryness (using a vortex evaporator). To the residue, 80 μl of methanol were added, the mixture was vortexed and 30 μl were injected to the HPLC.

HPLC conditions: HPLC column, Hypersil ODS (C-18) 5 μm, 4.6 × 150 mm; wavelength, 258 nm; mobile phase, 60% water, 40% methanol and 0.01% trifluoroacetic acid.

The same assay was used for monitoring *N*-benzyloxycarbonylglycinamide (IX) in dog plasma and dog urine using *N*-acetyl,*N*′-benzylglycinamide as an internal standard.

2.3.2. HPLC assay for monitoring Z-glycine (VIII) in dog plasma and urine

Plasma samples are allowed to reach room temperature, vortexed and centrifuged. The plasma was then assayed as follows:

To 0.5 ml of plasma, 10 μg of internal standard (*Z*-GABA), 100 μl of hydrochloric acid solution (1 N) and 5 ml of *tert*-butylmethylether were added. The mixture was vigorously vortexed for 30 s, centrifuged for 10 min at $3000 \times g$, the organic phase was separated and evaporated to dryness (using a vortex evaporator). To the residue, 100 μl of mobile phase were added, the mixture was vortexed and 30 μl were injected to the HPLC.

Urine samples was allowed to reach room temperature, vortexed and centrifuged. The urine was then assayed as follows:

To 0.1 ml of urine, 30 μg of internal standard (*Z*-GABA) and 5 ml of *tert*-butylmethylether were added. The mixture was vigorously vortexed for 30 s, centrifuged for 10 min at $3000 \times g$, the organic phase was separated off. To the aqueous phase, 0.5 ml of heptane sulfonic acid solution (0.5%, w/v) and 5 ml of *tert*-butylmethylether were added. The mixture was vigorously vortexed for 30 s, centrifuged for 10 min at $3000 \times g$, the organic phase was separated and evaporated to dryness (using a vortex evaporator). To the residue, 100 μl of mobile phase were added, the mixture was vortexed and 30 μl were injected to the HPLC.

HPLC conditions: HPLC column, Lichrocart 250-4, Lichrosphere 100 RP-18, 5 μm; wavelength, 258 nm; mobile phase, 60% buffer acetate (pH 3), 40% methanol.

A linear response was observed for compounds VII and IX at a concentration range of 2–40 mg/l and for compound VIII at a concentration range of 2–80 mg/l. The inter-day percentage coefficient of variation (%CV) among replicates ranged between 2.6 and 11.1% for *N*-acetyl,*N*′-benzylglycinamide (VII), 3.2 and 11.5% for *Z*-glycine (VIII) and 2.7 and 10.5 for *Z*-glycinamide (IX) with 18.4% CV at lowest limits of quantification (LOQ) of 2 mg/l only for *Z*-glycinamide.

2.4. Pharmacokinetic analysis

The linear terminal slope (β) of $\log C$ (drug plasma concentration of compounds VII, VIII or IX) versus t (time) was calculated by the method of least squares. The terminal half-life of each compound ($t_{1/2}\beta$) was calculated from the quotient 0.69/terminal slope. The AUC (area under the C vs t curve) was calculated by using the trapezoidal rule with extrapolation to infinity. The total body clearance (CL) of the investigated compounds was calculated by using the quotient of the i.v. dose (D) and the AUC. The volume of distribution (V_β) was calculated using the quotient of the clearance and the linear terminal slope. The volume of distribution at steady state (V_{ss}) and the mean residence time (MRT) were calculated by classical methods (Yamaoka et al., 1978; Benet and Galeazzi, 1979; Gibaldi and Perrier, 1982; Yamaoka, 1986).

The fraction metabolized (f_m) of *Z*-glycinamide (IX) to *Z*-glycine (VIII) was calculated from the quotient of AUCs of *Z*-glycine (VIII) as a metabolite of *Z*-glycinamide (IX) to the AUC of *Z*-glycine obtained after its i.v. administration to the same dogs.

The fraction excreted unchanged (f_e) of compounds VII, VIII and IX was calculated from the ratio of the cumulative amount excreted unchanged in the urine (U_∞) to the dose. The cumulative amount of metabolite excreted in the urine (M_u) was also caluclated.

2.5. Partitioning and stability studies

The blood–plasma concentration ratio of compounds VII and IX (partitioning study) was carried out at room temperature (25°C) by spiking known amounts of the compound in six samples of fresh blood taken from a dog prior to drug administration. *N*-acetyl,*N*′-benzylglycinamide and *Z*-glycinamide concentrations were 5, 10, 15, 20, 30 and 40 mg/l. Each blood sample was centrifuged immediately after spiking and the separation of the plasma was carried out according to the procedure mentioned above. Plasma levels of the two compounds VII and IX were determined by HPLC.

A blood stability study of compounds VII and IX was carried out by incubating 600 μg of each compound in 30 ml of dog blood (placed in heparinized test tubes) at 37°C with continuous shaking. Blood samples (4 ml) were then collected at the following times: 0, 1, 2, 3, 4, 5 and 6 h. Plasma was immediately separated and the compound concentrations in the plasma assayed by HPLC.

3. Results

Stability studies showed that compound VII was unstable in dog blood for 6 h at physiological conditions. Compound IX was stable in dog blood for 6 h at physiological conditions. Subsequently, the stability of compound VII was tested in dog blood at 20°C. The results were that: compound VII was unstable at 20°C in dog blood, and was stable at 37 and 20°C in dog plasma; compounds VII and IX were evenly distributed between blood and plasma with a plasma to blood ratio of 0.87 ± 0.11 and 0.95 ± 0.05, respectively.

Fig. 1 describes the glycine and glycinamide derivatives investigated in this study. The dipeptides (glycylglycine (I), glycylglycinamide (II) and gaboylglycinamide (III)) did not show anticonvulsant activity following i.p. administration (300 mg) to mice. We therefore decided to derivatize glycinamide and the above dipeptides by synthesizing their *N*-acetyl and *N*′-benzyl derivatives (compounds IV–VIII). Out of the derivatized compounds (compounds IV–VIII), only *N*-acetyl,*N*′-benzylglycinamide (VII) showed anticonvulsant activity following i.p. administration of 100 mg/kg to mice. Compound VII did not

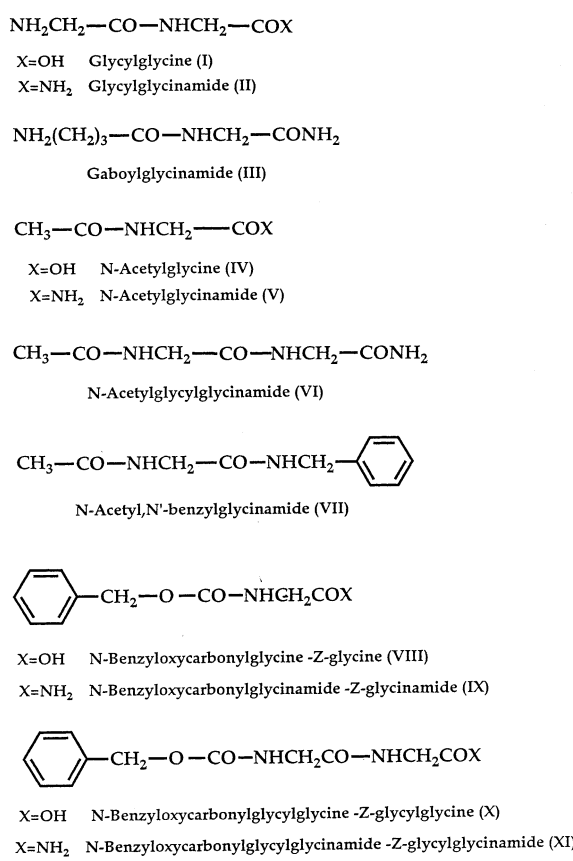

Fig. 1. Chemical structures of the glycine and glycinamide derivatives developed and evaluated in this study.

Table 1
Anticonvulsant activity and neurotoxicity of *N*-acetyl,*N*′-benzylglycinamide (VII) and *N*-benzyloxycarbonylglycinamide (IX) following i.p. administration to mice in comparison to phthaloylglycinamide and *N*-valproylglycinamide[a]

	N-Acetyl,*N*′-benzylglycinamide	*Z*-Glycinamide	Phthaloylglycinamide	Valproylglycinamide
MES, ED_{50} (mg/kg)	88 (81–99)[b]	46 (32–59)[b]	94 (72–130)[b]	152 (133–165)[b]
s.c. Met, ED_{50} (mg/kg)	>200	95 (63–153)[b]	>400	127 (97–195)[b]
Neurotoxicity, TD_{50} (mg/kg)	>200	230 (203–270)[b]	>600	369 (341–404)[b]
PI, MES	2.3	5.0	>6.4	2.4
PI, s.c. Met	—[c]	2.4	—[c]	2.9

MES, maximal electroshock; s.c. Met, chemically induced shock obtained following s.c. injection of metrazole; ED_{50}, effective dose in 50% of the test animals; TD_{50}, neurotoxic dose in 50% of the test animals; PI, protective index (the ratio of the TD_{50} to the ED_{50}).
[a] The data for phthaloylglycinamide and valproylglycinamide are taken from Abu Salach et al. (1994) and Hadad and Bialer (1995), respectively.
[b] Each range inside parentheses represents the 95% confidence interval.
[c] PI values could not be calculated due to lack activity at the s.c. Met test.

show neurotoxic signs at a dose as high as 300 mg/kg. Therefore, this compound was further analyzed quantitatively and its ED_{50} data and protective indices in mice and rats in comparison to phthaloylglycinamide (Abu Salach et al., 1994) and valproylglycinamide (Hadad and Bialer, 1995) are presented in Tables 1 and 2. *N*-Benzyloxycarbonylglycinamide (IX) also showed anticonvulsant activity in mice and rats, and its ED_{50} values are presented in Tables 1 and 2. As a consequence of their anticonvulsant activity, the pharmacokinetics of compounds VII and IX were investigated following i.v. administration (400 mg) to six dogs, and the mean plasma levels of compounds VII and IX are presented in Figs. 2 and 3, respectively.

Z-Glycinamide (IX) metabolized mainly to *Z*-glycine (VIII) (Fig. 3). Therefore, the pharmacokinetics of *Z*-glycine was investigated following its i.v. administration to the same dogs (Fig. 4) in order to calculate the fraction metabolized (f_m) of *Z*-glycinamide to *Z*-glycine. The mean f_m value was $80 \pm 14\%$. The inter-dog coefficient of variation (%CV) for compounds VII, VIII and IX ranged from 9 to 35% at all measured concentrations (Figs. 2–4). Table 3 describes the mean pharmacokinetic parameters of compounds VII, VIII and IX in comparison to that of phthaloylglycinamide and valproylglycinamide. *Z*-glycinamide and *N*-acetyl,*N*′-benzylglycinamide were eliminated from the body mainly by metabolic clearance. *Z*-Glycinamide had higher CL and *V* values than *N*-acetyl,*N*′-benzylglycinamide and, therefore, both compounds have similar mean $t_{1/2}$ and MRT values in dogs, of 2.3 and 3.3 h, respectively.

4. Discussion

Pharmacokinetic analysis of *N*-acetyl,*N*′-benzylglycinamide (VII) and *Z*-glycinamide (IX) in dogs showed that these two compounds have similar pharmacokinetic parameters. Compounds VII and IX have the following mean ($\pm$S.D.) pharmacokinetic parameters, respectively: CL, 6.8 ± 0.84 and 10.7 ± 4.3 l/h; V_{ss}, 21 ± 4 and 33 ± 10 l; $t_{1/2}$, 2.1 ± 0.5 and 2.3 ± 1.3 h; and MRT, 3.0 ± 0.6 and 3.3 ± 1.8 h. The mean fraction excreted unchanged (f_e) in the urine of compound VII was $9.8 \pm 5.4\%$ and the f_e value of *Z*-glycinamide was $2.3 \pm 1.3\%$. Both compounds are eliminated from the body mainly by metabolism which probably occurs primarily in the liver. Therefore, their hepatic extraction ratios (*E*) are 1.25 and 2%, respectively, indicating that these

Table 2
Anticonvulsant activity and neurotoxicity of *N*-acetyl,*N*′-benzylglycinamide (VII) and *N*-benzyloxycarbonylglycinamide (IX) following oral administration to rats in comparison to phthaloylglycinamide and *N*-valproylglycinamide[a]

	N-Acetyl,*N*′-benzylglycinamide	*Z*-Glycinamide	Phthaloylglycinamide	Valproylglycinamide
MES, ED_{50} (mg/kg)	37 (23–59)[b]	64 (43–89)[b]	31 (18–49)[b]	73 (65–83)[b]
s.c. Met, ED_{50} (mg/kg)	>250	>250	>250	>250
Neurotoxicity, TD_{50} (mg/kg)	>500	>500	>500	>1000
PI, MES	>13.8	>7.8	>16.1	>13.7
PI, s.c. Met	—[c]	—[c]	—[c]	—[c]

MES, maximal electroshock; s.c. Met, chemically induced shock obtained following s.c. injection of metrazole; ED_{50}, effective dose in 50% of the test animals; TD_{50}, neurotoxic dose in 50% of the test animals; PI, protective index (the ratio of the TD_{50} to the ED_{50}).
[a] The data for phthaloylglycinamide and valproylglycinamide are taken from Abu Salach et al. (1994) and Hadad and Bialer (1995), respectively.
[b] Each range inside parentheses represents the 95% confidence interval.
[c] PI values could not be calculated due to lack activity at the s.c. Met test.

compounds will not undergo liver first-pass effect or hepatic presystemic metabolism upon oral administration. In comparison to other glycinamide derivatives (Table 2), such as phthaloylglycinamide (Abu Salach et al., 1994) and *N*-valproylglycinamide (Hadad and Bialer, 1995). *Z*-Glycine (VIII) has a similar CL value, but its V_{ss} was the lowest (0.2 l/kg) and therefore its $t_{1/2}$ was the shortest. As *Z*-glycinamide was mainly metabolized to the inactive *Z*-glycine it indicates that the parent compound is the active entity.

The other four compounds have a low CL, a volume of distribution of about 1–2.3 l/kg and a mean half-life of more than 2 h. The better pharmacokinetic profile of compounds VII and IX may contribute to their anticonvulsant activity and to the lack of activity of *Z*-glycine (VIII).

Generally it seems better to investigate the pharmacokinetics and pharmacodynamics (anticonvulsant activity and neurotoxicity) of drugs in the same animal species due to a possible different metabolic pathway. However, previous studies with glycinamide derivatives showed that their pharmacokinetics was similar in dogs and rats (Abu Salach et al., 1994; Hadad and Bialer, 1995; Blotnik et al., 1997). In addition, the dog is a common animal model for crossover comparative pharmacokinetic analysis, while the rodents are the classical animal models for preclinical anticon-

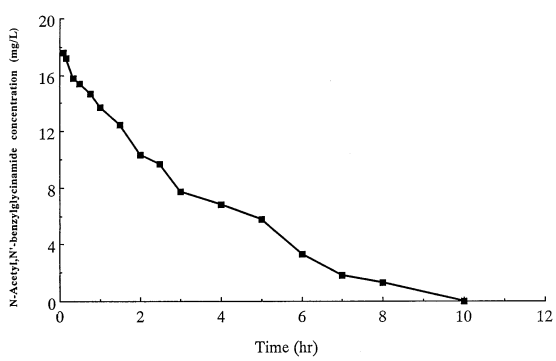

Fig. 2. Mean plasma levels of *N*-acetyl,*N*′-benzylglycinamide (VII) obtained following its i.v. administration (400 mg) to six dogs.

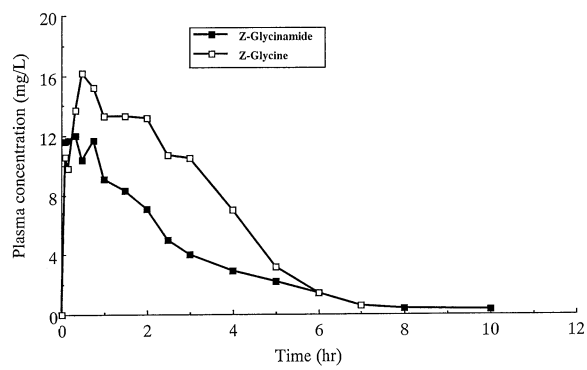

Fig. 3. Mean plasma levels of *N*-benzyloxycarbonylglycine (VIII) and *N*-benzyloxycarbonylglycinamide (IX) following i.v. administration of *N*-benzyloxycarbonylglycinamide to six dogs.

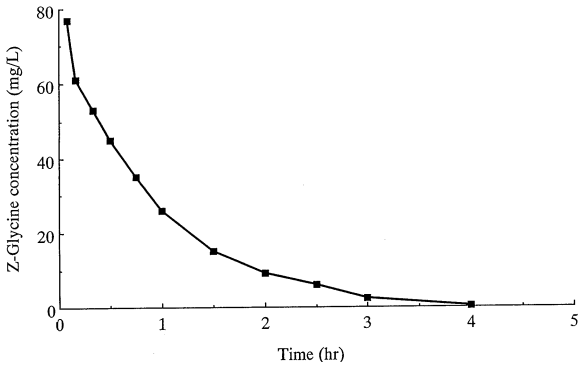

Fig. 4. Mean plasma levels of *N*-benzyloxycarbonylglycine (VIII) following its i.v. administration to six dogs.

vulsant screening. These facts makes this pharmacokinetic pharmacodynamic study in animals of good feasibility to be scaled up to humans and with potential clinical relevance.

Kohn and co-workers demonstrated that *N*-acetyl,*N*′-benzyl-DL-phenylglycinamide and *N*-acetyl,*N*′-benzyl-DL-alaninamide demonstrated a marked anticonvulsant activity at the MES test in mice (Conley and Kohn, 1987; Kohn et al., 1988). For both compounds, the anticonvulsant activity was due to the D-stereoisomer, as the L-stereoisomer was virtually inactive. An analogous compound *N*-acetyl,*N*′-benzylglycinamide (VII) was found to be active at a higher dose of 300 mg/kg (Conley and Kohn, 1987). Heterocyclic derivatives such as (*R*,*S*)-2-acetamido-*N*-benzyl-2-pyrolacetamide were more potent than the analogous alanine and phenylglycine derivatives (Kohn et al., 1990, 1991). From the above SAR study, the authors concluded that stringent steric and electronic requirements exist for obtaining maximal anticonvulsant activity in this class of amino acid derivatives. Unlike glycine, the *N*-methylated glycine derivatives, betaine(*N*,*N*,*N*-trimethylglycine), dimethylglycine and sarcosine(*N*-methylglycine), antagonized strychnine-induced seizures in mice (Freed, 1985). Betaine was also found to block the induction of convulsions by electroshock, metrazole and homocysteine (Freed et al., 1979).

Of the compounds investigated in this study, all dipeptides (compounds I–III, VI, X and XI) were found to be inactive as anticonvulsants. At this stage it is hard to judge whether the lack of anticonvulsant activity of the dipeptide derivatives and the activity of compounds VII and IX is due to their pharmacodynamic properties or pharmacokinetic profile. The only two active compounds that emerged from this study were lipophilic derivatives of glycinamide (compounds VII and IX), which demonstrated similar pharmacokinetic profiles. The major pharmacokinetic parameters of compounds VII and IX were similar to those of other active glycinamide derivatives, phthaloylglycinamide and *N*-valproylglycinamide (Abu Salach et al., 1994; Hadad and Bialer, 1995; Blotnik et al., 1997), which have the potential to became new antiepileptics.

The current study provides clues regarding the structural requirement for anticonvulsant-active glycine derivatives. The primary conclusion is that glycinamide derivatives have a better potential to be active than their corresponding glycine analogues. Unlike Lambert et al. (1994), *Z*-glycine was found to be inactive in our study. However, its glycinamide analogous (IX) was found to be active and possess better pharmacokinetic properties. Therefore, *Z*-glycinamide (IX) is currently undergoing further testing. Nevertheless, glycinamide alone is inactive and it is not a magic moiety. Only when it is combined with lipophilic anchor such as benzyl and acetyl or benzyloxycarbonyl (*Z*-group) it is evolved into an anticonvulsant active entity.

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Table 3
Mean pharmacokinetic parameters of *N*-acetyl,*N*′-benzylglycinamide (VII), *N*-benzyloxycarbonylglycinamide (IX), *N*-benzyloxycarbonylglycine (VIII), phthaloylglycinamide and *N*-valproylglycinamide obtained following i.v. administration to dogs

Pharmacokinetic parameters	*N*-Acetyl,*N*′-benzyl glycinamide	*Z*-Glycinamide	Phthaloylglycinamide[a]	Valproylglycinamide[b]	*Z*-Glycine
$t_{1/2}$ (h)	2.1 ± 0.4	2.3 ± 1.3	3.4 ± 0.7	2.7 ± 0.5	0.6 ± 0.14
CL (l/h)	6.4 ± 1.4	11 ± 3	9 ± 2	3 ± 0.8	5.4 ± 0.5
V_{ss} (L)	20 ± 4	33 ± 10	46 ± 13	12 ± 3	4.9 ± 0.6
V_{β} (L)	19 ± 6	33 ± 10	48 ± 13	11 ± 3	4.5 ± 0.8
MRT (h)	3.3 ± 0.8	3.3 ± 1.8	4.6 ± 0.9	4.6 ± 0.7	0.8 ± 0.1
f_e (%)	9.8 ± 5.4	2.3 ± 1.3	7.0 ± 0.7	8.4 ± 2.6[c]	48 ± 1.2
f_m (%)[d]					80.1 ± 13.6
Mu/*D* (%)[e]					20 ± 19.8

$t_{1/2}$, half-life; CL, total body clearance; V_{ss}, Volume of distribution at steady state; V_{β}, volume of distribution; MRT, mean residence time; f_e, fraction excreted unchanged in the urine; Mu, cumulative amount of the metabolite *Z*-glycine excreted in the urine
[a] Data taken from Abu Salach et al. (1994).
[b] *N*-Valproylglycinamide was given at a dose equivalent to 400 mg of valproic acid; data taken from Hadad and Bialer (1995).
[c] New data which are not taken from Hadad and Bialer (1995).
[d] Fraction of *Z*-glycinamide metabolized to *Z*-glycine.
[e] Mu/*D*, the fraction of *Z*-glycine excreted in the urine as a metabolite of *Z*-glycinamide.

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