Antiepileptic drug pharmacokinetics and neuropharmacokinetics in individual rats by repetitive withdrawal of blood and cerebrospinal fluid: milacemide

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1 The kinetics and metabolism of milacemide have been studied in an animal model which allows the simultaneous investigation of the temporal inter-relationships of drugs and metabolites in blood (pharmacokinetics) and cerebrospinal fluid (CSF, neuropharmacokinetics) in individual freely moving rats.

2 Milacemide dose-dependently increased CSF glycine and glycinamide (intermediary metabolite) concentrations. This confirms that milacemide is a CNS glycine prodrug.

3 Pretreatment with L-deprenyl (2 mg kg⁻¹), a specific inhibitor of monoamine oxidase type B (MAO-B), almost completely prevented the formation of glycinamide and increased milacemide accumulation in CSF. T_{max} and $t_{1/2}$ were significantly increased and C_{max} and AUC values were decreased for glycinamide compared to controls. Pretreatment with clorgyline (5 mg kg⁻¹), a specific inhibitor of MAO-type A, only moderately decreased glycinamide C_{max} and AUC values.

4 After milacemide administration (100, 200 and 400 mg kg⁻¹, i.p.) serum and CSF milacemide concentrations rose linearly and dose-dependently. Serum glycinamide concentrations exhibited small dose-dependent rises but these were not linearly related. In contrast, CSF glycinamide concentrations rose linearly and dose-dependently with C_{max} values 2.5, 3.2 and 4.1 times greater than the corresponding values for serum glycinamide after giving 100, 200 and 400 mg kg⁻¹ respectively of milacemide.

5 Serum glycine concentrations were unaffected but CSF concentrations increased dose-dependently and these were significant at the higher milacemide doses (200 and 400 mg kg⁻¹). Animals given 400 mg kg⁻¹ milacemide had glycine values which were still significantly elevated 7 h later.

6 In conclusion, serum milacemide rapidly enters and equilibrates with the CNS compartment where it is metabolised primarily by MAO-B to glycinamide and finally to glycine. Metabolism in the peripheral compartment is negligible.

Keywords: Milacemide; glycine; cerebrospinal fluid; pharmacokinetics; neuropharmacokinetics; monoamine oxidase

Introduction

Glycine, a non-essential and the simplest amino acid known, was first proposed as a spinal inhibitory neurotransmitter by Aprison & Werman, (1965) and subsequently has been found to have numerous functions within the central nervous system (CNS). It is a major postsynaptic inhibitor, an essential modulator of the N-methyl-D-aspartate (NMDA) receptor and it is also involved in the metabolism of proteins, peptides, one-carbon units and nucleotides (Brody *et al.*, 1976; Borman *et al.*, 1987; Johnson & Asher, 1987). As glycine is considered to play a role in disorders such as epilepsy, Alzheimer's disease, Huntington's disease, stroke and schizophrenia, its pharmacological manipulation may have therapeutic potential (Greenamyre *et al.*, 1985; Rothman & Olney, 1987; Deutsch *et al.*, 1989).

The influx of glycine into the brain from the blood under physiological conditions is negligible (Oldendorf, 1971) but very large oral or intraperitoneal doses can increase brain glycine concentrations significantly (Richter & Wainer, 1971; Toth & Lajtha, 1981) and have an anticonvulsant effect in numerous seizure models (Toth *et al.*, 1983; Spenos & Darby, 1984). Glycine also enhances the anticonvulsant effect of other drugs (Liu *et al.*, 1990; Paterson, 1991). However, in man it is not known whether the accompanying elevation in extracerebral glycine is harmful (Truong & Fahn, 1988; Waziri, 1988).

Milacemide (2-n-pentylaminoacetamide), a glycinamide derivative, is considered to be a glycine prodrug and has anticonvulsant, mood elevating and cognitive enhancing properties (van Dorsser *et al.*, 1983; Houtkooper *et al.*, 1986; Norton *et al.*, 1986; Saletu *et al.*, 1986; Handelman *et al.*, 1989; Schwartz *et al.*, 1992). Its exact mechanism of action is unclear but glycine, γ -aminobutyric acid (GABA) and dopamine may play a role (de Vorebeke *et al.*, 1983; Semba *et al.*, 1992). Milacemide readily crosses the blood-brain-barrier and is converted by N-dealkylation to glycinamide and then to glycine. The first step is considered to be catalysed by the enzyme monoamine oxidase type B (MAO-B; de Vorebeke *et al.*, 1989). As rat brain glycine levels can be elevated by oral milacemide administration (Christophe *et al.*, 1983) it may have potential in the treatment of disorders associated with glycine dysfunction.

In order that the therapeutic potential of a drug can be optimally realised, it is essential to devise a rational dosing regimen based on its kinetics. Therefore, we have used a procedure (Patsalos *et al.*, 1992) based on earlier CSF studies (Hutson *et al.*, 1985; Sokomba *et al.*, 1988) which permits simultaneous investigation of the temporal inter-relationship of drugs and metabolites in blood (pharmacokinetics) and cerebrospinal fluid (CSF; neuropharmacokinetics) in individual freely moving rats to study the kinetics of milacemide and its metabolism. Furthermore, using selective inhibitors of MAO-B and MAO type A (MAO-A) we have determined their respective contributions to the metabolism of milacemide and thus their possible importance in modulating its pharmacological activity.

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Methods

Male Sprague-Dawley rats (Charles River, Margate, Kent) weighing 250–300 g were individually housed under a 12 h light-dark cycle (lights on 06 h 00 min) with free access to a normal laboratory diet (SDS R and M number 1 expanded, Scientific Dietary Services, Witham, Essex) and water.

Surgical procedures, CSF and blood sampling

Rats were anaesthetized with pentobarbitone (60 mg kg⁻¹, i.p.) and catheters implanted in the cisterna magna, for CSF sampling, and the right jugular vein, for blood sampling, as previously described (Patsalos *et al.*, 1992).

Three days after surgery, CSF and blood catheters were checked for patency and CSF (30 µl) and blood (200 µl) samples simultaneously collected every 30 min for 1.5 h; the first two samples were not used for analysis and were discarded.

Animals were then injected with milacemide (100, 200 or 400 mg kg⁻¹ dissolved in 0.154 M NaCl; Continental Pharma Inc., Belgium) intraperitoneally and CSF and blood were withdrawn at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4, 5, 6, 7 and 8 h later for milacemide, glycinamide and glycine determination. CSF samples were collected in 0.5 ml polypropylene tubes, (Treff Lab, Dagersheim, Switzerland) and blood samples in 1.5 ml polypropylene tubes (Bio-Medical Laboratories Supplies, Birmingham, UK). After clot retraction, sera were separated by centrifugation. Sera and CSF were stored at -20°C until analysis.

Milacemide and glycinamide analysis

Milacemide and glycinamide concentrations in sera (10 µl) and CSF (5 µl) were determined by high performance liquid chromatography (h.p.l.c.) with u.v. and spectrofluorometric detection respectively as described by Semba *et al.* (1991).

Glycine analysis

Glycine concentrations in CSF and sera were determined by h.p.l.c. and based on a modification of the method of Turnell & Cooper (1982) as follows:

CSF By use of a Gilson autoinjector (model 231), 2.5 µl CSF, 10 µl homoserine (internal standard; 60 mM constituted in 100 mM borate buffer pH 9.5 and containing mercaptoethanol 60 µl per 25 ml) and 10 µl *o*-phthalaldehyde solution (50 mg *o*-phthalaldehyde dissolved in 1 ml methanol and made up to 10 ml with 50 µl mercaptoethanol and 100 mM borate buffer pH 9.5) were pipetted into a plastic autosampling vial. After mixing, derivatisation was allowed to proceed for 1 min and then 10 µl of the mixture was injected into a Gilson liquid chromatography apparatus controlled by a Hewlett-Packard Vectra EC/12 computer with data processing capability. A 20 µl loop and a Kratos fluorescence detector (model GM970, Ramsey, NJ, U.S.A.) set at excitation wavelength 395 nm and emission filter cut-off 470 nm were used. The analytical column was Microsorb C18 (5 µm particle size, 15 cm × 4.6 mm i.d., Rainin Instrument Company Inc, Woburn, MA, U.S.A.) attached to a 1.5 cm × 4.6 mm i.d. guard column packed with the same material (Rainin Instrument Company, Inc). The mobile phase comprised: Solvent A—640 ml of 250 mM sodium dihydrogen orthophosphate, 190 ml acetonitrile made up to 1900 ml with water (h.p.l.c. grade), buffered to pH 6.5 with 5 M NaOH and made to a final volume of 2 litres with water. Solvent B—560 ml acetonitrile, 480 ml methanol and 460 ml water. All solvents were filtered (0.45 µm membrane, HVLP 04700, Millipore U.K. Ltd, Watford, U.K.) and degassed with helium (15–20 min) before use. The gradient programme used was: 100%A (0–10 min), 92% A/ 8%B (10–22 min) and the retention times for homoserine and glycine were 15 and

17 min respectively. The flow rate was set at 2.0 ml min⁻¹.

Serum The procedures used for serum were essentially the same as for CSF except that deproteinisation was first undertaken as follows: 5 µl serum, 10 µl homoserine (internal standard) and 50 µl deproteinisation reagent (comprising 5 ml acetonitrile and 10 µl mercaptoethanol), were pipetted into a plastic microcentrifuge tube and shaken for 2 min. After centrifugation for 2 min the supernatant was removed and 10 µl were mixed with 10 µl *o*-phthalaldehyde derivatisation mixture (described above) and 7 µl injected into the h.p.l.c. apparatus. The solvent gradient programme used was: 100%A (0–4 min), 85%A/15%B (4–22.2 min) and the retention times for homoserine and glycine were 14 and 16 min respectively.

Effect of selective monoamine oxidase inhibitors on milacemide metabolism

By use of clorgyline (May and Baker, Dagenham, U.K.) and L-deprenyl, specific inhibitors of MAO-A and MAO-B respectively, the contribution of these enzymes to the metabolism of milacemide to glycinamide was examined. Rats were cannulated for CSF sampling only and clorgyline (5 mg kg⁻¹), L-deprenyl (2 mg kg⁻¹) or saline vehicle (0.154 M NaCl) were administered intraperitoneally (*n* = 4–7 per treatment group). Two hours later the rats were given milacemide (400 mg kg⁻¹, i.p.) and CSF (30 µl) samples collected at 30 min intervals for 4 h.

Pharmacokinetic analysis

Milacemide and glycinamide versus time data for both sera and CSF were analysed according to a one compartment model using PCNONLIN 3.0 programme (Metzler & Weiner, 1988) with a weighting factor of 1/concentration. Initial parameter estimates were obtained using JANA (Dunn, 1985) and both programmes were implemented on an Amstrad 2286/40 computer. The following parameters were computed: time to maximum concentration (*T*_{max}), maximum concentration (*C*_{max}), area under the concentration versus time curve (AUC) and apparent elimination half-life (*t*_{1/2}).

Statistics

Statistical analysis of the pharmacokinetic parameters was made by summary measures and analysed by one-way ANOVA followed by Neuman-Keuls multiple comparison test.

Results

Blood pharmacokinetics

Figure 1 shows the serum concentration-time profiles of milacemide and glycinamide after milacemide administration (100, 200 and 400 mg kg⁻¹). Milacemide concentrations rose linearly and dose-dependently and had already peaked at the time of the first blood sample (0.25 h). Concentrations then fell exponentially. Glycinamide concentrations exhibited small dose-dependent rises, but these were not linearly related.

Table I shows the apparent pharmacokinetic constants for individual rats together with the mean values, as calculated from log concentration versus time plots. The pharmacokinetic constants for individual rats showed moderate variability within each dose group with values mostly varying over a 2–3 fold range. Mean *C*_{max} values for milacemide increased proportionately with increasing dose. However, whilst mean *t*_{1/2} values were indistinguishable for the groups

Table 1 Pharmacokinetic constants for blood (serum)

Rat No.	Milacemide			$T_{\max}$ (h)	Glycinamide		
	$C_{\max}$ ($\mu\text{mol l}^{-1}$)	AUC ($\mu\text{mol l}^{-1} \text{ h}^{-1}$)	$t_{1/2}$ (h)		$C_{\max}$ ($\mu\text{mol l}^{-1}$)	AUC ($\mu\text{mol l}^{-1} \text{ h}$)	$t_{1/2}$ (h)
A 100 mg kg ⁻¹							
1	118	92	0.6	—	—	—	—
2	199	90	0.3	0.3	85	61	0.6
3	71	104	0.1	0.6	69	98	0.5
4	42	54	0.9	0.5	66	92	0.7
5	191	105	0.4	0.6	51	84	0.9
6	143	77	0.4	0.3	54	75	1.0
7	108	71	0.5	0.3	61	70	0.7
8	76	169	1.5	0.9	47	105	1.1
Mean	118	95	0.7	0.5	62	84	0.8
± s.e.mean	± 20	± 12	± 0.1	0.2	5	6	0.1
B 200 mg kg ⁻¹							
1	139	104	0.5	0.3	75	79	0.8
2	111	56	0.3	0.3	28	31	0.8
3	257	327	0.9	0.5	58	128	1.4
4	171	247	1.0	0.6	80	174	1.6
5	274	266	0.7	0.6	96	160	0.6
6	211	180	0.6	0.4	98	131	0.9
7	152	130	0.6	0.9	47	105	1.1
Mean	188	187	0.7	0.5	69	126	1.0
± s.e.mean	± 23	± 37	± 0.1	0.1	10	13	0.1
C 400 mg kg ⁻¹							
1	387	845	1.5	1.2	49	189	1.4
2	443	692	1.1	0.6	68	208	1.9
3	193	458	1.6	0.9	77	237	1.5
4	550	947	1.2	0.3	98	263	1.9
5	300	582	1.3	0.5	79	211	2.0
6	475	1166	1.7	0.6	82	261	2.0
7	391	1002	1.8	1.3	70	259	1.4
8	487	769	1.1	0.8	55	214	2.4
Mean	403	807	1.4**	0.8	72	230	1.8**
± s.e.mean	± 40	± 8	± 0.1	0.1	5	10	0.1

*T*_{max} = time to maximum concentration; *C*_{max} = maximum concentration; AUC = area under the serum concentration versus time curve; *t*_{1/2} = apparent elimination half-life. ***P* < 0.01 compared with 100 mg kg⁻¹ and 200 mg kg⁻¹ milacemide-treated groups: one-way ANOVA followed by Neuman-Keuls multiple comparison test.

Figure 1 Serum milacemide (a) and glycinamide (b) after i.p. administration of milacemide. Values are mean ± s.e.mean of 7–8 rats. Milacemide 100 mg kg⁻¹ (●), 200 mg kg⁻¹ (▼) and 400 mg kg⁻¹ (■).

Figure 2 Cerebrospinal fluid milacemide (a) and glycinamide (b) after i.p. administration of milacemide. Values are mean $\pm$ s.e. mean of 7–8 rats; Milacemide 100 mg kg⁻¹ (●); 200 mg kg⁻¹ (▼) and 400 mg kg⁻¹ (■).

given 100 and 200 mg kg⁻¹ milacemide, the value was substantially and significantly higher ($P < 0.001$) in the group given 400 mg kg⁻¹ milacemide. This difference was reflected in the high AUC value for the latter group.

Mean serum glycinamide concentrations peaked a little later than milacemide (mean $T_{\max}$ range, 0.5–0.8 h). The $C_{\max}$ value was approximately half that of milacemide in the group given 100 mg kg⁻¹ milacemide. As glycinamide concentrations rose only slightly with increasing milacemide dose (Figure 1b), mean $C_{\max}$ values for glycinamide after 200 and 400 mg kg⁻¹ milacemide were respectively 0.37 and 0.18 of the corresponding values for the precursor. Mean $t_{1/2}$ values for glycinamide increased with dose but statistical significance ($P < 0.001$) was only achieved at the highest dose.

Figure 3 Cerebrospinal fluid glycine after i.p. administration of milacemide. Values are mean $\pm$ s.e. mean of 7–8 rats; Milacemide 100 mg kg⁻¹ (●); 200 mg kg⁻¹ (▼) and 400 mg kg⁻¹ (■). * $P < 0.05$; ** $P < 0.01$ compared with baseline values (one-way ANOVA followed by Neuman-Keuls multiple comparison test).

Figure 4 Cerebrospinal fluid milacemide (a) and glycinamide (b) after i.p. administration of milacemide (400 mg kg⁻¹) to rats pretreated with L-deprenyl (■); clorgyline (▼) or 0.154 M saline (●). Values are mean of 7–8 rats.

Table 2 Neuropharmacokinetic constants for cerebrospinal fluid

Rat No.	Milacemide				Glycinamide			
	$T_{\max}$ (h)	$C_{\max}$ ($\mu\text{mol l}^{-1}$)	AUC ($\mu\text{mol l}^{-1} \text{h}^{-1}$)	$t_{1/2}$ (h)	$T_{\max}$ (h)	$C_{\max}$ ($\mu\text{mol l}^{-1}$)	AUC ($\mu\text{mol l}^{-1} \text{h}$)	$t_{1/2}$ (h)
A 100 mg kg⁻¹								
1	0.4	94	111	0.5	1.0	167	469	0.5
2	0.2	135	113	0.5	1.1	437	437	0.6
3	0.3	99	97	0.6	1.2	165	559	0.7
4	0.6	41	71	0.7	1.2	112	395	0.8
5	0.4	96	124	0.6	1.1	161	487	0.8
6	0.3	109	129	0.6	1.1	171	492	0.7
7	0.3	95	108	0.6	1.1	141	423	0.7
8	0.7	95	158	0.5	1.1	157	493	0.8
Mean	0.4	95	114	0.6	1.1	153	469	0.7
$\pm$ s.e.mean	0.1	9	9	0.1	0.1	7	18	0.04
B 200 mg kg⁻¹								
1	0.5	197	251	0.5	1.1	219	687	0.8
2	0.4	194	178	0.4	1.1	215	640	0.7
3	0.5	212	332	0.6	1.3	244	837	0.9
4	0.5	196	432	1.3	1.6	234	1025	1.3
5	0.5	213	267	0.5	1.2	227	610	0.8
6	0.5	243	330	0.5	1.2	233	800	0.9
7	0.5	233	350	0.6	1.4	175	666	0.9
Mean	0.5	213	306	0.6	1.3	221	752	0.9
$\pm$ s.e.mean	0.1	7	31	0.1	0.1	8	55	0.1
C 400 mg kg⁻¹								
1	0.7	621	1583	1.2	1.3	217	982	0.4
2	0.2	690	1028	1.0	1.9	275	1376	1.3
3	0.4	471	854	1.0	1.4	271	1305	0.5
4	0.7	604	1422	1.1	1.5	289	1189	0.7
5	0.7	436	1083	1.2	1.4	328	1250	0.6
7	0.5	587	1176	1.1	1.5	327	1397	0.7
8	0.5	407	905	1.2	1.3	323	1239	0.6
Mean	0.6	533	1149	1.1**	1.5*,**	294	1255	0.7
$\pm$ s.e.mean	0.1	37	88	0.1	0.1	14	46	0.1

For abbreviations see Table 1.

* $P < 0.05$; ** $P < 0.05$; *** $P < 0.01$, compared with 200 mg kg⁻¹, 100 mg kg⁻¹ and 100 mg kg⁻¹ milacemide-treated groups respectively: one-way ANOVA followed by Neuman-Keuls multiple comparison test.

CSF neuropharmacokinetics

Figure 2 shows the corresponding CSF concentration time profiles of milacemide and glycinamide. As observed for sera (Figure 1a), CSF milacemide concentrations rose linearly and dose-dependently peaking ($T_{\max}$) at approximately 0.5 h after milacemide administration and then falling exponentially. In contrast to sera (Figure 1b), CSF glycinamide concentrations also rose linearly and dose-dependently.

The apparent neuropharmacokinetic constants for individual rats, and the mean values, are shown in Table 2. Mean $C_{\max}$ values for CSF milacemide were fairly comparable to those in serum, though the CSF/serum ratio rose with milacemide dosage (0.81, 1.13 and 1.32 at 100, 200 and 400 mg kg⁻¹). CSF mean $t_{1/2}$ and AUC values mirrored those observed in serum.

CSF glycinamide concentrations peaked somewhat later (mean $T_{\max}$, 1.1–1.5 h) than milacemide (mean $T_{\max}$, 0.4–0.5 h) and $C_{\max}$ values were 2.5, 3.2 and 4.1 times greater than the corresponding values for serum glycinamide after giving 100, 200 or 400 mg kg⁻¹ respectively of milacemide. Further, $C_{\max}$ values for CSF glycinamide were approximately 1.5 fold higher, equivalent and almost half those of milacemide after the same doses. Mean $t_{1/2}$ values for glycinamide did not vary appreciably with milacemide dosage.

Glycine concentrations

Glycine concentrations in sera were unaffected by milacemide administration (data not shown). Figure 3 shows the glycine

concentration profiles in CSF for the animals described previously. Values rose linearly and dose-dependently, but were only significantly elevated at the higher milacemide doses (200 and 400 mg kg⁻¹), with a $T_{\max}$ of 3.5 h. Glycine values for rats given milacemide (400 mg kg⁻¹) rose to about 150% of control values. Glycine concentrations after 200 mg kg⁻¹ milacemide returned to baseline by 6 h after administration but animals given 400 mg kg⁻¹ of the drug had values that were still significantly elevated ($P < 0.001$) 7 h later.

Effect of monoamine oxidase inhibition

Figure 4 shows milacemide and glycinamide concentration profiles in CSF of rats given 400 mg kg⁻¹ milacemide and given saline (vehicle), 5 mg kg⁻¹ clorgyline or 2 mg kg⁻¹ L-deprenyl 2 h previously. L-Deprenyl prevented almost completely the formation of glycinamide and increased the accumulation of milacemide (Table 3). In contrast, clorgyline did not significantly affect milacemide concentrations. However, it caused a small but significant reduction in mean glycinamide $C_{\max}$ though mean AUC was unaffected.

Table 3 shows a comparison of the various apparent neuropharmacokinetic constants obtained. L-Deprenyl pretreatment significantly ($P < 0.01$) increased $T_{\max}$ and $t_{1/2}$ and significantly decreased $C_{\max}$ and AUC for glycinamide compared to controls. Milacemide $t_{1/2}$ and AUC values were also significantly increased by L-deprenyl pretreatment. In contrast clorgyline pretreatment affected only glycinamide $C_{\max}$ and AUC values ($P < 0.05$).

Figure 5 Schematic representation of the inter-relationship of milacemide, glycineamide and glycine in blood and brain (cerebrospinal fluid). BBB = blood-brain-barrier.

Table 3 Effect of pretreatment with selective monoamine oxidase type A (MAO-A) and type B (MOA-B) inhibitors on cerebrospinal fluid milacemide and glycineamide neuropharmacokinetic constants

	<i>n</i>	<i>T</i> _{max} (h)	<i>C</i> _{max} (μmol l ⁻¹)	AUC (μmol l ⁻¹ h)	<i>t</i> _{1/2} (h)
<i>Milacemide</i>					
Vehicle	(4)	0.3 ± 0.1	568 ± 53	639 ± 50	0.9 ± 0.1
Clorgyline	(5)	0.6 ± 0.1	513 ± 35	716 ± 52	0.7 ± 0.1
L-Deprenyl	(7)	1.2 ± 0.3	457 ± 50	1150 ± 140*	1.9 ± 0.4*
<i>Glycinamide</i>					
Vehicle	(4)	1.4 ± 0.1	326 ± 40	1253 ± 99	1.0 ± 0.1
Clorgyline	(5)	1.3 ± 0.2	263 ± 14**	976 ± 66*	0.9 ± 0.1
L-Deprenyl	(7)	3.0 ± 0.3**	8 ± 1**	81 ± 23**	1.5 ± 0.3

Values are expressed as mean ± s.e.mean.

P* < 0.05; *P* < 0.01; compared with vehicle-treated (0.154 M saline) group; one-way ANOVA followed by Neuman-Keuls multiple comparison test. For abbreviations see Table 1.

Discussion

Pharmacokinetic considerations are important for the design of dosage regimens in clinical pharmacotherapy, including therapeutic drug monitoring which is performed routinely for several antiepileptic drugs. The adjustment of drug dosage based on the monitoring of serum or plasma concentration of antiepileptic drugs, assumes that these concentrations reflect those at the CNS site of action or biophase. As this assumption is not easy to investigate in man, relevant animal studies are useful.

As concentrations of drugs in CSF are indices of their concentrations at central sites of action and the CSF is kinetically indistinguishable from the central site of action of various drugs, including numerous antiepileptic drugs (Danhof & Levy, 1984; Dingemans *et al.*, 1987; Klockowski & Levy, 1988; Sokomba *et al.*, 1988), studies of the inter-relationship between CSF and blood kinetics are desirable. The technique for the serial and simultaneous sampling of CSF and blood compartments in freely behaving rats (Patsalos *et al.*, 1992) has been found to be useful for the evaluation of the antiepileptic drug, milacemide, as much useful kinetic data was obtained on a small number of rats.

Furthermore, kinetic values were obtained for individual animals.

Our main findings concern the temporal distribution, kinetics and metabolism of milacemide in serum and CSF. Significant divergences between the blood and CSF compartments were observed. The dose-dependent increase in CSF glycineamide and glycine concentrations (Figures 2b and 3) after milacemide administration (100, 200 and 400 mg kg⁻¹) largely confirms that milacemide is a CNS glycine prodrug with intermediary metabolism to glycineamide.

After intraperitoneal administration, milacemide rapidly appeared in both serum (*T*_{max} < 0.25 h) and CSF (mean *T*_{max} 0.5 h; range 0.2–1.0 h). These fairly comparable values suggest ready penetration of the blood-brain-barrier.

The dosages of the MAO inhibitors L-deprenyl (2 mg kg⁻¹) and clorgyline (5 mg kg⁻¹) were chosen to inhibit selectively MAO-B and MAO-A activity respectively (Johnston, 1968; Kalir *et al.*, 1981; Knoll & Magyar, 1972). Therefore, as L-deprenyl but not clorgyline significantly increased CSF milacemide *t*_{1/2} and AUC (Figure 4, Table 3) and decreased CSF glycineamide AUC by 93% while clorgyline only decreased it by 22%, it can be concluded that the conversion of milacemide to glycineamide is largely mediated by MAO-B.

These CNS data confirm *in vitro*, *ex vivo* and indirect urine findings (de Varebeke *et al.*, 1983; 1989). Also, CSF glycineamide $C_{\max}$ values (Tables 1 and 2) after 100 mg kg⁻¹ milacemide administration were 2.5 fold higher than serum values which is consistent with peripheral (liver) MAO-B activity being lower than in the CNS. This agrees with *in vitro* evidence that K_m and $V_{\max}$ values for glycineamide formation from milacemide were 37% higher for rat brain than liver MAO-B (de Varebeke *et al.*, 1988). The dose-dependent increase in CSF but not in serum glycineamide, and the similar glycineamide $t_{1/2}$ values for both sera and CSF suggest that peripheral but not central MAO-B activity is rate-limiting and essentially saturated after 100 mg kg⁻¹ milacemide administration. Further, the similar $t_{1/2}$ values for milacemide and glycineamide suggests that glycineamide elimination is formation rate-limiting (Rowland & Tozer, 1989).

The distribution of milacemide and glycineamide and the contribution of the peripheral blood compartment to the CSF compartment need some comment. The rapid and dose-dependent increase in CSF milacemide indicates that blood-brain-barrier transport occurs readily and is not rate-limiting over the concentration-range used. Glycineamide can cross the blood-brain-barrier (Christophe *et al.*, 1983). However, since its concentrations in sera were 60–75% lower than in CSF (Figures 1b and 2b), blood glycineamide probably contributes minimally to CSF glycineamide and the latter is largely produced by brain MAO-B.

Previous *in vitro* data suggest that milacemide may be both a substrate and an inhibitor of MAO-B (de Varebeke *et al.*, 1989). The disproportionate increase and divergence in CSF milacemide neuropharmacokinetic parameters ($C_{\max}$ and AUC) compared to the serum pharmacokinetic parameters is consistent with this. Inhibition of liver MAO-B by milacemide would result in more milacemide becoming available for transport into the brain.

An increase of serum glycine after milacemide administration was not detected. In contrast, CSF glycine increased dose-dependently and significantly at the higher doses (200 and 400 mg kg⁻¹). After intraperitoneal administration at high doses, glycine (40 mmol kg⁻¹) readily enters the brain (presumably by diffusion) and increases significantly in the CSF for up to 8 h (unpublished data). Further, the apparent elimination $t_{1/2}$ for glycine in blood and CSF in these experiments were essentially the same (approximately 2 h) suggesting that the elimination of glycine in both compart-

ments is similar. Thus the absence of a significant increase in serum glycine in the present study further suggests that peripheral metabolism to glycine plays a minor role in the elimination of milacemide. As might be expected the $t_{1/2}$ for glycine as a metabolite of milacemide is larger (approximately 6 h, Figure 3) than that for glycine as a substrate (approximately 2 h, unpublished data).

Whether an elevation of brain glycine concentrations when milacemide is used clinically has pharmacologically relevant effects is unknown. Although milacemide has been used to treat patients with epilepsy or myoclonus, the overall response was marginal (Houtkooper *et al.*, 1986; Norton *et al.*, 1986; Brown *et al.*, 1991). Its evaluation in the management of schizophrenia and Huntington's Chorea has similarly been disappointing (Rosse *et al.*, 1991; Giuffra *et al.*, 1992). The maximum dose used in these patients was approximately 35 mg kg⁻¹ but as oral bioavailability of milacemide in man is limited by a first pass effect of 90% (Roba *et al.*, 1986), its effective maximum dose used was probably not more than 3.5 mg kg⁻¹. Further, as MAO-B is present in endothelial cells of the blood-brain-barrier (Minn *et al.*, 1991), the low milacemide doses used clinically probably lead to milacemide blood concentrations too low for effective blood-brain-barrier transport. As milacemide is generally well tolerated clinically, the present data suggest that milacemide might be evaluated clinically at a much higher dose and that its measurement in blood would be helpful in the optimization of therapy since this is an index of CNS concentrations.

In summary, Figure 5 shows schematically the inter-relationship between blood and brain of milacemide, glycineamide and glycine. Milacemide rapidly equilibrates between blood and brain and it is largely metabolized by MAO-B to glycineamide which is subsequently metabolized to glycine. Cerebral metabolism via MAO-B is more important than peripheral metabolism and although peripherally produced glycineamide can cross the blood-brain-barrier, its contribution to cerebral glycineamide is small. As serum glycine was not increased in the present study, the rise in CSF glycine on milacemide administration is derived exclusively from cerebral metabolism.

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References

- APRISON, M.H. & WERMAN, R. (1965). The distribution of glycine in cat spinal cord and roots. *Life Sci.*, **4**, 2075–2083.
- BORMAN, J., HAMILL, O.P. & SAKMANN, B. (1987). Mechanism of anion permeation through channels gated by glycine and γ -aminobutyric acid in mouse cultured neurones. *J. Physiol.*, **385**, 243–288.
- BRODY, T., SHIN, Y.S. & STOKSTAD, E.L. (1976). Rat brain folate identification. *J. Neurochem.*, **27**, 409–413.
- BROWN, P., THOMPSON, P.D., ROTHWELL, J.C., DAY, B.L. & MARDEN, C.D. (1991). A therapeutic trial of milacemide in myoclonus and Stiff-Man syndrome. *Movement Disord.*, **6**, 73–75.
- CHRISTOPHE, J., KUTZNER, R., NGUYEN-BUI, W.D., DAMIEN, C., CHATELAIN, P. & GILLET, L. (1983). Conversion of orally administered 2-n-pentylaminoacetamide into glycineamide and glycine in the rat brain. *Life Sci.*, **33**, 533–541.
- DANHOF, M. & LEVY, G. (1984). Kinetics of drug action in disease states. I. Effect of infusion rate on phenobarbital concentrations in serum, brain and cerebrospinal fluid of normal rats at onset of loss of righting reflex. *J. Pharmacol. Exp. Ther.*, **229**, 44–50, 1984.
- DEUTSCH, S.I., MASTROPAOLO, J., SCHWARTZ, B.L., ROSSE, R.B. & MORIHISA, J.M. (1989). A 'glutamatergic' hypothesis of schizophrenia. Rationale for pharmacotherapy with glycine. *Clin. Neuropharmacol.*, **12**, 1–13.
- DE VAREBEKE, J.P., CAVALIER, R., DAVID-REMACLE, M. & YODIM, M.B.H. (1988). Formation of the neurotransmitter glycine from the anticonvulsant milacemide is mediated by brain monoamine oxidase B. *J. Neurochem.*, **50**, 1011–1016.
- DE VAREBEKE, J.P., NIEBES, P., PAUWELS, G., ROBA, J. & KORF, J. (1983). Effect of milacemide, a glycineamide derivative, on the rat brain γ -aminobutyric acid system. *Biochem. Pharmacol.*, **32**, 2751–2755.
- DE VAREBEKE, J.P., PAUWELS, G., BUYSE, C., DAVID-REMACLE, M., DE MEY, J., ROBA, J. & YODIM, M.B.H. (1989). The novel neuropsychotropic agent milacemide is a specific enzyme-activated inhibitor of brain monoamine oxidase B. *J. Neurochem.*, **53**, 1109–1116.
- DINGEMANSE, J., VAN BREE, J.B.M.M. & DANHOF, M. (1987). Pharmacokinetic modelling of the anticonvulsant action of phenobarbital in rats. *J. Pharmacol. Exp. Ther.*, **249**, 601–608.
- DUNN, A. (1985). A new iterative polyexponential curve fitting program. *Comput. Meth. Prog. Biomed.*, **20**, 269–275.
- GIUFFRA, M.E., MOURADIAN, M.M. & CHASE, T.N. (1992). Glutamatergic therapy for Huntington's Chorea. *Clin. Neuropharmacol.*, **15**, 148–151.

- GREENAMYRE, J.D., PENNEY, J.B., YOUNG, A.B., D'AMATO, C.J., HICKS, S.P. & SHOULSON, I. (1985). Alterations in L-glutamate binding in Alzheimer's and Huntington's diseases. *Science*, **227**, 1496–1498.
- HANDELMANN, G.E., NEVINS, M.E., MUELLER, L.L., AANOUE, S.M. & CORDI, A.A. (1989). Milacemide, a glycine prodrug, enhances performance of learning tasks in normal amnesic rodents. *Pharmacol. Biochem. Behav.*, **34**, 823–828.
- HOUTKOOPER, M.A., VAN OORSCHOT, C.A.E.H., RENTMEESTER, T.W., HOPPENER, P.J.E.A. & ONKELINX, C. (1986). Double-blind study of milacemide in hospitalised therapy-resistant patients with epilepsy. *Epilepsia*, **27**, 255–262.
- HUTSON, P.H., SARNA, G.S., KANTAMANENI, B.D. & CURZON, G. (1985). Monitoring the effect of a tryptophan load on brain indole metabolism in freely moving rats by simultaneous cerebrospinal fluid monitoring and brain dialysis. *J. Neurochem.*, **44**, 1266–1274.
- JOHNSON, J.W. & ASCHER, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature*, **325**, 529–531.
- JOHNSTON, J.P. (1968). Some observations upon a new inhibitor of monoamine oxidase in brain tissues. *Biochem. Pharmacol.*, **17**, 1285–1297.
- KALIR, A., SABBAGH, A. & YODIM, M.B.H. (1981). Selective acetylenic 'suicide' and reversible inhibitors of monoamine oxidase type A and type B. *Br. J. Pharmacol.*, **73**, 55–64.
- KLOCKOWSKI, P.M. & LEVY, G. (1988). Kinetics of drug action in disease states. XXIV. Pharmacodynamics of diazepam and its active metabolites in rats. *J. Pharmacol. Exp. Ther.*, **244**, 912–918.
- KNOLL, J. & MAGYAR, K. (1972). Some puzzling pharmacological effects of monoamine oxidase inhibitors. *Adv. Biochem. Psychopharmacol.*, **5**, 393–408.
- LIU, Z., SEILER, N., MARESCAUX, C., DEPAULIS, A. & VERGNES, M. (1990). Potentiation of γ -vinyl GABA (vigabatrin) effects by glycine. *Eur. J. Pharmacol.*, **182**, 109–115.
- MINN, A., GHERSI-EGEA, J.F., PERRIN, R., LEININGER, B. & SIEST, G. (1991). Drug metabolising enzymes in the brain and cerebral microvessels. *Brain Res. Rev.*, **16**, 65–82.
- NORTON, L.C., LAXER, K.D., SCHOMER, D. & OSBORN, P. (1986). Phase II study to evaluate the efficacy and safety of milacemide (CP 1552S) in the treatment of complex partial seizures in adult patients. *Epilepsia*, **27**, 648.
- OLDENDORF, W.H. (1971). Brain uptake of radiolabeled amino acids, amines and hexoses after arterial injection. *Am. J. Physiol.*, **221**, 1629–1639.
- PATERSON, S.L. (1991). Glycine potentiation of anticonvulsant drugs in pentylenetetrazol seizures in rats. *Brain Res. Bull.*, **26**, 43–47.
- PATSALOS, P.N., ALAVIJEH, M.S., SEMBA, J. & LOLIN, Y.I. (1992). A freely moving and behaving rat model for the chronic and simultaneous study of drug pharmacokinetics (blood) and neuropharmacokinetics (cerebrospinal fluid): hematological and biochemical characterisation and kinetic evaluation using carbamazepine. *J. Pharmacol. Toxicol. Method.*, **28**, 21–28.
- RICHTER, J.J. & WAINER, A. (1971). Evidence for separate systems for the transport of neutral and basic amino acids across the blood-brain-barrier. *J. Neurochem.*, **18**, 613–620.
- ROBA, J., CAVALIER, R., CORDI, A., GORISSEN, H., HERIN, M., DE VAREBEKE, P.J., ONKELINX, C., REMACLE, M. & VAN DORSSER, W. (1986). Milacemide. In *New Antiepileptic Drugs*. Meldrum, B.S. & Porter, R.J. pp.179–190. London: John Libbey & Company Ltd.
- ROSSE, R.B., SCHWARTZ, B.L., DAVIS, R.E. & DEUTSCH, S.I. (1991). An NMDA intervention strategy in schizophrenia with 'low dose' milacemide. *Clin. Neuropharmacol.*, **14**, 268–272.
- ROTHMAN, S.M. & OLNEY, J.W. (1987). Excitotoxicity and the NMDA receptor. *Trends Neurosci.*, **10**, 299–302.
- ROWLAND, M. & TOZER, T.N. (1989). *Clinical Pharmacokinetics. Concepts and Application*. 2nd Ed. Philadelphia: Lea and Febiger.
- SALETU, B., GRUNBERGER, J. & LINZMAYER, L. (1988). Acute and subacute central nervous system effects of milacemide in elderly people. Double-blind placebo controlled quantitative electroencephalography and psychometric investigation. *Arch. Gerontol. Geriatr.*, **5**, 165–182.
- SCHWARTZ, B.L., HASHTROUDI, S., HERTING, R.L. & DEUTSCH, S.I. (1992). The effects of milacemide on item and source memory. *Clin. Neuropharmacol.*, **15**, 114–119.
- SEMBA, J., DOHENY, M., PATSALOS, P.N., SARNA, G. & CURZON, G. (1992). Effect of milacemide on extracellular and tissue concentrations of dopamine and 5-hydroxytryptamine in rat frontal cortex. *Br. J. Pharmacol.*, **105**, 59–62.
- SEMBA, J., RATNARAJ, N. & PATSALOS, P.N. (1991). Simple and rapid micro-analytical procedures for the estimation of milacemide and its metabolite glycylamide in rat plasma and cerebrospinal fluid by high-performance liquid chromatography. *J. Chromatogr. Biomed. Applic.*, **565**, 357–362.
- SOKOMBA, E.N., PATSALOS, P.N., LOLIN, Y.I. & CURZON, G. (1988). Concurrent monitoring of central carbamazepine and transmitter amine metabolism and motor activity in individual unrestrained rats using repetitive withdrawal of cerebrospinal fluid. *Neuropharmacol.*, **27**, 409–415.
- SPENOS, G. & DALY, E.C. (1984). Activity of glycine, dimethylglycine and their metabolites against 3-mercaptopropionic acid induced seizures in mice. *Neurology*, **34**, (Suppl. 1) 22.
- TOTH, E. & LAJTHA, A. (1981). Elevation of cerebral levels of non-essential amino acids in vivo by administration of large doses. *Neurochem. Res.*, **6**, 1309–1317.
- TOTH, E., LAJTHA, A., SARHAN, S. & SEILER, N. (1983). Anticonvulsant effects of some inhibitory neurotransmitter amino acids. *Neurochem. Res.*, **8**, 291–302.
- TRUONG, D.D. & FAHN, S. (1988). Therapeutic trial with glycine in myoclonus. *Movement Disord.*, **3**, 222–232.
- TURNELL, D.C. & COOPER, J.C.H. (1982). Rapid assay for amino acids in serum or urine by pre-column derivatisation and reversed-phase liquid chromatography. *Clin. Chem.*, **28**, 527–531.
- VAN DORSSER, W., BARRIS, D., CORDI, A. & ROBA, J. (1983). Anticonvulsant activity of milacemide. *Arch. Int. Pharmacodyn.*, **266**, 234–249.
- WAZIRI, R. (1988). Glycine therapy of schizophrenia. *Biol. Psychiatry*, **23**, 210–211.

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