Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 796 (2003) 35–44
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Chiral liquid chromatography resolution and stereoselective pharmacokinetic study of the enantiomers of a novel anticonvulsant, *N*-(4-chlorophenyl)-1-(4-pyridyl)ethylamine, in rats

X. Tao [a], P.K. Kadaba [b], I.P. Nnane [a,*]

[a] *Temple University School of Pharmacy, 3307 N, Broad Street, Philadelphia, PA 19140, USA*
[b] *K & K Biosciences Inc., Chadds Ford, PA, USA*

Received 20 March 2003; received in revised form 22 July 2003; accepted 29 July 2003

Abstract

A selective chiral high performance liquid chromatographic (HPLC) method was developed and validated to separate and quantify the enantiomers of a novel anticonvulsant agent, *N*-(4-chlorophenyl)-1-(4-pyridyl)ethylamine (AAP-Cl), in rat plasma. After extraction of the plasma samples with ethyl acetate, the separation was accomplished by an HPLC system consisting of a Chirex chiral column (250 mm × 4.6 mm i.d.) and a mobile phase of hexane:ethanol:tetrahydrofuran (280:20:40 (v/v)) containing trifluroacetic acid (0.3% (v/v)) and triethylamine (0.018% (v/v)) at a flow rate of 0.8 ml/min with UV detection. Male Sprague-Dawley rats were given (+)-AAP-Cl (10 and 20 mg/kg), (−)-AAP-Cl (10 mg/kg) or the racemic mixture (20 mg/kg) by i.v. bolus injection and serial blood samples were collected at different times after drug administration. (+)-AAP-Cl and (−)-AAP-Cl were separated with a resolution factor, R_s, of at least 1.4, and a separation factor, α, greater than 1.09. Linear calibration curves were obtained over the concentration range of 0.5–30 μg/ml in plasma for both (+)-AAP-Cl and (−)-AAP-Cl ($R^2 \geq 0.996$) with a limit of quantitation of 100 ng/ml and the recovery was greater than 80% for both enantiomers. The accuracy and precision for both enantiomers ranged from 96 to 102% (±0.2–7%) at upper and lower concentrations. The plasma concentration–time profiles of the enantiomers of AAP-Cl were best described by a two-compartment open model with a mean terminal half-life of about 5 h, volume of distribution at steady state of 3 l/kg and clearance of about 0.6 l/(h kg) in rats. There was no significant difference between the pharmacokinetic parameters of (+)-AAP-Cl and (−)-AAP-Cl, suggesting that the disposition of AAP-Cl in rats is not enantioselective. In addition, no chiral inversion of (+)-AAP-Cl to (−)-AAP-Cl or vice versa was observed. The results of this investigation have shed some light on the mechanism of action and disposition of AAP-Cl in rats.

Keywords: Enantiomer separation; Pharmacokinetics; *N*-(4-Chlorophenyl)-1-(4-pyridyl)ethylamine

1. Introduction

Epilepsy is a leading neurological disorder with 1–4 million Americans and 20–40 million people

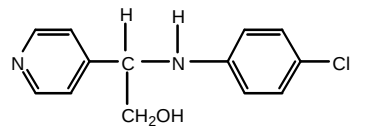
* Corresponding author. Tel.: +1-215-707-6917; fax: +1-215-707-3678.
E-mail address: ivo.nnane@temple.edu (I.P. Nnane).

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S1570-0232(03)00611-1

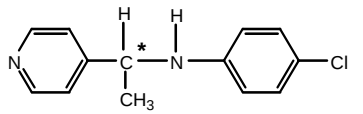
worldwide suffering from some form of epilepsy [1]. The number of antiepileptic drugs used for the treatment of epilepsy is remarkably small, and the currently available drugs, irrespective of the mechanism of action, are unable to control seizures in about 20% of epileptic cases [2,3].

The triazoline heterocycles is a new family of anticonvulsant agents with a unique mechanism of action quite distinct from the traditional anticonvulsant drugs [1]. 1-(4-Chlorophenyl)-5-(4-pyridyl)-1,2,3-triazoline (ADD17014) is a representative member of this group, and compares well with currently available antiepileptic drugs in rodent models of epilepsy [4]. It has been suggested that ADD17014 exhibits a 'dual' mechanism of action; while the parent triazoline impairs presynaptic release of L-glutamate and L-aspartate, the transmitters of excitatory amino acid (EAA) receptors, its active metabolite, 2-(4-chlorophenyl)amino-2-(4-pyridyl)ethanol or the primary β-amino alcohol (β-AA, Fig. 1), appears to block postsynaptic EAA receptors [5].

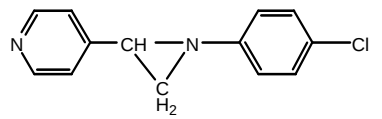
The identification of the pharmacophore of the triazoline anticonvulsants has led to the emergence of the aminoalkylpyridines as a new generation of triazoline metabolite analogues. *N*-(4-Chlorophenyl)-1-(4-pyridyl)ethylamine (AAP-Cl) (Fig. 1) is a representative member of the aminoalkylpyridine anticonvulsants, where the hydroxylmethyl group in β-AA (Fig. 1) is replaced by a non-polar methyl group in AAP-Cl. It has been demonstrated that AAP-Cl interacts with the receptors of the EAA and is orally active and more potent than the triazolines for seizure protection in both mice and rats. In addition, AAP-Cl has a long duration of action and no apparent signs of motor toxicity, and does not show the inherent chemical instability exhibited by the triazolines in neutral and acidic pH [5,6].



βAA



AAP-Cl



AZI-Cl

Fig. 1. Chemical structures of *N*-(4-chlorophenyl)-1-(4-pyridyl) ethylamine (AAP-Cl), 1-(4-chlorophenyl)-2-(4-pyridyl)aziridine (AZI-Cl, IS) and 2-(4-chlorophenyl)amino-2-(4-pyridyl)ethanol (β-AA). (*) Chiral center.

AAP-Cl has a chiral center and may be administrated as a racemic mixture of (+) and (−) enantiomers (Fig. 1). Although both (+) and (−) enantiomers of AAP-Cl afford protection in animal models of seizure, the (+) enantiomer shows greater potency and much higher protective index values than the parent racemic mixture or the (−) enantiomer. (+)-AAP-Cl is also highly orally effective and is a non-neurotoxic excitatory amino acid antagonist that holds promise for commercial development as a treatment for epilepsy and other neurological disorder such as Parkinson's disease in humans [1].

Although some reports concerning the analysis of AAP-Cl have been published [7], a chiral HPLC method for separation of the enantiomers of AAP-Cl has not been reported until now. This study was carried out to develop and validate a chiral HPLC method for the analysis of the enantiomers of AAP-Cl and to investigate their disposition in rats. The results of the present investigation may lead to a better understanding of the mechanism of action of AAP-Cl and the importance of stereochemistry in its disposition.

2. Experimental

2.1. Materials and reagents

The racemic mixture of *N*-(4-chlorophenyl)-1-(4-pyridyl)ethylamine (AAP-Cl) and its enantiomers, (+)-AAP-Cl and (−)-AAP-Cl, and 1-(4-chlorophenyl)-2-(4-pyridyl)aziridine (AZI-Cl) were generously provided by Dr. P.K. Kadaba (K & K Biosciences Inc., Chadds Ford, PA, USA). Water was

obtained from an in-house NANOpure® apparatus (Barnstead International, Dubuque, IA, USA). Ethyl acetate, acetonitrile, hexane, ethanol and tetrahydrofuran were purchased from Fisher Scientific (Pittsburg, PA, USA). Trifluroacetic acid, triethylamine and hydroxypropyl-β-cyclodextrin (HPβCD) were obtained from Sigma–Aldrich (St. Louis, MO, USA). All chemicals and solvents were of analytical or HPLC grade. Polyethylene tubing (0.58 mm i.d., 0.96 mm o.d.) and silastic medical grade tubing (0.025 in. i.d., 0.047 in. o.d.) were purchased from Harvard Apparatus (Newark, NJ, USA). The anaesthetic agents, ketamine (100 mg/ml) and xylazine (20 mg/ml) were obtained from Dodge Animal Health (Fort Dodge, IA, USA) and Phoenix Pharmaceuticals Inc. (St. Joseph, MO, USA), respectively.

2.2. Preparation of standard solutions

Stock solutions of all analytes, (+)-AAP-Cl, (−)-AAP-Cl and the internal standard (IS), AZI-Cl, were prepared in acetonitrile (1.0 mg/ml). The solutions were kept in capped test tubes, and stored at −20 °C. Working standard solutions containing (+)-AAP-Cl, (−)-AAP-Cl (5–300 μg/ml) or AZI-Cl (10 μg/ml) were obtained by dilutions of the stock solutions in acetonitrile.

2.3. Sample preparation

Whole blood (∼250 μl) was collected via the jugular vein from male Sprague-Dawley rats into heparinized Eppendorf centrifuge tubes. The blood was immediately centrifuged (13,000 rpm × 5 min) and plasma was transferred into clean Eppendorf centrifuge tubes with snap cap. The plasma samples were analyzed immediately or stored at −20 °C until when required. Aliquots (100 μl) of the plasma sample, spiked with the IS (10 μg/ml, 100 μl), were denatured by adding acetonitrile (200 μl) and extracted with ethyl acetate (500 μl) using a vortex mixer for 2 min and centrifugation at 13,000 rpm for 3 min. The supernatant was washed with water (100 μl), shaken for 1 min and centrifuged for 3 min at 13,000 rpm. The organic phase was transferred into clean test tubes and evaporated to dryness under nitrogen at room temperature. The residues were then reconstituted in the mobile phase (100 μl) for HPLC analysis.

2.4. Chiral HPLC analysis

An HP®-1050 HPLC system (Agilent Technologies, Wilmington, DE, USA), consisting of a standard vacuum degasser, quaternary pump, auto-sampler, column oven, and a multi-wavelength detector were used to conduct the analysis. Chromatographic separation of the enantiomers of AAP-Cl and the internal standard, AZI-Cl, was achieved on a Chirex (*S*)-LEU and (*R*)-NEA column (250 mm × 4.6 mm i.d.) protected by a guard column (30 mm × 4.6 mm i.d.) packed with the same packing material (Phenomenex Inc., Torrance, CA, USA). The composition of the mobile phase was hexane:ethanol:tetrahydrofuran (280:20:40 (v/v)) containing trifluroacetic acid (0.3% (v/v)) and triethylamine (0.018% (v/v)) at a flow rate of 0.8 ml/min. The UV detector was set at 247 nm and the temperature was maintained at 30 °C. Agilent ChemStation® software was used for data acquisition and integration.

2.5. Calibration curves and assay validation

Plasma (90 μl) obtained from untreated rats was spiked with 10 μl of working standard solutions in order to generate concentrations of the enantiomers ranging from 0.5 to 30 μg/ml. The calibration samples were spiked with the IS (10 μg/ml, 100 μl) and prepared as described above. The calibration curves were generated by plotting the ratios of the peak area of each enantiomer to the peak area of the internal standard versus the concentration of the enantiomer spiked in the samples. Linear regression analysis was performed using Microsoft® Excel. The precision and accuracy of the assay were obtained by comparing the predicted concentration (obtained from the calibration curve) to the actual concentration of each enantiomer spiked in blank plasma. The standard deviation (S.D.) and the coefficient of variation (CV = S.D./mean) were calculated over the entire calibration range. The limit of detection (LOD) for each enantiomer was considered as the concentration that produced a signal-to-noise (S/N) ratio of 3. The recovery was calculated from the ratio of the peak area of each enantiomer after extraction from plasma to the peak area of an equivalent amount of the standard solution. The calibration curves and assay validation studies were all performed in duplicate on three separate occasions ($n = 3$).

2.6. Preliminary pharmacokinetic studies

Male Sprague-Dawley rats (280–300 g) were obtained from Charles River Laboratories (Wilmington, MA, USA). The animals were maintained in a controlled environment of constant temperature (20 °C), 50% relative humidity and 12 h light/dark cycles for 7 days prior to use. The health of all animals was evaluated throughout the study by monitoring changes in body weights. The rats were surgically prepared, under ketamine (90 mg/kg) and xylazine (10 mg/kg) anaesthesia, by implanting a silastic medical grade catheter (0.025 in. i.d., 0.047 in. o.d.) into the jugular vein 24 h prior to drug administration and blood sampling. The pure enantiomers or the racemic mixture of AAP-Cl, which was composed of 50% of each enantiomer, was dissolved in hydroxypropyl-β-cyclodextrin (40% in saline) to produce concentrations of 10 or 20 mg/ml. All compounds were administered as a single i.v. bolus injection through the jugular vein. (+)-AAP-Cl was given at a dose level of 10 and 20 mg/kg, while (−)-AAP-Cl was dosed at 10 mg/kg and the racemic mixture of AAP-Cl at 20 mg/kg ($n = 3$). Blood samples (∼250 μl) were collected in heparinized tubes before drug administration, and post-dose at 5, 15, 30, 45, 60, 90 min, and 2, 4, 6, 24 h. Heparinized saline (250 μl) was injected into the animals immediately after the collection of each sample.

2.7. Data analysis

The resolution factor (R_s), the separation factor (α) and the asymmetry factors (A_s) were calculated based on the following equations:

$$R_s = 1.176\frac{t_{R_2} - t_{R_1}}{[W_{1/2}]_1 + [W_{1/2}]_2},$$

$$\alpha = \frac{t_{R_2} - t_0}{t_{R_1} - t_0}, \qquad A_s = \frac{b}{a}$$

where t_{R_1} is the retention time of (+)-AAP-Cl, and t_{R_2} the retention time of (−)-AAP-Cl; $[W_{1/2}]_1$ and $[W_{1/2}]_2$ are the peak widths at half peak height for (+)-AAP-Cl and (−)-AAP-Cl, respectively; t_0 the dead time of the column; a the width of the front half of the peak, and b the width of the back half of the peak measured at 10% of the peak height from the leading or trailing edge of the peak to a line dropped perpendicularly from the peak apex.

Pharmacokinetic data analysis was performed by both noncompartmental approach and compartmental modeling using WinNonlin (Pharsight Corporation Inc., Mountain View, CA, USA). In the simple noncompartmental model, a logarithmic trapezoidal method was used to calculate the areas under the plasma concentration versus time curve with extrapolation to infinity ($AUC_{0-\infty}$). The $AUC_{t_1-t_2}$ of each segment was calculated by the following equation:

$$\mathrm{AUC}\int_{t_1}^{t_2} = \delta t\frac{C_2 - C_1}{\ln(C_2/C_1)}$$

where C_1, C_2 are the plasma concentrations at time t_1, t_2, respectively, and δt is $(t_2 - t_1)$. The terminal elimination half-life ($t_{1/2,\,\mathrm{terminal}}$) was calculated by $0.693/\lambda_z$, where λ_z is the first-order rate constant associated with the terminal linear portion of the log concentration versus time curve. Other noncompartmental model pharmacokinetic parameters such as clearance (CL) and the volume of distribution at steady state (V_{ss}) were obtained from the WinNonlin output.

The one- and two-compartment analyses, with various weighting schemes, were also evaluated to determine the best model. The goodness of fit was based on random distribution of residuals and the Aikaike's information criteria. An open two-compartment model best described the plasma concentration versus time data for AAP-Cl enantiomers, after intravenous administration, according to the following general expression:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

where C_p is the concentration of drug in plasma at any time (t), the coefficients A and B are the extrapolated intercepts of the line of residuals and the linear terminal elimination phase at time zero, respectively. The rate constants α and β represent the hybrid first-order rate constants for the distribution and the elimination process, respectively. The half-lives for the distribution ($t_{1/2,\,\alpha}$) and elimination ($t_{1/2,\,\beta}$) phases were calculated by the following equations, $0.693/\alpha$ and $0.693/\beta$, respectively. The volume of the central compartment (V_p), the initial concentration (C_{max}), the volume of distribution at steady state (V_{ss}), the areas under the plasma concentration versus time curves (AUC) and

the clearance (CL) were calculated using the following equations:

$$Vp = \frac{\text{dose}}{A + B}, \qquad C_{max} = \frac{\text{dose}}{Vp},$$

$$V_{ss} = Vp + \frac{k_{12}}{k_{21}} Vp, \qquad \text{AUC} = \frac{A}{\alpha} + \frac{B}{\beta},$$

$$\text{CL} = \frac{\text{dose}}{\text{AUC}}$$

where k_{12} is the first-order rate constant for transfer of drug from the central compartment to the tissue compartment; k_{21} the first-order rate constant for transfer of drug from the tissue compartment to the central compartment. The plasma concentration versus time data obtained from each rat after intravenous administration of AAP-Cl or its enantiomers were fitted separately and the parameters were reported as means ± standard deviations of values obtained from separate fits for each dose level.

The analysis of variance (ANOVA) module on SAS system for Windows version 8 (SAS Institute Inc., Cary, NC, USA) was used to determine the statistical differences between pharmacokinetic parameters of (+)-AAP-Cl and (−)-AAP-Cl. A *P*-value of less than 0.05 was considered as statistically significant.

3. Results and discussion

3.1. Chiral HPLC

The chiral HPLC procedure was suitable for the separation of (+)-AAP-Cl, (−)-AAP-Cl and the internal standard (AZI-Cl). The retention times for AZI-Cl, (+)-AAP-Cl and (−)-AAP-Cl were 16.8, 19.2 and 20.5 min, respectively. A resolution factor, R_s, of at least 1.4, and a separation factor, α, greater than 1.09 for AAP-Cl enantiomers was obtained (Fig. 2).

The chromatographic results show that trifluroacetic acid (TFA) is critical for chiral separation of AAP-Cl enantiomers. The resolution factors increased as the percentage of TFA (up to 4%) in the mobile phase was increased (Table 1). Accordingly, 3% of TFA was used as the optimum concentration in the mobile phase. Addition of triethylamine (TEA) in the mobile phase was effective in reducing peak tailing. In addition, increasing the concentration of TEA in the mobile phase, decreased the retention times and peak widths although a loss of resolution was also observed (Table 2). An optimum concentration of TEA of 0.018% in the mobile phase was chosen in order to improve peak shape and minimize loss of resolution. Table 3 shows the effects of some organic solvents on the resolution, retention times and peak shape of AAP-Cl enantiomers. Although addition of methanol (MeOH) in the mobile phase resulted to shorter retention times and sharper peaks, it also decreased the resolution dramatically. Furthermore, although addition of either chloroform ($CHCl_3$) or ethylene chloride (CH_2Cl_2), improved separation of the enantiomers to some extent, it also produced long retention times, serious band broadening and peak tailing, resulting to lower sensitivity. Addition of tetrahydrofuran (THF) increased the R_s, and retention times resulting in band broadening. However, when TEA was added into the mobile phase in

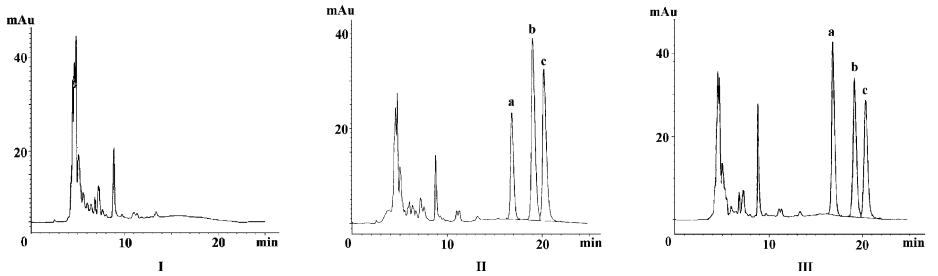


Fig. 2. Chiral HPLC separation of the enantiomers of *N*-(4-chlorophenyl)-1-(4-pyridyl)ethylamine (AAP-Cl) and 1-(4-chlorophenyl)-2-(4-pyridyl)aziridine (AZI-Cl, IS). (I) Ethyl acetate extracts of blank rat plasma, (II) blank plasma spiked with standards (5 μg/ml of each enantiomer) and (III) rat plasma sample drawn at 15 min after intravenous administration of 20 mg/kg of the racemic mixture of AAP-CL. Where (a) is AZI-Cl, (b) is (+)-AAP-Cl, (c) is (−)-AAP-Cl. Chiral separation was achieved using the optimum HPLC conditions described in this study.

Table 1
The effect of TFA on the resolution of the enantiomers of *N*-(4-chlorophenyl)-1-(4-pyridyl)ethylamine (AAP-Cl)

TFA in mobile phase (%)	Retention time (min)		$W_{1/2}$ (min)		α	R_s	A_s
	(+)-AAP-Cl	(−)-AAP-Cl	(+)-AAP-Cl	(−)-AAP-Cl			
0	7.10	7.10	0.145	0.145	1	NA	NA
0.1	13.75	14.57	0.294	0.330	1.09	1.30	1.1
0.3	17.20	18.36	0.366	0.409	1.09	1.47	1.1
0.4	18.31	19.53	0.398	0.442	1.09	1.45	1.1

Column: Chirex (*S*)-LEU and (*R*)-NEA (250 mm × 4.6 mm i.d.). Mobile phase: hexane/EtOH/THF (280/20/40 (v/v)), with 0.018% (v/v) of TEA. $W_{1/2}$ is the peak width at half peak height, α the separation factor, R_s the resolution factor, and A_s the asymmetry factors as defined in Section 2.7. NA, not applicable.

Table 2
The effect of TEA on the resolution of the enantiomers of *N*-(4-chlorophenyl)-1-(4-pyridyl)ethylamine (AAP-Cl)

TEA in mobile phase (%)	Retention time (min)		$W_{1/2}$ (min)		α	R_s	A_s
	(+)-AAP-Cl	(−)-AAP-Cl	(+)-AAP-Cl	(−)-AAP-Cl			
0	23.88	26.14	0.547	0.673	1.12	1.85	2.4
0.012	17.91	19.17	0.395	0.442	1.09	1.49	1.2
0.018	17.20	18.36	0.366	0.409	1.09	1.47	1.1
0.024	16.94	18.05	0.365	0.409	1.09	1.44	1.04

Column: Chirex (*S*)-LEU and (*R*)-NEA (250 mm × 4.6 mm i.d.). Mobile phase: hexane/EtOH/THF (280/20/40 (v/v)), with 0.3% (v/v) of TFA, $W_{1/2}$ is the peak width at half peak height, α the separation factor, R_s the resolution factor, and A_s the asymmetry factors as defined in Section 2.7.

Table 3
The effect of some organic solvents on the resolution of the enantiomers of *N*-(4-chlorophenyl)-1-(4-pyridyl)ethylamine (AAP-Cl)

Other organic solvent in mobile phase	Retention time (min)		$W_{1/2}$ (min)		α	R_s	A_s
	(+)-AAP-Cl	(−)-AAP-Cl	(+)-AAP-Cl	(−)-AAP-Cl			
3% of MeOH	21.89	22.74	0.416	0.443	1.04	1.16	1.9
3% of $CHCl_3$	27.39	29.01	0.610	0.685	1.07	1.25	1.9
3% of CH_2Cl_2	27.26	28.72	0.582	0.639	1.06	1.20	2.2
3% of THF	23.93	25.38	0.506	0.560	1.08	1.37	2.3
6% of THF	24.19	25.93	0.556	0.628	1.09	1.48	2.3
12% of THF (with 0.1% of TEA in THF)	18.84	20.10	0.405	0.449	1.09	1.48	1.1

Column: Chirex (*S*)-LEU and (*R*)-NEA (250 mm × 4.6 mm i.d.). Mobile phase: hexane/EtOH (280/20 (v/v)) with 0.3%, (v/v) of TFA. $W_{1/2}$ is the peak width at half peak height, α the separation factor, R_s the resolution factor and A_s the asymmetry factors as defined in Section 2.7.

combination with THF, peak tailing was diminished, the retention time were shortened and the peaks were sharp and symmetrical. Accordingly, the optimum mobile phase for the chiral resolution of AAP-Cl enantiomers consisted of hexane:ethanol:THF (280:20:40 (v/v)) containing 0.3% (v/v) TFA and 0.018% (v/v) TEA. A satisfactory chiral HPLC separation of AAP-Cl enantiomers in rat plasma samples following administration of the racemic mixture of the compound was achieved using the optimum analytical conditions.

3.2. Assay validation

Linear calibration curves were obtained over the concentration range of 0.5–30 μg/ml in plasma for both (+)-AAP-Cl ($y = 0.288x - 0.178$, $R^2 = 0.996$) and (−)-AAP-Cl ($y = 0.287x - 0.182$, $R^2 = 0.997$). The accuracy and precision of the assay, for both enantiomers, ranged from 96 to 102% (±2–6%) over the entire calibration range (Table 4). The recoveries of both enantiomers were concentration dependent and ranged from 82.07 ± 1.48 to 95.28 ±

Table 4
The accuracy and precision of the chiral HPLC method for measurement of the enantiomers of *N*-(4-chlorophenyl)-1-(4-pyridyl)ethylamine (AAP-Cl)

Concentration (μg/ml)	(+)-AAP-Cl		(−)-AAP-Cl	
	Calculated concentration (μg/ml)	CV (%)	Calculated concentration (μg/ml)	CV (%)
1	0.97 ± 0.055	5.6	0.96 ± 0.06	6.3
2.5	2.57 ± 0.17	6.6	2.50 ± 0.12	4.9
7.5	7.41 ± 0.13	1.8	7.35 ± 0.14	1.9
10	9.67 ± 0.38	4.0	9.53 ± 0.48	5.0
30	30.47 ± 0.91	2.9	30.62 ± 0.08	0.25

The experiments were performed in duplicate on three separate occasions ($n = 3$). Values represent the means ± standard deviations. CV (%) = 100 (S.D./mean).

4.74% (Table 5). The LOD was 100 ng/ml for both enantiomers.

3.3. Pharmacokinetics

The concentrations of both enantiomers of AAP-Cl in rat plasma showed a biexponential decline after i.v. administration of either (+)-AAP-Cl (10 and 20 mg/kg), (−)-AAP-CL (10 mg/kg) (Fig. 3) or the racemic mixture of AAP-Cl (20 mg/kg), (Fig. 4). Pharmacokinetic parameters estimated by the non-compartmental approach and two-compartmental approaches are listed in Tables 6 and 7, respectively. The values for the pharmacokinetic parameters such as $t_{1/2,\,\text{terminal}}$, CL and V_{ss} obtained from the noncompartmental approach were similar to corresponding parameters such as $t_{1/2,\,\beta}$, CL and V_{ss} calculated by the two-compartmental open model. The enantiomers of AAP-Cl were rapidly distributed with very short distribution half-lives ($t_{1/2,\,\alpha}$) of about 3–5 min. The terminal elimination half-lives ($t_{1/2,\,\beta}$), for both enantiomers, were approximately 5 h at both dose levels examined. The clearance (CL) and volume of distribution at steady state (V_{ss}) for both enantiomers were 0.5–0.7 l/(h kg) and 3.5–4.2 l/kg, respectively, in rats (Tables 6 and 7). The relatively high apparent volume of distribution at steady state suggests that both enantiomers of AAP-Cl are distributed extensively into tissues. This was expected for a lipophilic compound such as AAP-Cl, which has to cross the blood brain barrier (BBB) in order to interact with the excitatory amino acids receptors in the rat brain to produce its anticonvulsant effects [4].

Table 5
The recovery of the enantiomers of *N*-(4-chlorophenyl)-1-(4-pyridyl)ethylamine (AAP-Cl) from rat plasma

Concentration (μg/ml)	Recovery (%)	
	(+)-AAP-Cl	(−)-AAP-Cl
1	93.93 ± 6.42	95.28 ± 4.74
7.5	82.07 ± 1.48	85.01 ± 1.64
10	86.77 ± 3.48	88.19 ± 4.53
30	86.87 ± 2.60	88.11 ± 0.22

The experiments were performed in duplicate on three separate occasions ($n = 3$). Values represent the means ± standard deviations.

Although both the (+) and (−) enantiomers of AAP-Cl afford protection in animal models of seizure, the (+) enantiomer shows greater potency than the (−) enantiomer. As a result, we examined the pharmacokinetics of the (+) enantiomer at two dose levels in order to assess the influence of the dose given on the observed profile. The pharmacokinetic parameters of (+)-AAP-Cl were not significantly different when the i.v. dose was increased from 10 to 20 mg/kg in rats. In addition, the $AUC_{0-\infty}$ was proportional to the dose of (+)-AAP-Cl administered (Tables 6 and 7). These observations indicate that (+)-AAP-Cl exhibits linear pharmacokinetics at i.v. doses of up to 20 mg/kg.

Although the concentrations following administration of the (+)-AAP-Cl appear marginally higher than those of the (−)-AAP-Cl when given at the same dose (10 mg/kg), the pharmacokinetic parameters (e.g. CL, V_{ss}) obtained from the two profiles were not significantly different. Furthermore, there were also no significant differences in the pharmacokinetic parameters obtained for (+)-AAP-Cl and (−)-AAP-Cl, following

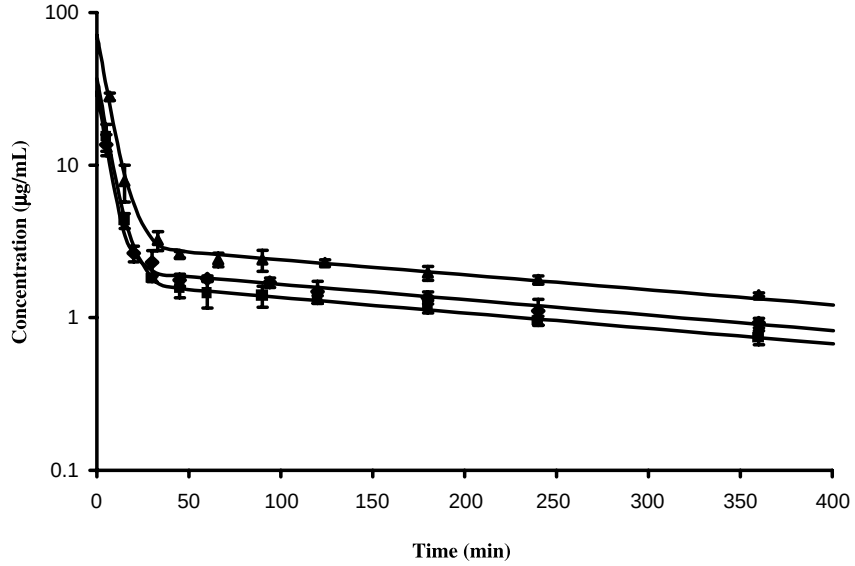


Fig. 3. Mean concentrations of *N*-(4-chlorophenyl)-1-(4-pyridyl)ethylamine (AAP-Cl) enantiomers in plasma following single intravenous doses of the pure enantiomer in rats. (▲) (+)-AAP-Cl at dose 20 mg/kg, (◆) (+)-AAP-Cl at dose 10 mg/kg, (■) (−)-AAP-Cl at dose 10 mg/kg. Values represent means ± standard deviations from three rats. The two-compartment open model was used to describe the data and fit the lines.

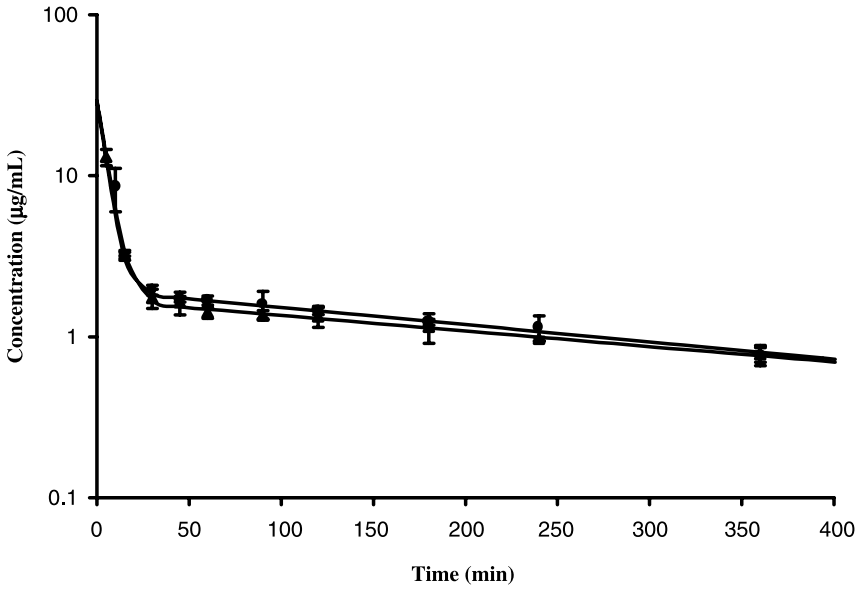


Fig. 4. Mean concentrations of *N*-(4-chlorophenyl)-1-(4-pyridyl)ethylamine (AAP-Cl) enantiomers in plasma following single intravenous doses of the racemic mixture (20 mg/kg), which consists of 50% of each enantiomer, in rats. (●) (+)-AAP-Cl, (▲) (−)-AAP-Cl. Values represent means ± standard deviations from three rats. The two-compartment open model was used to describe the data and fit the lines.

Table 6
The pharmacokinetic parameters of the enantiomers of *N*-(4-chlorophenyl)-1-(4-pyridyl)ethylamine (AAP-Cl) after administration of single i.v. doses in rats by noncompartmental approach

Parameter	Administration of pure enantiomer			Administration of racemic mixture (20 mg/kg)		*P*-value*
	(+)-AAP-Cl		(−)-AAP-Cl (10 mg/kg)	(+)-AAP-Cl	(−)-AAP-Cl	
	20 mg/kg	10 mg/kg				
$t_{1/2,\ terminal}$ (h)	6.28 ± 1.71	5.13 ± 1.32	4.98 ± 1.55	4.68 ± 0.58	5.27 ± 0.61	>0.05
CL (l/(h kg))	0.60 ± 0.12	0.57 ± 0.08	0.64 ± 0.14	0.66 ± 0.11	0.65 ± 0.08	>0.05
V_{ss} (l/kg)	4.08 ± 0.47	3.56 ± 0.40	3.70 ± 0.34	3.85 ± 0.32	4.14 ± 0.54	>0.05
$AUC_{0-\infty}$ (μg/ml h)	33.32 ± 7.01	17.53 ± 3.78	15.52 ± 3.06	15.21 ± 1.35	15.38 ± 3.17	>0.05

Values are expressed as means ± standard deviations in three rats.
* *P*-value for comparison of (+)-AAP-Cl to (−)-AAP-Cl at 10 mg/kg dose of the pure enantiomer or at 20 mg/kg dose of the racemic mixture using ANOVA.

Table 7
The pharmacokinetic parameters of the enantiomers of *N*-(4-chlorophenyl)-1-(4-pyridyl)ethylamine (AAP-Cl) after administration of single i.v. doses in rats by compartmental approach

Parameter	Administration of pure enantiomer			Administration of racemic mixture (20 mg/kg)		*P*-value*
	(+)-AAP-Cl		(−)-AAP-Cl (10 mg/kg)	(+)-AAP-Cl	(−)-AAP-Cl	
	20 mg/kg	10 mg/kg				
$t_{1/2,\ \alpha}$ (min)	4.18 ± 0.32	3.53 ± 0.72	4.25 ± 0.34	3.30 ± 0.58	3.76 ± 0.20	>0.05
$t_{1/2,\ \beta}$ (h)	5.11 ± 0.68	4.98 ± 1.23	5.16 ± 1.68	4.68 ± 0.63	5.19 ± 0.32	>0.05
V_p (l/kg)	0.28 ± 0.07	0.33 ± 0.04	0.30 ± 0.03	0.35 ± 0.05	0.34 ± 0.03	>0.05
C_{max} (μg/ml)	70.99 ± 5.41	30.02 ± 2.27	32.69 ± 1.55	28.84 ± 3.62	29.29 ± 1.34	>0.05
CL (l/(h kg))	0.69 ± 0.08	0.58 ± 0.08	0.63 ± 0.13	0.65 ± 0.08	0.66 ± 0.09	>0.05
V_{ss} (l/kg)	3.90 ± 0.32	3.57 ± 0.38	3.79 ± 0.31	3.81 ± 0.26	4.13 ± 0.48	>0.05
$AUC_{0-\infty}$ (μg/ml h)	28.98 ± 6.34	17.38 ± 3.27	15.79 ± 3.22	15.29 ± 1.20	15.63 ± 3.51	>0.05

Values are expressed as means ± standard deviations in three rats.
* *P*-value for comparison of (+)-AAP-Cl to (−)-AAP-Cl at 10 mg/kg dose of the pure enantiomer or at 20 mg/kg dose of the racemic mixture using ANOVA.

i.v. administration of the racemic mixture (Tables 6 and 7). These findings suggest that the disposition of AAP-Cl in rats is not enantioselective. Moreover, no chiral inversion of (+)-AAP-Cl to (−)-AAP-Cl or vice versa was observed in rats.

In other studies, we have observed that about 1–2% of the administered dose of AAP-Cl (100 mg/kg, i.p.) is recovered unchanged in rat urine within 0–24 h with only traces detected in 24–48 h samples [7]. Three unidentified metabolites were also detected in the urine samples collected after administration of AAP-Cl in rats. These observations suggest that AAP-Cl is cleared from the systemic circulation as a result of biotransformation and renal excretion. An exhaustive account of the elimination pathways of AAP-CL will be described separately.

4. Conclusion

The chiral HPLC method described in this investigation was suitable for the study of the stereoselective pharmacokinetics of AAP-Cl in rats. However, stereoselective disposition of AAP-Cl was not observed in rats. Thus, it would appear that the pharmacokinetic behavior of (+)-AAP-Cl is not responsible for its greater potency in animal model of epilepsy. It is plausible that the pharmacodynamic interaction of

(+)-AAP-Cl with the EAA receptors may account for its higher potency in rats. Nevertheless, further research needs to be carried out, on the pharmacodynamic interaction between AAP-Cl enantiomers and EAA receptors, in order to ascertain the exact explanation for the observed differences in the potencies of AAP-Cl enantiomers in animal models of epilepsy.

Acknowledgements

This work was financially supported by a grant from K & K Biosciences Inc.

References

[1] P.K. Kadaba, Optional resolution of racemic aminoalkylpyridine anticonvulsant and (+)aminoalkylprytidine enantiomers as highly potent orally effective anticonvulsant drugs and excitationory amino acid antagonists, US Patent 6,083,964 (2000).
[2] M.A. Rogawski, R.J. Poter, Pharmacol. Rev. 42 (1990) 223.
[3] N.R. Temkin, A.D. Jarell, G.D. Anderson, Drugs 61 (2001) 1045.
[4] P.K. Kadaba, J.T. Slevin, Epilepsia 29 (1988) 330.
[5] P.K. Kadaba, P.J. Stevenson, I.P. Nnane, L.A. Damani, Bioorg. Med. Chem. 4 (1996) 165.
[6] T.R. Deshmukh, P.K. Kadaba, Med. Chem. Res. 3 (1993) 223.
[7] G. Lin, I.P. Nnane, L.A. Damani, K.K. TsE, A.H. Chow, P.K. Kadaba, J. Pharm. Biomed. Anal. 18 (1998) 403.