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The pharmacokinetics of vigabatrin in rat blood and cerebrospinal fluid

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Summary

Purpose: Data on the blood pharmacokinetics of vigabatrin, an antiepileptic drug with a unique and novel mechanism of action, in the rat are sparse. Additionally, little is known of the kinetics of vigabatrin in the central cerebrospinal fluid (CSF) compartment. We therefore investigated the rate of penetration into and the inter-relationship between serum and CSF compartments following systemic administration of vigabatrin in the rat.
Methods: Sprague—Dawley rats were implanted with a jugular vein catheter and a cisterna magna catheter for blood and CSF sampling, respectively. Vigabatrin was administered by intraperitonial injection at three different doses (250, 500 and 1000 mg/kg) and blood and CSF collected at timed intervals up to 8 h. Vigabatrin concentrations in sera and CSF were determined by high performance liquid chromatography.
Results: Vigabatrin concentrations in blood and CSF rose linearly and dose-dependently and the time to maximum concentration (T_{max}) was 0.4 and 1.0 h, respectively. Vigabatrin is not protein bound in serum and its elimination from serum (mean $t_{1/2}$ values, 1.1—1.4 h) is rapid and dose-independent. The efflux of vigabatrin from CSF was significantly slower than that seen for serum (mean $t_{1/2}$ values, 2.2—3.3 h).
Conclusions: The kinetics of vigabatrin are linear with rapid entry into CSF. However, although vigabatrin CSF kinetics parallel that seen in serum, CSF vigabatrin concentrations represent only 2% of concentrations seen in serum and do not reflect free drug concentrations in serum.

Introduction

Vigabatrin (γ-vinyl GABA), an analogue of the major inhibitory neurotransmitter of the brain

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γ-aminobutyric acid (GABA), is the first of a series of 10 novel antiepileptic drugs that have entered clinical use since 1989.[1] Although a successful and well-tolerated drug with particular efficacy in partial seizures[2,3] and is the treatment of first choice in infantile spasms,[4,5] it is now evident that long-term vigabatrin treatment is associated with persistent visual field problems.[6–9] That the effects may be irreversible and the fact that as many as 65% of children and 40% of adults prescribed vigabatrin present with concentric visual field constrictions, has resulted in a substantial decline in its use.

Vigabatrin is effective in a variety of seizure models including audiogenic seizures and seizures induced by electroconvulsive shock, strychnine and β-carbolines.[10–12] The mechanism of action of vigabatrin is considered to be that of an irreversible inhibition of GABA-transaminase, the enzyme responsible for GABA catabolism.[13–14] The resulting increase in brain GABA concentrations parallels the anticonvulsant effects of vigabatrin.[15]

Although the blood pharmacokinetics of vigabatrin in man is well-characterised such data are scarce in the rat.[16] Thus, whilst Valdizan et al.[17] have reported on the time course of vigabatrin in rat plasma after single and multiple dose vigabatrin administration, Sills et al.[18,19] have reported plasma vigabatrin concentrations at a single time point after a single dose. The time course of vigabatrin has also been reported in rat CSF[20] and brain[21] and single time point CSF (after multiple dose vigabatrin administration)[22] and brain (after single dose vigabatrin administration)[18,19] vigabatrin concentrations has also been documented. However, the exact relationship between the central brain and peripheral blood pharmacokinetics of vigabatrin is not known. In patients with epilepsy, analysis of CSF obtained by single lumber puncture after single dose vigabatrin ingestion has shown vigabatrin to be present at 6 h post-ingestion and for CSF vigabatrin concentrations to be approximately 10–15% of blood concentrations.[23,24] CSF vigabatrin concentrations increase dose-dependently and after multiple-doses CSF vigabatrin concentration show a tendency to accumulate.[25] Whilst elevated CSF GABA concentrations are measurable at 3-days post-vigabatrin administration, vigabatrin is not detectable at that time.

Although the CSF compartment is considered to be kinetically indistinguishable from that of the biophase or site of brain action of drugs that are active in the brain,[26–29] this has not been confirmed for vigabatrin. Furthermore, CSF concentrations may not necessarily reflect regional concentration differences within the brain.[30] Nevertheless, CSF concentrations can provide insight as to what is occurring in the brain and the present study used a well-established and validated rat model[31–33] so as to determine the blood and CSF kinetic interrelationship of vigabatrin after single dose vigabatrin (250, 500 and 1000 mg/kg) administration.

Materials and methods

Animals

Male Sprague–Dawley rats (Charles River, Margate, Kent, U.K.) weighing 300–350 g were used. They were group housed in contiguous cages under a 12-h light:12-h dark cycle with free access to water and a normal laboratory diet (SDS R and M number 1 expanded. Scientific Dietary Services, Witham, Essex, U.K.). An ambient temperature of 25 °C was maintained and all rats were allowed to acclimatise to their new environment for about one week prior to experimentation. All animal procedures strictly followed Home Office regulations and were performed under the Animal (Scientific Procedures) Act 1986.

Surgical procedures, CSF and blood sampling

Rats were anaesthetised with 2% halothane (Merial Animal Health Ltd., Dublin, Ireland) and catheters implanted in the cisterna magna for CSF sampling, and the right jugular vein for blood sampling, as previously described.[31] Post-surgery, animals were individual housed in perspex cages. Two days later, CSF and blood catheters were checked for patency and CSF (20 μl) and blood (100 μl) samples were simultaneously collected every 30 min for 1 h. Then animals were randomly assigned to one of three vigabatrin administration groups and administered with vigabatrin (250, 500 and 1000 mg/kg constituted in saline) by intraperitonial (i.p.) injection. Blood samples were withdrawn at 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 h. In order to prevent the development of hypovolaemia, an equivalent volume of heparinised saline was administered after each blood sampling. CSF samples were collected at 30 min intervals for 8 h. Blood samples were collected in 0.5 ml polypropylene tubes (Treff Lab, Dagersheim, Switzerland), vortex mixed and centrifuged at 11,000 rpm for 5 min (Sigma, 2K15). Supernatant sera and CSF were stored frozen (−70 °C) until analysed for vigabatrin content.

Measurement of vigabatrin concentration

Vigabatrin concentrations in serum and CFS were measured by a modification of the high performance

liquid chromatography (HPLC) technique described by Ratnaraj and Patsalos.[34] A Gilson HPLC system (Anachem Ltd., Luton, Beds) comprising of the following modules was used: two pumps (a Gilson 302 pump and a Gilson 305 pump), an autoinjector (Gilson model 234) and a Gilson 811C dynamic mixer, and a Perkin-Elmer flurescence detector (Perkin-Elmer Ltd., Beaconsfield, Bucks.) which was set 250 nm (excitation) and 550 nm (emission). A Hypersil BDS-C18, 3 μm, 125 mm × 3 mm column (Hewlett-Packard, Stockport, Cheshire, UK) and a LiChrospher select B 4 × 4 (5 μm) pre-column (Hewlett-Packard) were used. Chromatograms were run at 35 °C using a model 7955 column chiller (Jones Chromatography Ltd., Hengoed, Mid. Glamorgan). *O*-pthalaldehyde, the derivatization agent, and L-norvaline, the internal standard were, purchased from Sigma (Poole, Essex; BDH, Dagenham, Essex). Vigabatrin S(+) was supplied by Marion Merrill Dow, Inc (Cincinnati, OH, USA). All other chemicals were of analytical grade. The lower limit of quantitation of the assay, as determined by precision and accuracy, and whereby a coefficient of <10% was targeted, was 1 μmol/l. Based on a signal-to-noise ratio of 2:1, the limit of detection was 0.25 μmol/l. The coefficient of variation of the within-batch precision was 2% and for the between-batch precision the value was <4%. There is no interference from any other antiepileptic drug that is currently licensed for clinical use.

The procedure used for the determination of the free non-protein-bound serum vigabatrin concentration was the same as for total vigabatrin concentrations except that samples were first filtered through an Amicon Cetrifree Micropartition System (Amicon, Stonehouse, UK) using a Sigma 2K15 centrifuge with a temperature setting of 25 °C.

Data analysis

Pharmacokinetic parameters were calculated using individual concentration versus time data sets based on a one-compartment model. Time to maximum concentration (T_{max}) and maximum concentration (C_{max}) were determined by visual inspection of the concentration versus time curves. The area under the concentration versus time curve (AUC) to the last sample (serum or CSF) concentration was obtained using the linear trapezoidal rule and extrapolation to infinity by dividing the last concentration by the rate constant phase (β). The elimination half-life ($t_{1/2}$) was calculated from the slope (λz), estimated by log linear regression of the terminal phase of the concentration versus time curve. Results are presented as mean ± S.E.M. Data were compared using Student's *t*-test and multiple comparisons were corrected for in the statistical analysis.

Results

Blood pharmacokinetics

Table 1 shows the pharmacokinetic parameters for vigabatrin in serum for individual rats together with the mean values, as calculated from concentration versus time plots after 250, 500 and 1000 mg/kg vigabatrin administration. Vigabatrin concentrations rose linearly and dose-dependently. Rapid absorption was demonstrated by the fact that C_{max} values were achieved at time of first sampling (15 min post-dose). Concentrations subsequently declined rapidly and the decline was exponential (Fig. 1). Both C_{max} and AUC values increased linearly and dose-dependently. Vigabatrin $t_{1/2}$ values were

Table 1 Pharmacokinetic parameters for serum after 250, 500 and 1000 mg/kg vigabatrin (VGB) administration

Rat	T_{max} (h)	C_{max} (μmol/l)	AUC (μmol h/l)	$t_{1/2}$ (h)
VGB 250 mg/kg				
1	0.3	1030.7	1455.7	1.0
2	0.7	585.7	893.8	1.2
3	0.3	1101.2	1765.8	1.3
4	0.3	1105.8	1820.5	1.2
5	0.3	925.8	1539.0	1.0
Mean	0.4	949.8	1495.0	1.1
±S.E.M.	0.07	96.7	166.0	0.1
VGB 500 mg/kg				
1	0.3	1803.8	4022.3	0.8
2	0.3	1578.4	3276.9	1.4
3	0.3	1528.5	3634.9	1.2
4	0.3	1289.4	2786.5	1.1
5	0.3	1558.7	3275.1	1.2
6	0.7	1814.2	3507.1	1.0
Mean	0.4	1595.5	3417.1	1.1
±S.E.M.	0.06	79.8	169.2	0.1
VGB 1000 mg/kg				
1	0.3	2744.1	6311.0	1.6
2	0.3	3412.5	7818.4	1.6
3	0.3	3310.8	8394.8	1.7
4	0.3	2992.3	6871.7	1.4
5	0.7	2733.8	7169.2	1.3
6	0.7	2581.0	7394.5	1.5
7	0.3	2815.0	6914.6	1.0
Mean	0.4	2941.3	7267.7	1.4
±S.E.M.	0.06	118.4	258.0	0.1

C_{max}, apparent maximum concentration; T_{max}, apparent time to maximum concentration; AUC, area under the serum concentration-time curve; $t_{1/2}$, terminal half-life.

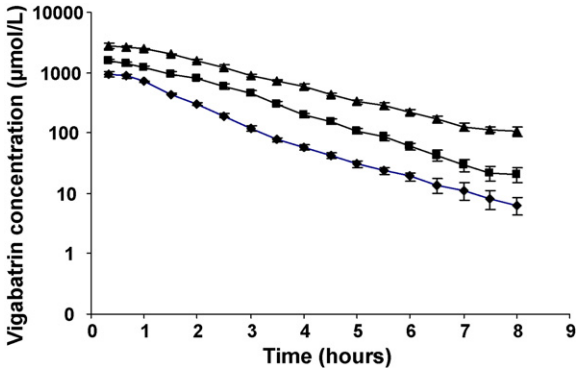

Figure 1 Vigabatrin concentration vs. time profiles in serum after 250 mg/kg (◆), 500 mg/kg (■) and 1000 mg/kg (▲) vigabatrin administration. Data are mean ± S.E.M. of 6—7 animals.

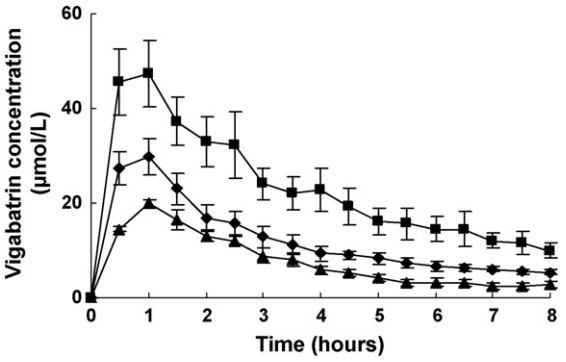

Figure 3 Vigabatrin concentration vs. time profiles in CSF after 250 mg/kg (▲), 500 mg/kg (◆) and 1000 mg/kg (■) vigabatrin administration. Data are mean ± S.E.M. of 6—7 animals.

1.1 ± 0.1, 1.1 ± 0.1 and 1.4 ± 0.1 after 250, 500 and 1000 mg/kg administration, respectively, and were not statistically different ($p > 0.05$).

A total of nine random sera, chosen to reflect a range of sampling times (0.25—8 h) and vigabatrin concentrations (13—1378 µmol/l), were analysed for content of free non-protein-bound vigabatrin. The free/total serum of vigabatrin concentration ratio varied between 0.93 and 1.07 (mean ± S.E.M. = 0.995 ± 0.01) and was not time or concentration dependent (Fig. 2).

CSF neuropharmacokinetics

Fig. 3 shows the corresponding CSF concentration versus time profiles of vigabatrin. After drug administration, vigabatrin rapidly and readily penetrated into the CSF compartment and vigabatrin was detectable in CSF at the time of first sampling; 30 min post-dose. The calculated mean ± S.E.M. kinetic constants foe CSF, as calculated from the concentration versus time plots, can be seen in Table 2. The kinetic constants for individual rats showed moderate variability within the three dose groups. CSF vigabatrin concentrations peaked somewhat later (mean T_{max}, 0.8—1.0 h) than serum vigabatrin (mean T_{max}, 0.4 h), and were dose-independent. C_{max} and AUC values increased linearly and dose-dependently and CSF $t_{1/2}$ values although

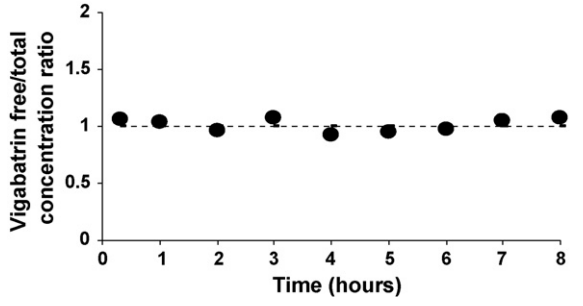

Figure 2 Vigabatrin free/total concentration ratio at different vigabatrin concentrations (13—1378 µmol/l) over time.

Table 2 Neuropharmacokinetic parameters for CSF after 250, 500 and 1000 mg/kg vigabatrin (VGB) administration

Rat	T_{max} (h)	C_{max} (µmol/l)	AUC (µmol h/l)	$t_{1/2}$ (h)
VGB 250 mg/kg				
1	1.0	19.5	61.7	1.9
2	1.5	21.9	56.6	1.3
3	1.0	17.8	83.8	2.7
4	1.0	15.2	72.8	2.7
5	1.0	22.4	85.0	2.4
6	0.5	14.8	68.1	2.1
Mean	1.0	18.6	71.8	2.2
±S.E.M.	0.2	1.3	4.4	0.2
VGB 500 mg/kg				
1	0.5	20.7	95.8	3.4
2	1.0	30.4	108.2	2.8
3	1.0	46.6	189.6	2.8
4	1.0	27.3	99.8	4.3
5	0.5	36.4	130.8	2.5
6	1.0	29.5	114.0	3.2
Mean	0.8	31.8	123.0	3.2
±S.E.M.	0.1	3.6	14.2	0.3
VGB 1000 mg/kg				
1	1.0	37.2	180.6	3.5
2	1.0	31.1	129.4	2.9
3	1.0	80.1	412.7	3.6
4	1.0	47.0	267.4	4.1
5	0.5	45.2	190.8	2.9
6	0.5	63.3	239.1	2.9
Mean	0.8	50.6	236.7	3.3
±S.E.M.	0.1	7.4	40.3	0.2

C_{max}, apparent maximum concentration; T_{max}, apparent time to maximum concentration; AUC, area under the serum concentration-time curve; $t_{1/2}$, terminal half-life.

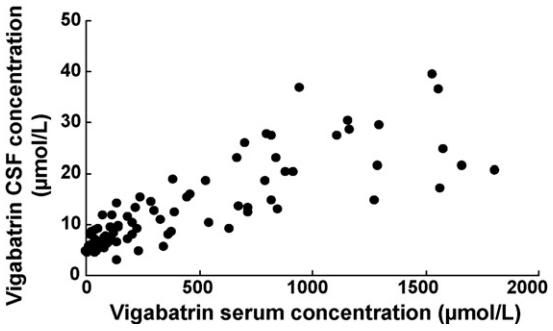

Figure 4 Relationship between vigabatrin concentrations in serum and CSF. Data are those from 500 mg/kg vigabatrin administration. The equation for linear regression is $y = 0.0149 + 5.8625$; $r^2 = 0.7106$.

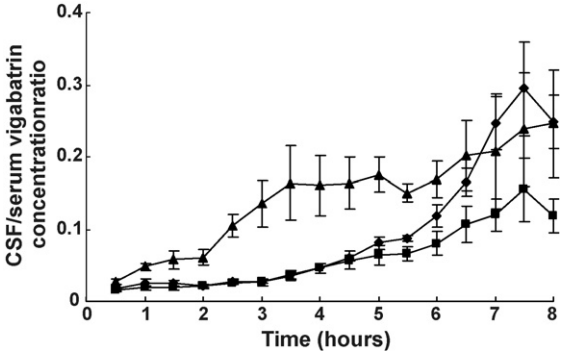

Figure 5 CSF/serum vigabatrin concentration ratios vs. time after 250 mg/kg (■)), 500 (◆) and 1000 (▲) mg/kg vigabatrin administration. Data are mean ± S.E.M. of 6—7 animals.

dose-independent were significantly longer than that seen in serum ($p < 0.001$). Compared to CSF, mean C_{max} and AUC serum vigabatrin values were significantly larger and CSF/serum C_{max} ratios ranged 0.019 and 0.017. Nevertheless, CSF and serum vigabatrin concentrations were linearly related (Fig. 4; $r^2 = 0.7106$).

Fig. 5 shows the vigabatrin CSF/serum concentration ratio over time after 250, 500 and 1000 mg/kg vigabatrin administration. It can be seen that equilibration between the blood and CSF compartments (as measured by a constant CSF/serum vigabatrin concentration ratio) was not achieved although there was a tendency towards equilibration by 7—8 h post-vigabatrin administration. For each sampling point, the mean CSF/serum vigabatrin concentration ratio was significantly lower than that of the corresponding free/total serum (free fraction) vigabatrin concentration ratio ($p > 0.001$).

Discussion

There is a sparcity of data on the pharmacokinetics of vigabatrin in animal species and those that are published in relation to the rat represent composite values obtained from individual animals killed at different time points after drug administration. Furthermore, the temporal pharmacokinetic inter-relationship of vigabatrin in blood and CSF has not been studied. Therefore we investigated the inter-relationship using a rat model which allows serial sampling of blood and CSF under conditions that involve minimal animal handling and thus pharmacokinetic parameters are obtained under physiological conditions.

Vigabatrin was administered as a single dose (250, 500 or 1000 mg/kg) by intraperitonial injection and the major (serum and CSF) pharmacokinetic findings of this study are that: (1) the pharmacokinetics of vigabatrin in serum are linear and dose-dependent; (2) vigabatrin is not protein bound in serum; (3) the elimination of vigabatrin from serum is rapid (mean $t_{1/2}$ values, 1.1—1.4 h) and independent of dose; (4) vigabatrin rapidly (mean T_{max} values, 0.8—1.0 h) penetrated the blood-brain barrier and was detectable at time of first CSF sampling (30 min post-dose); (5) although the CSF pharmacokinetics of vigabatrin paralleled that seen in serum, CSF vigabatrin concentrations represented only 2% of serum vigabatrin concentrations and did not reflect free drug concentrations in serum; (6) the efflux of vigabatrin from the CSF compartment was significantly slower (mean $t_{1/2}$ values, 2.2—3.3 h) than that suggested by serum values (mean $t_{1/2}$ values, 1.1—1.4 h).

That the efflux of vigabatrin from the CSF compartment was significantly slower (mean $t_{1/2}$ values, 2.2—3.3 h) than that suggested by serum values (mean $t_{1/2}$ values, 1.1—1.4 h), could be attributable to a number of factors. One possibility is that vigabatrin concentrations were below the lower limit of quantitation so that the estimation of CSF half-life values was unreliable. However, this was not the case since the lower limit of quantitation (1 μmol/l) was below vigabatrin concentrations measured in CSF (concentration range: 4.8—63.2 μmol/l). A second possibility is that if one were to use a one compartment model instead of a two compartment model for fitting the serum concentration versus time data, the serum elimination half-life would be underestimated. However, the vigabatrin data presented in Fig. 1 are well-described by a one compartment model analysis and therefore this possibility can be excluded.

Although there are reports of single point (at 2 h of 4 h post-dose) concentration analysis of serum vigabatrin in the rat after acute administration of 250, 500 and 1000 mg/kg vigabatrin, such data do

not allow any calculation of pharmacokinetic parameters.[18,19] The study of Valdizan et al.[17] reported on the serum vigabatrin concentration versus time profile after a single intraperitonial administration of 200 mg/kg vigabatrin. Blood samples were collected by heart puncture (thus, requiring a single rat per time point) and samples were collected at 4, 24, 48 and 72 h post-dose. Although the calculated $t_{1/2}$ value from these data is ~11 h, the fact that vigabatrin was not detectable at 48 h post-dose and that the $t_{1/2}$ value is based on only two time points (4 and 24 h) would suggest that the value cannot be considered as accurate. In the present study mean $t_{1/2}$ values for serum vigabatrin, as calculated from vigabatrin concentration versus time profiles characterised by 16 time point over a sampling period of 8 h, varied between 1.1 and 1.4 h (Table 1).

That vigabatrin enters the CSF compartment was confirmed by Halonen et al.[22] who reported on cisternal CSF concentrations 24 h after vigabatrin administration (100 or 250 mg/kg) for 4, 8 and 12 consecutive days. A further study by the same group[20] reported on cisternal CSF vigabatrin concentrations after a single vigabatrin dose (1000 mg/kg) administered intraperitoneally. CSF samples were collected at 5, 24, 48 and 96 h after vigabatrin administration and showed that vigabatrin concentrations were at their highest at 5 h and interestingly vigabatrin was still measurable in CSF samples collected at 96 h. Unfortunately, the sparcity of the data do not allow for any meaningful calculation of CSF pharmacokinetic data. However, comparing the CSF vigabatrin concentrations achieved at 5 h post-dose with concentrations observed in the present study (Fig. 3) vigabatrin concentrations are significantly different (88 μmol/l versus 18 μmol/l for the present study). The fact that the two studies used different rat strains may in part explain the difference.

Vigabatrin is an ethyl analogue of GABA and as such, and unlike GABA, passes through the blood brain barrier and enters the brain where it elevates brain GABA concentration via an inhibitory effect on GABA-transaminase. Two physiochemical characteristics which determine the rate and degree of entry into the CSF and brain are lipid solubility and the degree of serum protein binding, respectively. Because vigabatrin has high water solubility, it would not be expected to gain rapid entry into the brain. Nevertheless, vigabatrin did rapidly enter the central compartment since vigabatrin was detectable in CSF at time of first sampling (30 min; Fig. 3). Additionally, the rate of entry into the CSF was not dose-dependent, as there was no difference in T_{max} values after vigabatrin dose administration (250, 500 and 1000 mg/kg). Thus, the transport of vigabatrin across the blood-CSF barrier in the choroids plexus was not rate-limiting at the concentration ranges achieved in the present study. Indeed, serum and CSF vigabatrin concentrations were linearly related (Fig. 4), although the individual vigabatrin data points at the highest vigabatrin concentrations do show a tendency to scatter downwards which would suggest that there is still distribution of vigabatrin from the serum to the CSF at these points.

As the serum protein binding of a drug is a determinant of the available drug that can enter the brain/CSF, it might be expected that vigabatrin, which is not protein bound in serum, would be located in high concentrations in these central compartments. However, this is not the case with CSF concentrations representing but a small fraction (2%) of the concentrations observed in serum. In man the equivalent figure is 10%.[24] The reason for this is unknown, but it should be noted that free drug concentrations in blood and CSF will only be the same at steady-state, and interestingly these data with vigabatrin parallel that observed with another GABA acting drug, tiagabine.[35] In contrast, CSF concentrations of the antiepileptic drugs carbamazepine, lamotrigine, phenobarbitone, phenytoin and primidone are a direct reflection of the free (non-protein-bound) concentrations in serum.[26–28,36]

In conclusion, this is the first study to investigate the temporal pharmacokinetic inter-relationship of vigabatrin in rat blood and CSF and shows that vigabatrin has linear peripheral and central kinetics and with rapid penetration into the CSF compartment. Although vigabatrin is not protein bound in serum, CSF concentrations are only ~2% of those in serum and thus do not reflect free drug concentrations.

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