Influence of administration vehicles and drug formulations on the pharmacokinetic profile of lamotrigine in rats

M. M. Castel-Branco[a], I. V. Figueiredo[a], A. C. Falcão[a]*, T. R. A. Macedo[b], M. M. Caramona[a]

[a]Laboratory of Pharmacology, Faculty of Pharmacy, Coimbra University, Coimbra, Portugal [b]Institute of Experimental Pharmacology and Therapeutics, Faculty of Medicine, Coimbra University, Coimbra, Portugal

ABSTRACT

Given that administration vehicles and drug formulations can affect drug bioavailability, their influence on the pharmacokinetic profile of lamotrigine (LTG), a new-generation anti-epileptic drug, was studied in rats. Three different formulations administered intraperitoneally at a dose of 10 mg/kg were used: (1) LTG suspended in a 0.25% methylcelulose solution, (2) LTG dissolved in a 50% propylene glycol solution, and (3) LTG isethionate dissolved in distilled water. Plasma and brain homogenate levels were determined in order to evaluate vehicle-dependent drug absorption. The results demonstrated rapid absorption of LTG when it was administered as an aqueous solution, in contrast to a slower and more erratic absorption after the injection of either the lipophilic solution or the suspension. A plasma peak was achieved 15 min post-dose with the aqueous solution, with a brain peak being achieved 15 min later, while with the other formulations both plasma and brain homogenate peaks were reached 2 h after LTG administration. This study suggests that LTG isethionate dissolved in distilled water is the most suitable formulation for successful LTG pharmacokinetic studies in rats.

INTRODUCTION

Lamotrigine (LTG) [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine] is a new-generation anti-epileptic drug which has been shown to be effective against partial and secondarily generalized tonic-clonic seizures, either as adjunctive treatment in patients with refractory epilepsy or as monotherapy [1]. The first mechanism of action of LTG described in the literature was similar to that presented by the anti-epileptics carbamazepine and phenytoin and involved the stabilization of the presynaptic membrane through blockade of voltage-sensitive sodium channels, which resulted in inhibition of the release of excitatory neurotransmitters, particularly glutamate and aspartate [1]. However, taking into account the fact that LTG is effective against more forms of epilepsy than carbamazepine and phenytoin, it has been suggested that LTG acts via additional mechanisms other than inhibition of voltage-dependent sodium channels alone. Thus, it has been proposed that LTG also inhibits high voltage-activated calcium currents, consequently interacting with the vesicular release of transmitters [2–5]. Modulation of potassium currents via blockade of potassium channels was also proposed as a possible mechanism [4]. In spite of contrary results obtained by Waldmeier et al. on LTG modulation of GABA (γ-aminobutyric acid) release [6], Cunningham and Jones suggested that the reciprocal modulation of the background release of the major excitatory and inhibitory transmitters (inhibition of glutamate and enhancement of GABA release) may explain the anticonvulsive effect of LTG [5]. A recent study by Hassel et al. showed an increase in rat hippocampal GABA shunt activity and an elevation in cerebral taurine levels after chronic treatment with LTG [7]. The first mechanism proposed to explain the anticonvulsant activity of LTG

seems to be doubtful as it was shown that the blockade of sodium channels was not a prerequisite for inhibition of glutamate release [5,6].

A linear relationship appears to exist between the dose of LTG administered and the respective plasma concentration [8], although its interrelation with the induced pharmacological response remains unknown. Tentative target ranges of 1–4 mg/L have been proposed, but subsequent observations have indicated that some patients may tolerate much higher therapeutic concentrations (> 10 mg/L) without clinical toxicity [9]. In the light of current knowledge, it seems evident that, in order to assess the relationship between plasma concentration and clinical effect of LTG, further studies are required. As LTG needs to cross the blood–brain barrier to exercise its therapeutic effect, the basis for the interpretation of the LTG plasma levels requires that these levels reflect the drug concentrations at the neuronal sites of action. From this perspective, the neuropharmacokinetic characterization of LTG would necessarily have to include a parallel study of the evolution of LTG concentrations in the blood and in the brain over time if we wish to establish the relationship between the two types of curve. For ethical and logistical reasons, this type of work would have to resort to animal experimentation for the determination of the pharmacokinetic profile at the level of the central nervous system. However, although most hypotheses on the fundamental mechanisms of human epileptic phenomena derive from investigations carried out on experimental animal models, research on patients with epilepsy is essential to validate the relevance of the emerging data. Nevertheless, ethical considerations limit experimental paradigms severely, suggesting that parallel investigations involving patients and experimental animals need to be designed [10].

One of the initial problems in a laboratory drug study is the choice of an adequate vehicle and formulation, since administration vehicles and drug formulations could have consequences for drug bioavailability [11]. Bearing in mind that anti-epileptic drugs have to cross the blood–brain barrier to exercise their therapeutic effect in the central nervous system, most anti-epileptic drugs are lipophilic [12]. Consequently, water insolubility becomes a common problem in the laboratory evaluation of these drugs. To resolve this problem, it often becomes necessary to resort to a suspension or a lipophilic vehicle to inject the drug into the laboratory animal. However, if this choice is incorrect, low or retarded absorption may occur and consequently the

results of the study may be misinterpreted. For this reason, the choice of an adequate vehicle and formulation becomes a critical factor in the development of laboratory pharmacokinetic and pharmacodynamic studies of this class of drug.

Several experimental studies have been carried out over the last 20 years to elucidate the anticonvulsant profiles or the mechanisms of action of LTG, but few studies have characterized the pharmacokinetic profile of this drug in the blood and brains of laboratory animals [13,14]. To complete the characterization of the neuropharmacokinetics of LTG in order to better understand the relationship between LTG plasma levels and its pharmacological response, further studies are necessary. Upon consultation of the literature for how to administer LTG to the rats, we found that a suspension was the most frequently used formulation. Therefore we performed a preliminary study to determine the most appropriate administration vehicle and drug formulation for successful LTG pharmacokinetic and pharmacodynamic studies [15].

MATERIALS AND METHODS

Animals

This study was carried out on adult male Wistar rats (250–320 g) housed in a local bioterium with a controlled dark/light cycle (12 h/12 h). Animals were allowed free access to food and water until the experiment began. The experiments were performed at 22–23 °C. Animal experimentation in this study was conducted in accordance with European guidelines for the care and use of laboratory animals (86/609/EEC) and the protocol accredited by the Portuguese Veterinary General Division.

Drugs

Lamotrigine and lamotrigine isethionate were kindly provided by Wellcome Research Laboratories (Cardiff, UK). Three different LTG formulations for intraperitoneal (i.p.) injection were used at a dose of 10 mg/kg (5 mg/mL): (1) LTG suspended in 0.25% methylcelulose in distilled water (suspension); (2) LTG dissolved in 50% propylene glycol in distilled water (lipophilic solution), and (3) LTG isethionate dissolved directly in distilled water (aqueous solution). Ketamine hydrochloride (7.7 mg/kg) (Parke-Davis, Pfizer Laboratories, Scixal, Portugal) and chlorpromazine (2.3 mg/kg) (Vitória Laboratories, Amadora, Portugal) were used for anaesthesia. Reagents and columns used in the chromato-

graphic analysis were purchased from Merck (Merck KGaA, Darmstadt, Germany).

Experimental design

The animals were divided into three groups of 30 animals, each group receiving intraperitoneally, at the same time in the morning, one of the three formulations referred to above. Sample collection occurred at predetermined times. Subgroups of five animals were used at each data point. The blood samples were obtained by open cardiac puncture and collected in citrated tubes at 15 min, 30 min, 2 h, 12 h, 24 h and 48 h post-dose. This procedure was carried out under anaesthesia intramuscularly injected 10 min before the predetermined sampling time. Immediately afterwards, the animals were decapitated to remove the brain. The brain homogenization was performed with 5 mL of phosphate buffer (pH = 7.4) per g of brain tissue at 4 °C. Plasma and brain homogenate were immediately frozen at −25 °C until analysis.

Lamotrigine quantification

Lamotrigine levels in plasma and brain homogenates were determined according to a high-performance liquid chromatography (HPLC) method previously described [16]. Briefly, to 1 mL of plasma, 100 μL of a 40 mg/L internal standard solution, 1 mL of 2 M NaOH and 5 mL of ethyl acetate were added. After centrifugation, the upper organic layer was transferred to a clean 10-mL conical glass tube and evaporated to dryness. The brain homogenate extraction included a previous deproteinization step: to 1 mL of brain homogenate, 100 μL of a 20 mg/L internal standard solution and 100 μL of a 20% trichloroacetic acid solution were added. After centrifugation, the supernatant was transferred to a 10-mL glass tube and submitted to a liquid-liquid extraction into ethyl acetate after basification as described for plasma. The residues obtained were reconstituted with 200 μL of mobile phase and injected into the HPLC system. Chromatographic separation was carried out on a LiChrospher 100 RP-18 (5 μm) LiChroCART 125-4 (Merck KGaA) for 10 min. The mobile phase, consisting of 35.0% methanol, 64.7% 0.1 M potassium dihydrogen phosphate aqueous solution and 0.3% triethylamine, was pumped at a flow rate of 1.0 mL/min. The detector was set at 306 nm. The linearity was demonstrated over a range of 0.1–15.0 mg/L for plasma and 0.1–5.0 mg/L for brain homogenate, with a LTG detection limit of 0.01 and 0.02 mg/L in plasma and brain homogenate, respectively. The results of the method validation were all in accordance with international recommendations, demonstrating the suitability of the method for LTG quantification in these biological matrices.

RESULTS

LTG plasma and brain homogenate concentration levels measured after i.p. administration of 10 mg/kg LTG in a suspension, lipophilic solution or aqueous solution are listed in *Table I(a,b)*. A rapid absorption of LTG was observed when it was administered as an aqueous solution, with a peak plasma value of 5.69 ± 0.71 mg/L at 15 min post-dose. Because no major differences were found between the brain homogenate values measured at 30 min and at 2 h post-dose (ANOVA, $P > 0.05$), brain peak was considered to have been achieved at 30 min post-dose. The coefficient of variation for the values obtained with the aqueous solution ranged from 12 to 31% for plasma measurements and from 16 to 18% for brain homogenate levels. When LTG was administered dissolved in a propylene glycol solution or suspended in a methylcelulose solution, the plasma and brain homogenate peak values were reached only at 2 h post-dose. With these formulations, larger values of coefficient of variation were observed.

No significant differences were found amongst the values of area under the curve (AUC) calculated for the three formulations in each tissue (confidence limits at 95% significance level included all the three AUC values). However, statistical differences were observed amongst plasma values obtained with the three formulations at 15 min post-dose and between the values obtained with the aqueous solution and those obtained with the other formulations at 30 min post-dose. In the brain, statistical differences were also observed between the values obtained with the aqueous solution and those obtained with the other formulations before 2 h post-dose (ANOVA, $P < 0.05$) (*Figure 1*).

DISCUSSION

As mentioned above, the choice of an adequate vehicle and formulation is of the utmost importance to avoid misinterpretations of the results in pharmacokinetic and pharmacodynamic studies in laboratory animals. Having consulted the literature for which formulation is most frequently used to administer LTG to animals, three formulations were chosen and preliminary studies were performed in order to choose the best one.

Table I LTG plasma (a) and brain homogenate (b) concentrations after i.p. administration of 10 mg/kg LTG in a suspension, a lipophilic solution or an aqueous solution.

Time after administration (h)	LTG plasma concentration (mg/L)					
	Suspension	CV (%)	Lipophilic solution	CV (%)	Aqueous solution	CV (%)
(a)						
0.25	0.88 ± 0.266*	30	2.63 ± 2.015*	77	5.69 ± 0.706*	12
0.5	1.72 ± 0.104	06	2.45 ± 1.039	42	4.99 ± 1.099*	22
2	4.20 ± 0.932	22	4.37 ± 0.517	12	4.83 ± 0.916	19
12	3.91 ± 1.068	27	3.18 ± 0.460	14	3.44 ± 0.488	14
24	3.40 ± 1.522	45	2.75 ± 0.619	23	2.56 ± 0.567	22
48	1.50 ± 0.442	30	1.41 ± 0.218	15	1.33 ± 0.414	31
AUC_{0-48} (mg/L/h)	148.08		129.33		133.44	

Time after administration (h)	LTG brain homogenate concentration (mg/L)					
	Suspension	CV (%)	Lipophilic solution	CV (%)	Aqueous solution	CV (%)
(b)						
0.25	0.33 ± 0.070*	21	0.72 ± 0.569	79	1.08 ± 0.177*	16
0.5	0.52 ± 0.147	28	0.78 ± 0.387	49	1.64 ± 0.255*	16
2	1.62 ± 0.542	33	1.70 ± 0.272	16	1.67 ± 0.283	17
12	1.43 ± 0.179*	13	1.12 ± 0.191*	17	1.21 ± 0.205	17
24	1.20 ± 0.625	52	0.97 ± 0.126	13	0.75 ± 0.129	17
48	0.51 ± 0.363	72	0.57 ± 0.128	23	0.58 ± 0.105	18
AUC_{0-48} (mg/L/h)	53.30		47.26		45.08	

Data are mean ± SD for five rats; CV, coefficient of variation (SD/mean × 100); AUC, area under the curve (trapezoid rule); *statistically significant difference ($P < 0.05$) between values obtained using different formulations (ANOVA).

The vehicles and formulations chosen were the following: LTG suspended in a 0.25% methylcellulose solution, taking into consideration that this is the most frequently used formulation in experimental studies [e.g. 17,18]; LTG dissolved in a lipophilic solution of 50% propylene glycol in water, as used by Walker et al. [14]; LTG salt dissolved directly in distilled water, the unique hydrophilic form of LTG [13,19].

The final goal of our work was to ensure that the assessment of the relationship between plasma concentration and clinical effect of LTG in laboratory animals is not biased as a consequence of the use of an incorrect formulation for drug administration. From this perspective, the intraperitoneal route seems to be the most effective for successful outcomes in these studies, not only because the technique is simple and reproducible, but also, given the high vascularization existing in the peritoneum, because it can rapidly ensure that the total amount of the drug administered is in the circulation. Moreover, as a parenteral route it avoids the very slow absorption step inherent in oral drug administration.

The 10 mg/kg dose was chosen because this dose has previously been found to be within the anticonvulsive range in the rat [18]. Also, with this dose the plasma concentrations achieved were similar to the concentration range that has been proposed for epileptic patients [20].

We studied whether administration of LTG as suspension or solution, or as solution in different vehicles, resulted in differences in terms of LTG plasma and brain homogenate levels. The comparison amongst the calculated AUC values showed similar magnitudes of absorption irrespective of the formulations used. However, the significant differences detected amongst the LTG levels determined during the first 2 h revealed different absorption rates for the three formulations used. Compared to experiments with solutions, injection of LTG as suspension resulted in slower absorption. In fact, drug solutions are usually preferable to drug suspensions because of the retarded absorption which can be associated with administration of the drug as a suspension – a consequence of the greater difficulty of the drug particles in crossing the peritoneum [11]. Compared to
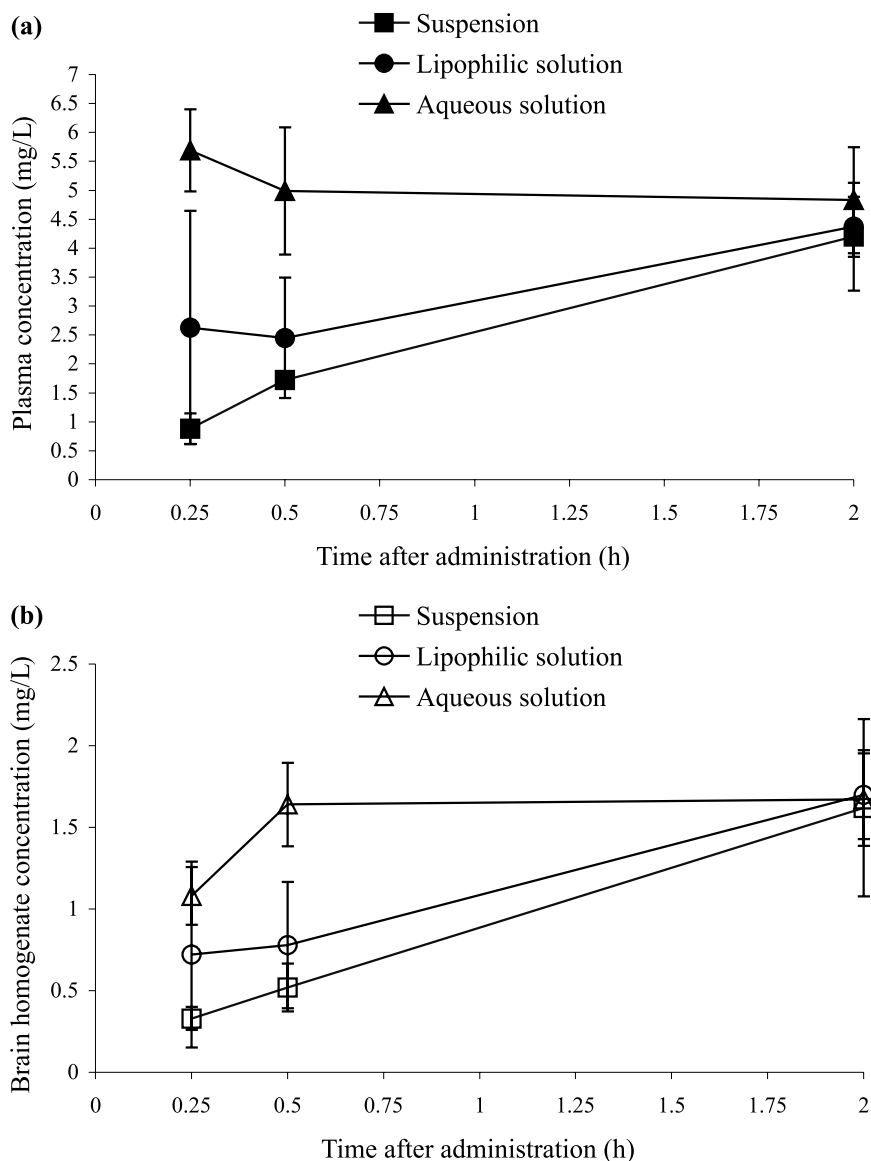
(a)

(b)

Figure 1 LTG concentration profiles in plasma (a) and brain homogenate (b) over the first 2 h (mean ± SD; *n* = 5).

administration of LTG as an aqueous solution, injection of LTG dissolved in a lipophilic vehicle also resulted in retarded absorption, as a result of the slower liberation of the drug from the vehicle in which it is dissolved. Moreover, the dispersion of the measurements at each sampling time was usually greater when LTG was administered as a suspension or a lipophilic solution, as reflected by higher values of coefficient of variation. This finding is important, bearing in mind that greater dispersion is associated with lower reproducibility of results.

As can be seen in *Figure 1*, LTG brain homogenate levels peaked at 2 h post-dose, following the same patterns observed with the respective plasma measurements in the case of the suspension and the lipophilic solution. These results were expected considering the slow entry of the drug from the peritoneum into the blood stream, which permitted the simultaneous passive diffusion of the drug from the blood into the brain tissue. Consequently, for entry of the drug into the brain, entry into the blood stream was a rate-limiting step. In the case of the aqueous solution it was possible to differentiate

between plasma and brain peaks, the latter being achieved 15 min after the plasma peak. Interestingly, an analysis of variance revealed no significant differences amongst the plasma levels measured during the first 2 h, which may suggest, although it is not a rate-limiting step, that drug entry from the peritoneum into the blood stream was only complete at 2 h post-dose. As a consequence, brain peak was achieved rapidly but the brain values were maintained while plasma values were not decreasing.

CONCLUSION

This study emphasizes that administration vehicle and drug formulation are critical factors in the laboratory evaluation of anticonvulsant drugs [11]. Plasma and brain level determinations are shown to be essential in evaluating the problems of vehicle-dependent drug absorption. From the results of this study it can be concluded that an aqueous solution of a LTG salt (LTG isethionate dissolved in distilled water) is the best formulation for successful LTG pharmacokinetic/pharmacodynamic experimental studies.

ACKNOWLEDGEMENTS

REFERENCES

1 Goa K.L., Ross S.R., Chrisp P. Lamotrigine – a review of its pharmacological properties and clinical efficacy in epilepsy. Drugs (1993) **46** 152–176.

2 Stefani A., Spadoni F., Sniscalchi A., Bernardi G. Lamotrigine inhibits Ca^{2+} currents in cortical neurons: functional implications. Eur. J. Pharmacol. (1996) **307** 113–116.

3 Stefani A., Spadoni F., Bernardi G. Differential inhibition by riluzole, lamotrigine, and phenytoin of sodium and calcium currents in cortical neurons. Implications for neuroprotective strategies. Exp. Neurol. (1997) **147** 115–122.

4 Grunze H., Wegerer J., Greene R.W., Walden J. Modulation of calcium and potassium currents by lamotrigine. Neuropsychobiology (1998) **38** 131–138.

5 Cunningham M.O., Jones R.S.G. The anticonvulsant lamotrigine decreases spontaneous glutamate release but increases spontaneous GABA release in the rat entorhinal cortex in vitro. Neuropharmacology (2000) **39** 2139–2146.

6 Waldmeier P.C., Baumann P.A., Wicki P., Feldtrauer J.J., Stierlin C., Schmutz M. Similar potency of carbamazepine,

7 Hassel B., Tauboll E., Gjerstad L. Chronic lamotrigine treatment increases rat hippocampal GABA shunt activity and elevates cerebral taurine levels. Epilepsy Res. (2001) **43** 153–163.

8 Peck A.W. Clinical pharmacology of lamotrigine. Epilepsia (1991) **32** S9–S12.

9 Bartoli A., Guerrini R., Belmonte A., Alessandrì M.G., Gatti G., Perucca E. The influence of dosage, age, and comedication on steady state plasma lamotrigine concentrations in epileptic children: a prospective study with preliminary assessment of correlations with clinical response. Ther. Drug Monit. (1997) **19** 252–260.

10 Engel J. Experimental models – experimental animal models of epilepsy: classification and relevance to human epileptic phenomena. Epilepsy Res. (1992) **8** (Suppl.) 9–20.

11 Löscher W., Nolting B., Fassbender C.P. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. I. The influence of administration vehicles. Epilepsy Res. (1990) **7** 173–181.

12 Löscher W., Frey H. Kinetics of penetration of common antiepileptic drugs into cerebrospinal fluid. Epilepsia (1984) **25** 346–352.

13 Walton N.Y., Jaing Q., Hyun B., Treiman D.M. Lamotrigine vs. phenytoin for treatment of status epilepticus: comparison in an experimental model. Epilepsy Res. (1996) **24** 19–28.

14 Walker M.C., Tong X., Perry H., Alavijeh M.S., Patsalos P.N. Comparison of serum, cerebrospinal fluid and brain extracellular fluid pharmacokinetics of lamotrigine. Br. J. Pharmacol. (2000) **130** 242–248.

15 Castel-Branco M.M., Almeida A.M., Gomes C.A. et al. Preliminary pharmacokinetic profiles of lamotrigine in rats: influence of administration vehicles and drug formulations. Fundam. Clin. Pharmacol. (2001) **15** 30.

16 Castel-Branco M.M., Almeida A.M., Falcão A.C., Macedo T.A., Caramona M.M., Lopez F.G. Lamotrigine analysis in blood and brain by high-performance liquid chromatography. J. Chrom. B (2001) **755** 119–127.

17 Miller A.A., Wheatley P., Sawyer D.A., Baxter M.G., Roth B. Pharmacological studies on lamotrigine, a novel potential antiepileptic drug. I. Anticonvulsant profile in mice and rats. Epilepsia (1986) **27** 483–489.

18 Wheatley P.L., Miller A.A. Effects of lamotrigine on electrically induced afterdischarge duration in anaesthetised rat, dog, and marmoset. Epilepsia (1989) **30** 34–40.

19 Conroy B.P., Black D., Lin C.Y. et al. Lamotrigine attenuates cortical glutamate release during global cerebral ischemia in pigs on cardiopulmonary bypass. Anesthesiology (1999) **90** 844–854.

20 Morris R.G., Black A.B., Harris A.L., Batty A.B., Sallustio B.C. Lamotrigine and therapeutic drug monitoring: retrospective survey following the introduction of a routine service. Br. J. Clin. Pharmacol. (1998) **46** 547–551.