ORIGINAL ARTICLE

Lamotrigine kidney distribution in male rats following a single intraperitoneal dose

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ABSTRACT

As it has been previously shown that lamotrigine (LTG) accumulates in the kidney of male rats, the purpose of the present investigation was to characterize the kidney profiles of LTG and its kidney distribution pattern in male rats, in order to confirm if a preferential distribution exists and to analyse if it does or does not affect the LTG systemic pharmacokinetics. Adult male Wistar rats were intraperitoneally injected with 5, 10 and 20 mg/kg of LTG. The concentration–time profiles of LTG in plasma and whole kidney were determined over 120 h postdose. The distribution of LTG in the rat kidney was investigated in another group of rats by measuring LTG levels in the renal cortex and medulla. The LTG plasma concentration–time profiles revealed a linear relationship with dose. However, a slight increase in the LTG elimination half-life with dose was observed. In contrast, a nonlinear relationship was established between LTG kidney levels and the dose administered. Consequently, nonparallel patterns were observed between LTG plasma and kidney profiles. The LTG kidney distribution pattern revealed an accumulation of LTG in the renal cortex. The present study demonstrated that LTG distributes preferentially to the kidneys of the male rat in a dose-dependent manner and suggests that such distribution may slightly affect the systemic kinetics of the drug.

INTRODUCTION

Lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine, LTG] is a new generation antiepileptic drug which has shown to be effective against partial and secondarily generalized tonic–clonic seizures, either as adjunctive treatment in patients with refractory epilepsy or when received as monotherapy [1]. Actually, LTG exhibits a relatively broad spectrum of efficacy, including some major seizure types, such as partial seizures (with or without secondary generalization), primarily generalized tonic–clonic seizures, absence seizures and drop attacks. Its efficacy in myoclonic seizures and infantile spasms is still unclear [2]. The first mechanism of action of LTG described in the literature was similar to that presented by carbamazepine and phenytoin and involved the stabilization of the presynaptic membrane through the blockade of the voltage-sensitive sodium channels, which resulted in inhibition of excitatory neurotransmitters release, particularly glutamate and aspartate [1]. More recently, it was proposed that LTG also inhibits high voltage-activated calcium currents, interacting consequently with the vesicular release of transmitters [3,4].

During the development of LTG, preclinical studies on its absorption, distribution and elimination were conducted in several animal species. The tissue distribution of LTG was expected to be good, bearing in mind the basic and lipophilic properties of the molecule. In the male rat, besides a good distribution of the antiepileptic drug through all organs and tissues, an accumulation of LTG in the kidneys was observed [5]. This accumulation was attributed to alterations in the renal handling of α-2U-globulin in this species and gender [6,7]. It was suggested that this effect could partly explain the maintenance of LTG serum concentrations over a long period of time [8].

The purpose of the present investigation was to characterize the kidney profiles of LTG in the male rat as well as its kidney distribution pattern, by analysing kidney samples at predetermined times after acute LTG administration, at various dosages. This study aims to confirm if such preferential distribution exists and if it does or does not affect the LTG systemic kinetics in the rat.

MATERIALS AND METHODS

Animals

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

Drugs

Lamotrigine, lamotrigine isethionate and the internal standard BW725C78 [3,5-diamino-6-(2-methoxyphenyl)-1,2,4-triazine] were kindly provided by Wellcome Research Laboratories (Cardiff, UK). Ketamine hydrochloride (7.7 mg/kg) (Pfizer Laboratories, Seixal, Portugal) and chlorpromazine (2.3 mg/kg) (Vitória Laboratories, Amadora, Portugal) were used to anaesthetize the animals before sample collection. Reagents and columns used in the chromatographic analysis were purchased from Merck (Merck KGaA, Darmstadt, Germany).

Experimental design

For the characterization of the plasma and kidney LTG profiles in the rat, three groups of 45 animals each were given LTG 5, 10 and 20 mg/kg in an aqueous solution of LTG isethionate, intraperitoneally (i.p.) [9]. Sample collection occurred at predetermined times. Subgroups of five animals were used at each data point. Blood samples were obtained by open cardiac puncture and collected in citrated tubes at 7.5, 15 and 30 min, 2, 12, 24, 48, 72 and 120 h postdose. Immediately afterwards, the kidneys were removed and homogenized in 5 mL of phosphate buffer (pH 7.4) per g of tissue at 4 °C. Blood collection was carried out under anaesthesia injected intramuscularly 10 min prior to the above-mentioned procedure. Plasma and kidney homogenates were immediately frozen at −25 °C until analysis.

For the study of the distribution of LTG in the rat kidney, another group of 30 animals was used. All the animals were also given LTG 10 mg/kg i.p. at the same time in the morning, and subjected to the same procedure. Kidney removal occurred at 12, 24 and 48 h postdose. Immediately afterwards the cortex and medulla of each kidney was dissected on ice. Taking into account the small dimension of the kidney medulla region and the need to obtain 1 mL of tissue homogenate as a minimum for the execution of the analytical technique, subgroups of 10 animals were used at each data point and a pool of each kidney region was formed from the 10 animals used. The resulting portions of kidney tissue were homogenized as mentioned above.

Lamotrigine quantification

LTG levels in plasma were determined according to a high-performance liquid chromatography (HPLC) method as previously described [10]. LTG levels in kidney homogenate were determined by the same analytical method adapted to this biological matrix. The method proved to be linear between 0.1 and 15.0 mg/L for kidney homogenate, with a detection limit of 0.025 mg/L. The mean coefficients of variation were 5.69% for intra-day and 8.76% for inter-day analyses. The bias varied between −10.10 and 7.92% for the intra-day assay and between −4.95 and 7.97% for the inter-day assay. The results of the method validation were all in accordance with international recommendations, providing the suitability of the method for the LTG quantification in this biological matrix.

RESULTS

Plasma and whole kidney profiles

Time courses of LTG plasma and whole kidney homogenate concentrations following i.p. administration of LTG 5, 10 and 20 mg/kg are shown in *Figure 1a,b*, respectively.

The plasma concentration vs. time profiles of LTG exhibited peak concentrations at 0.25–2.0 h postdose. Thereafter, LTG plasma levels decreased monoexponentially. The area under the plasma concentration vs. time curve (*AUC*) values had a linear relationship to dose ($r^2 = 0.999$). Good correlations were also found between the plasma concentrations vs. time plots at different dosages ($r^2 > 0.95$, for all values following the first sampling time). *Table I* summarizes the pharmaco-

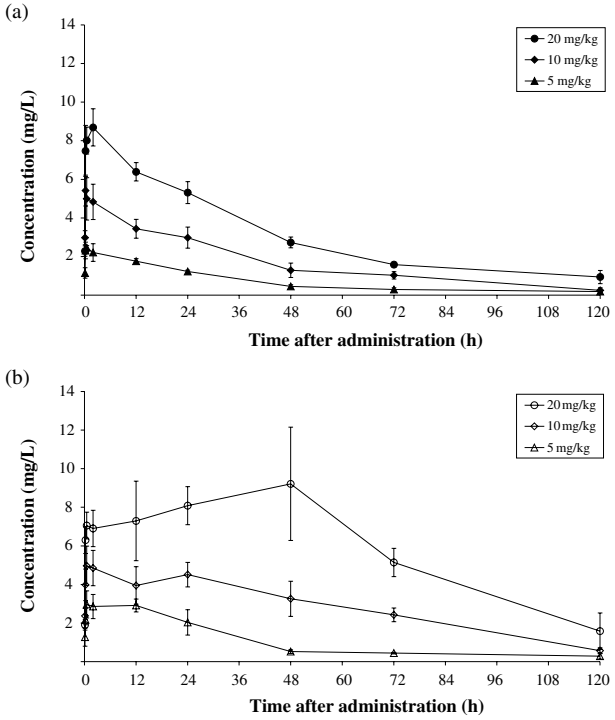

Figure 1 Plasma (a) and whole kidney homogenate (b) LTG concentration vs. time profiles after i.p. administration of LTG 5, 10 and 20 mg/kg. Data are mean ± standard deviation of five rats.

Table I Pharmacokinetic parameters after 5, 10 and 20 mg/kg LTG i.p. administration (noncompartmental analysis).

Parameters	5 mg/kg	10 mg/kg	20 mg/kg
k_{el} (h^{-1})	0.023	0.025	0.019
$t_{1/2el}$ (h)	29.90	28.02	36.12
AUC_0^∞/D (kg h/L)	18.19	20.82	20.92
t_{max} (h)	0.50	0.25	2.00
C_{max} (mg/L)	2.46	5.42	8.69
V_d (L/kg)	2.37	1.93	2.49
Cl (L/h/kg)	0.055	0.048	0.048
MRT (h)	43.38	39.67	51.66

k_{el}, Elimination rate constant; $t_{1/2el}$, elimination half-life; AUC_0^∞/D, area under the plasma concentration vs. time curve normalized with dose; t_{max}, peak concentration time; C_{max}, maximal concentration; V_d, volume of distribution; *Cl*, clearance; *MRT*, mean residence time.

kinetic parameters computed with WinNonlin® [11] according to a noncompartmental analysis.

In the LTG kidney profiles, the concentrations of the drug seemed to be constant for a dose given, corresponding to a plateau. In fact, an analysis of variance revealed no significant differences among the LTG kidney levels measured between 15 min and 12 h postdose for LTG 5 mg/kg, between 15 min and 24 h for LTG 10 mg/kg and between 15 min and 48 h for LTG 20 mg/kg (ANOVA, $P > 0.05$). Although *AUC* values had a linear relationship to dose ($r^2 = 0.999$), the correlations found between the kidney concentrations vs. time plots at different dosages did not reveal a linear relationship between kidney levels and dose ($r^2 = 0.719$ between LTG 5 and 10 mg/kg, $r^2 = 0.182$ between LTG 5 and 20 mg/kg and $r^2 = 0.605$ between LTG 10 and 20 mg/kg, for all values following 0.125 h).

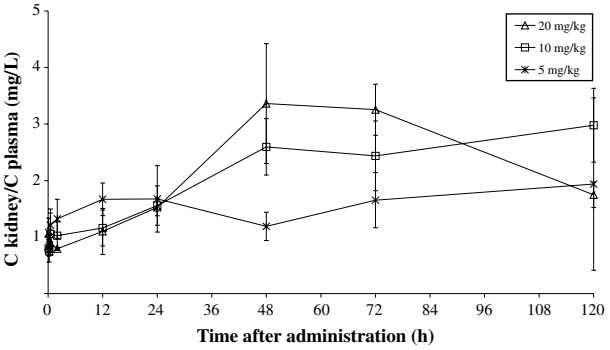

Figure 2 Whole kidney homogenate/plasma concentration ratios vs. time profiles after i.p. administration of LTG 5, 10 and 20 mg/kg. Data are mean ± standard deviation of five rats.

Kidney/plasma relationship

Nonlinear correlations were found between LTG plasma and kidney levels at each dose studied ($r^2 = 0.876$, $r^2 = 0.711$ and $r^2 = 0.218$ for LTG 5, 10 and 20 mg/kg, respectively, for all values following 0.125 h). The degree of drug uptake from plasma into kidney tissue was estimated from the ratio of the drug concentration in kidney homogenate over the plasma concentration (C_{kidney}/C_{plasma}). The time courses of these ratios at each dose studied are shown in *Figure 2*.

Regional kidney distribution

Table II shows the levels of LTG measured in the renal cortex and renal medulla after i.p. administration of LTG 10 mg/kg.

DISCUSSION

The present study was performed to characterize the LTG kidney distribution in the male rat following its acute

Table II LTG concentrations in renal cortex and renal medulla at 12, 24 and 48 h after 10 mg/kg LTG i.p. administration.

	Tissue concentration[a]			
	12 h	24 h	48 h	Regression equation
Renal cortex	5.53	4.82	4.80	$y = -0.0175x + 5.540$
Renal medulla	2.23	1.50	1.29	$y = -0.0236x + 2.335$

[a]The values presented result from a pool of 10 animals and are expressed as mg/L of tissue homogenate.

administration, as it was previously shown that LTG accumulates in the kidney of male rats. As far as we are aware, only Parsons et al. [5] quantified the degree of accumulation of LTG in the kidney of the male rat, presenting kidney/plasma ratios ranging from approximately 10 to 300, depending on the dosing regimen. Furthermore, there is no published data describing the distribution of LTG in the kidney tissue and this is the first report on the characterization of the LTG kidney distribution pattern.

The LTG plasma concentration vs. time profiles revealed that the drug rapidly appears in the plasma after i.p. administration and that, after reaching a maximum (at 15 min postdose), LTG concentrations exhibit a monoexponential decay. The LTG plasma concentration vs. time profiles exhibited a linear relationship to dose, which was confirmed by the regression analysis performed as well as by the similarity observed between the values estimated for some pharmacokinetic parameters at the three doses studied (*Figure 1a* and *Table I*). However, a more detailed analysis of *Table I* should consider a slight increase in the LTG elimination half-life for the highest dose used, which may suggest a tendency to nonlinearity.

Conversely, the LTG kidney concentration vs. time profiles exhibited a plateau during the initial sampling hours, which was revealed to be dose-dependent, since it was prolonged, the greater the dose administered. Consequently, a nonlinear relationship was established between LTG kidney values and the dose administered (*Figure 1b*). Accordingly, nonparallel patterns were observed between LTG plasma and kidney profiles, which can be illustrated by the nonlinear relationship established between drug in the plasma and drug in the kidney ($r^2 < 0.88$) and by the differences observed among kidney/plasma concentration ratio values calculated over time. In fact, *Figure 2* shows biphasic curves, with a ratio of approximately 1 between 0 and 24 h, whatever the dose, and a ratio higher than 1 between 24 and 120 h, whatever the dose, in accordance with standard deviations. Besides that, the study of the LTG kidney distribution pattern revealed an accumulation of LTG in the renal cortex, as demonstrated by the higher *y*-intercept value and by the smaller slope value of the regression equation obtained from the LTG levels measured in the renal cortex, when compared with those of the renal medulla. This accumulation is in accordance with the mechanistic studies that associate the LTG preferential distribution to the kidney with alterations in the renal handling of α-2U-globulin in the male rat. In fact, taking into account the basic and lipophilic properties of the molecule of LTG together with the carrier specificity of this protein for lipophilic ligands, it can be suggested that LTG, when bounded to the α-2U-globulin, makes it more resistant to hydrolysis and/or, when accumulated in the lysosomes, has a direct inhibitory effect on lysosomal function. This reduction of the proteolytic breakdown of α-2U-globulin occurs in the proximal tubule lysosomes, which are located in the cortical region of the kidney [6,7].

The comparison between the LTG plasma profiles and the correspondent kidney plots considered in this study is important to understand the influence of the LTG preferential distribution to the kidneys on the systemic kinetics of LTG in the rat, which is one of the laboratory animal species used most in experimental studies involving antiepileptics. Taking into account the slight increase observed in the LTG elimination half-life with dose, it should be considered that the preferential distribution of LTG to the kidney tissue may influence the systemic pharmacokinetic of this drug. In fact, an increase in the LTG elimination half-life can be a consequence of the drug redistribution from the kidneys to the plasma. Obviously, this increment will be greater with higher doses, as a result of the dose-dependent feature of the LTG kidney accumulation. The results obtained in this study are in accordance with the hypothesis suggested by Walker et al. [8]. However, taking into consideration the lack of urine data, it is not possible to exclude the fact that kidney levels only reflect the renal clearance. This seems to be the situation reported by Parsons et al. [5], when they attribute the marked difference observed in the plasma half-life between male and female rats to differences in the renal handling of α-2U-globulin in this species. Therefore, more studies should be performed to confirm the influence of the LTG kidney accumulation in the systemic kinetics of the drug in the rat.

CONCLUSION

The present investigation demonstrated that LTG distributes preferentially to the kidneys of the male rat and suggests that such a distribution may have some influence in the systemic kinetics of the drug in this species. Nevertheless, more studies involving multiple regimens and the collection of urine data should be performed to confirm the relevance of this phenomenon in the systemic kinetics of the drug.

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