Original Article

No impact of neuropathy on pharmacokinetic of lamotrigine in rat model

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Abstract

Purpose: The aim of the present research is to monitor any alteration in the serum concentrations of lamotrigine (LMT) in peripheral neuropathic conditions compared with normal conditions in a rat model. **Materials and Methods:** LMT concentrations were established at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h post dose by high performance liquid chromatography-ultraviolet. After per oral administration of 10 mg/kg drug, pharmacokinetic parameters were determined from plasma drug concentration. Later pharmacokinetic parameters of neuropathic pain induced rats were calculated in order to estimate the possible effect of neuropathic pain on pharmacokinetic parameters. **Results:** The regression coefficient determined for LMT calibration curve was 0.99 ± 0.001. The working range for LMT was 0.5 to 2.5 µg/ml Limit of Detection (LOD 0.2 µg/ml). The maximum drug concentration was found at 2 h. **Conclusion:** However, none of the pharmacokinetic parameter showed statistically significant alteration where the results were quite stimulating for the development of clinically useful oxcarbazepine dosage form to explore its activity on neuropathic pain.

Key words: Chronic constriction injury, lamotrigine, neuropathic pain, pharmacokinetics parameters

INTRODUCTION

Lamotrigine (LMT) is poorly water soluble drug[1] and highly soluble in 0.1N hydrochloric-acid. In all of the deeds, consideration of pharmacokinetics is paramount where it is the analysis of how the body affects a drug. In *in-vivo* the combined use pharmacokinetic assessment and animal models help to assess the compound efficacy.[2] Neuropathic pain is pain initiated by a primary lesion.[3] Newer antiepileptic drugs possess potential advantages of fewer drug-drug interactions[4] with suitable therapeutic modalities to relieve pain:[5] However, LMT can act as mono-therapy on neuropathic pain. Medical reports on drug kinetics in peripheral neuropathy are often hazy, because it is complicated to perform systematic pharmacokinetic studies in patients with neuropathic pain. This difficulty was due to inter-individual inconsistency in injury extent and location. Thus, experimental models appear to be a suitable strategy for understanding pharmacokinetic alteration and also that the minimal effectual exposure in rats is within 1-15 fold greater than in humans.[6] The present research was designed to study whether the pharmacokinetics of LMT were altered by peripheral neuropathy.

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MATERIALS AND METHODS

Chemicals

LMT active pharmaceutical ingredient was gifted by Novartis, Mumbai, India. Solvents used for quantification were of high performance liquid chromatography (HPLC) grade (Merck, India). Remaining all other chemicals and reagents were of analytical grade.

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Animals

Either sex of Wistar rats (180-210 g, n = 3) obtained from National Institute of Nutrition, Hyderabad, India was housed under 12:12 h light-dark cycle with food and water *ad libitum* and acclimatized for 1 week. The experimental protocol was approved by an Institutional Animal Ethics Committee of BITS-Pilani, Hyderabad (IAEC/RES/06/03).

Surgical procedures

Neuropathic pain was induced to rats by chronic constriction injury as previously described by Bennett.[7] Briefly, this surgical procedure involved tying four loose ligatures around the left sciatic nerve at the mid-thigh region. After this procedure, the animal developed a peripheral neuropathy, which resembles the human condition in its response to static, allodynia and hyperalgesia.

Experimental design

The human dose was extrapolated to animal dose using the US Food and Drug Administration dose calculator.[8] The animals were divided into two groups with three animals in each group. Group I contain healthy rats were as Group II consists of neuropathy induced rats.

Drug administration and blood sampling

During this investigation, LMT alone containing 10 mg/kg of the drug was administrated per oral (p.o.) to rats through a ball tipped needle using 1 mL of 20% polyethylene glycol solution as a vehicle. Blood samples (0.5 mL) were withdrawn prior to dosing and at 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 h post-dose from retro-orbitol plexus into centrifuge tubes containing 0.1 mL of 2.8% sodium citrate solution as an anticoagulant. Plasma was separated by centrifuging at 3,000 rpm for 3 min and stored at −20°C until further analysis.

Determination of LMT by HPLC

Plasma LMT was measured using a validated HPLC method as given by Torra *et al*.[9] Briefly the HPLC system (Water Co., Massachusetts), consisted of waters auto-sampler, a water 2691 separation module pump, 2487 dual lambda ultraviolet detector operated at 210 nm. The stationary phase was waters symmetry C_{18} column (150 mm *4.6 mm, 5 μm). Mobile phase used was 5 mM of pH 3 buffer: acetonitrile (55:45 v/v) at a flow rate of 0.8 mL/min.

Data analysis

Pharmacokinetic parameters were calculated by the non-compartment model using Try Kinetica PK-PD 5.0 program. The plasma LMT concentration versus time curves was used to determine the maximum plasma concentration (C_{max}) and time to achieve maximum plasma concentration (T_{max}) from the graph. Area under the plasma concentration-time (AUC_{0-t}) was calculated using the trapezoidal rule from zero to the last measured plasma concentration (C_{last}). The terminal elimination rate constant, β, was estimated using linear least square regression of log linear phase of concentration-time curves considering the last four experimental points. Area under the concentration-time curve to respective sampling point was calculated by adding C_{last}/β to AUC_{0-t}. Mean residence time of the drug (MRT) was calculated from the area under the plasma concentration and area under moment curve, half-life ($t_{1/2}$) and total body clearance (Cl_T) were also calculated.

Statistical analysis

The difference in pharmacokinetic parameters of LMT was evaluated by a graph-pad prism 5.0 version at $P < 0.05$ with 95% confidence intervals. Two-way ANOVA followed by Bonferroni *post hoc* multiple comparison test was performed to find the significance of LMT on normal and diseased rats.

RESULTS

Pharmacokinetics of LMT alone on normal rats

Plasma concentration versus time curve of LMT alone was depicted in Figure 1. Pharmacokinetic parameters of LMT were mentioned in Table 1. After p.o. administration of LMT alone the observed time to peak LMT levels was at 2 ± 0.001 h with 4.24 ± 0.003 μg/ml of maximum concentration. After initial absorption phase plasma concentration of LMT decreased gradually till 24 h. Time of drug residence in the body was found to be 33 h until 0.072 L of drug clearance per hour and 12 h of elimination $t_{1/2}$. The total volume of drug distributed in the body was 1.28 L.

Table 1: Categorization of drug promotional literatures according to pharmacological groups (*n*=100)

Pharmacological class of drugs	*n*=%
Antimicrobial agents	19
Cardiovascular agents	19
Agents acting on gastrointestinal tract	16
Agents affecting endocrine system	11
Agents affecting respiratory system	11
Analgesic agents	09
Agents acting on central nervous system	05
Agents affecting blood	04
Miscellaneous agents*	06
Total	100

*Miscellaneous agents included local anesthetic, multivitamins, interleukin-1 blocking agent, and antioxidants

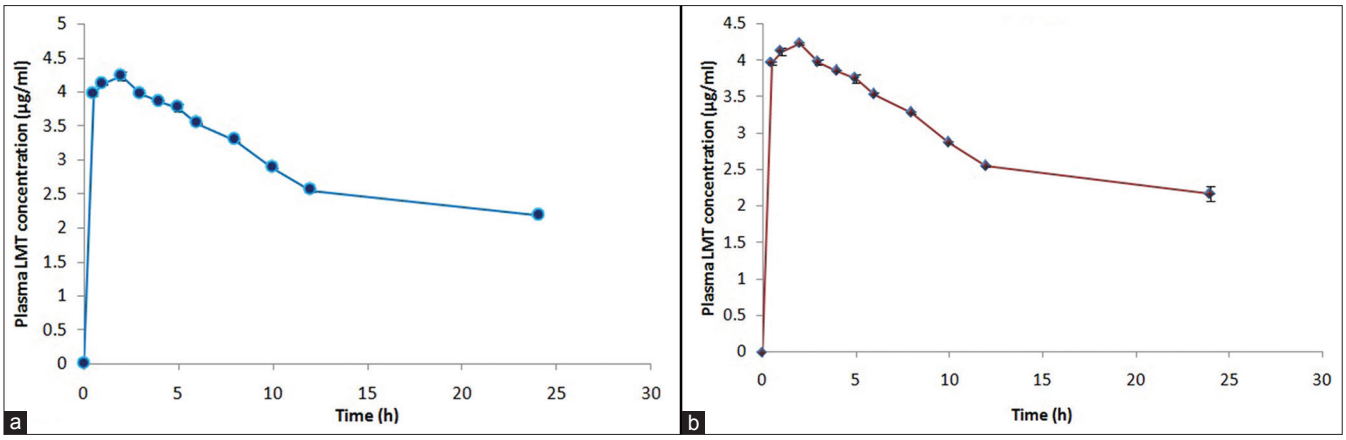

Figure 1: Plasma drug concentration vs. time plot for lamotrigine alone on (a) normal rats; (b) neuropathic rats. Plasma concentration time curves of lamotrigine following its oral administration at 10 mg/kg to Wistar rats. Data were expressed as mean ± SEM (*n* = 3)

Pharmacokinetics of LMT alone on neuropathic pain induced rats

Plasma concentration versus time curve of LMT alone and dosage forms on diseased rats were shown in Figure 1 Pharmacokinetic parameters of LMT alone in healthy and diseased rats were shown comparatively in Table 1. Upon p.o. administration of LMT for neuropathic pain induced rats, the observed time to peak LMT levels was at 2 h with a maximum plasma concentration of 4.23 ± 0.005 µg/mL. After initial absorption phase plasma concentration of LMT declined gradually. There was no significant alteration in pharmacokinetic parameters such as AUC_{0-12} was found to be 69.32 ± 0.16 h* µg/mL, $AUMC_{0-12}$ was found to be 726.81 ± 1.82 h^{2}* µg/mL, MRT was found to be 32.94 ± 0.5 h thus, there was no significant alteration in any of the pharmacokinetic parameters such as C_{max}, AUC and MRT of LMT alone on neuropathic pain induced rats compared to normal rats.

DISCUSSION AND CONCLUSION

This investigation was mainly intended to evaluate the pharmacokinetic profiles of LMT alone on normal rats and neuropathic pain induced rats. The various pharmacokinetic parameters were calculated by the optimal extra-vascular descriptive model fit utilizing the less available data and help to predict even most basic parameters such as MRT, AUC and C_{max}. Furthermore the results clears that there were no alterations in basic pharmacokinetic parameters such as the obtained elimination rate constant, elimination $t_{1/2}$ and Cl_T obtained results were in correlation with those of the results already reported. The data for LMT revealed that the maximum drug concentration obtained was found to be similar to that demonstrated by Theis *et al.*,[10] but the time to peak concentration was at 1h probably this was obtained from large population between 0.5 h and 2.5 h. From early trial phase 3 studies performed, the therapeutic anticonvulsant serum concentration was between 1-4 µg/mL and 3-14 µg/mL has proven to be quite safe but few[11-13] reported that concentration above 12 µg/mL was optimum probably such a high concentration may be required depending upon patients and their side effects. There was a direct relationship between daily dose, plasma level of LMT and analgesic effects, which was previously explained by Lunardi *et al.*[14] Although only a few animals were used per treatment group, the effects seen were consistent across the groups and based on ethical grounds. Butler *et al.*[15] demonstrated that oral dosing of LMT in rats in excess of the equivalent therapeutic dose in man (>10 mg/kg) does not produce the anticipated increase in the plasma concentration of drug in plasma. Our data clearly demonstrate that an oral dose of 10 mg/kg satisfied the absolute requirement for free drug/plasma concentration. The terminal $t_{1/2}$ obtained was 11-22 h in rats correlated with the results previously obtained.[16-18] The single dose of the drug was found to be sufficient to show the therapeutic efficacy as previously described by Garnett[19] as the pharmacokinetic profile found to be linear and kinetic parameters after multiple dosing were similar to those observed after a single dose. Because of the physiological properties of the drug it has become an interesting analgesic, because it inhibits the release of excitatory neurotransmitters; thus, it was used to study on neuropathic pain. Efficacy in pain models is reported following a single dose of the drug; however, many pain conditions are chronic in nature. Thus, we must ensure that the dosage form offer improved efficacy, enhanced pharmacodynamic effects and not altered pharmacokinetic parameters, such as accumulation, leading to increased drug $t_{1/2}$ or exposure. According to the pharmacokinetic data obtained from disease induced rats, there was no significant alteration in any of the key determinants, which include C_{max}, T_{max} and AUC likewise there was no alteration in other potential measures such as K_E, $t_{1/2}$ and Cl_T. Overall, the pharmacokinetic parameters of LMT alone on a rat

model explored the better PK profile during diseased conditions. Further, this study also served as an initial step towards identifying that there was no impact of peripheral neuropathy in alteration of pharmacokinetic parameters compared with normal conditions.

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