Original Article

Relationship between plasma levels and the anti-neuropathic pain effect of Lamotrigine in rat model

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ABSTRACT

Background: The study was undertaken to assess the pharmacokinetic and pharmacodynamic correlation of Lamotrigine (LMT) was determined following oral administration to prove that there was a direct relationship between daily dose, plasma level of LMT and analgesic effects.

Methods: Neuropathic pain was induced by chronic constriction injury of the sciatic nerve. This technique allows consecutive measurements of the paw withdrawal thresholds and paw withdrawal duration on hyperalgesia and allodynia respectively. Increase in threshold and decrease in the duration of paw withdrawals was used as an analgesic effect against neuropathy.

Results and discussion: The results demonstrate that there was a positive correlation between plasma correlation and pharmacodynamic effects in neuropathic pain.

Conclusion: Since there was a positive correlation between plasma correlation and pharmacodynamic effects in neuropathic pain, the plasma levels of Lamotrigine are good indicators of efficacy of the same in animal models of neuropathic pain.

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1. Introduction

Neuropathic pain is defined as pain initiated by a primary lesion or dysfunction of the nervous system. Few standard anti-epileptics though they show analgesic activity, they exhibited neurotoxicity. Currently there are no confronting each other trials of newer Anti-epileptic drugs (AED’s) on neuropathic pain, but due to its analogous patho-physiology such as sensitization, ectopic neuronal firing and sodium channel accumulation-redistribution-altered expression and also that both are caused by CNS injury. AED’s possess the prospective recompense of improved acceptability and fewer drug–drug interactions compared to standard treatments such as tri-cyclic antidepressants or established AED’s. Lamotrigine demonstrated efficacy in relieving pain associated with diabetes, HIV neuropathy and chemotherapy induced neuropathic pain. There is a need to research the role of Lamotrigine in treating the spinal cord injury pain and

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neuralgia after nerve section. A full pharmacokinetic profile is usually observed before compounds undergo extensive pain model testing. Various parameters in the determination of pharmacokinetic and pharmacodynamic relationships of various new pain drugs include the endpoint chosen (touch/pressure). It is always a rational approach to correlate the pharmacokinetic and pharmacodynamic data to draw meaningful conclusions. In this paper, for the peerless evidence we discuss the relationship of plasma drug concentration and the anti-neuropathic pain effect of Lamotrigine on rat.

2. Materials and methods

2.1. Materials

Lamotrigine active pharmaceutical ingredient (LMT-API) was obtained as a gift sample from Dr.Reddy’s Labs, Hyderabad. Remaining all other excipients, chemicals and solvents were procured from local suppliers.

2.2. Experimental design

Albino rats (National Institute of Nutrition, Hyderabad, India) of either sex, weighing 180–210 g were selected. The experimental protocol has been approved by Institutional Animal Ethical Care Committee (IAEC) of BITS-PILANI, Hyderabad (IAEC/RES/06/03) as per IAEC/CPCSEA. Human dose was extrapolated to animal dose using the USFDA dose calculator. In the study design for pharmacokinetics and pharmacodynamics assessment a number of nine Wistar rats were selected for drug administration. Three animals were used for pharmacokinetic studies and six animals for pharmacodynamic studies.

2.3. Assessment of pharmacokinetic data

All the animals in every group were administered drug with 1 ml of polyethylene glycol (vehicle). Blood was collected from the retro-orbital sinus after anaesthetizing animal. 0.1 ml of 2.8% sodium citrate was used as an anticoagulant. Blood samples were taken at regular time intervals from 0 h till 24 h following drug administration and plasma Lamotrigine concentration were determined using a validated HPLC method with minor modifications.

2.4. Data analysis

The various pharmacokinetic parameters were calculated by the optimal descriptive model fit using Try Kinetica PK-PD version 5.0 program (USA).

2.5. Assessment of pharmacodynamic data

2.5.1. Surgical procedures

Neuropathic pain was induced in rats by chronic constriction injury as previously described by Bennett and Xie. After this procedure, the animal developed a peripheral neuropathy which resembles the human condition in its response to static, allodynia and hyperalgesia.

2.5.2. Assessment of behavioral test procedures

For spontaneous pain, each rat was placed on a plantar test glass stand (lITC Life sciences, CA, USA) which was set at a

| Table 1 – Pharmacokinetics and pharmacodynamics of Lamotrigine. |
|-------------------|-------------------|-------------------|
| Time (h) | Plasma concentration (µg/mL) | Pharmacokinetic parameters | LMT (mean ± SEM) |
| 0 | 0.0 ± 0.0 | Cmax (µg/mL) | 4.24 ± 0.003 |
| 0.5 | 3.97 ± 0.02 | Tmax (h) | 2.0 ± 0.001 |
| 1 | 4.12 ± 0.01 | AUCC0- (h* µg/mL) | 139.57 ± 0.56 |
| 2 | 4.24 ± 0.06 | AUCC0- (h* µg/mL) | 4643.65 ± 29.68 |
| 3 | 3.97 ± 0.02 | MRT (h) | 33.27 ± 0.79 |
| 4 | 3.86 ± 0.01 | t1/2 (h) | 12.13 ± 0.06 |
| 5 | 3.77 ± 0.05 | Cl (mL/min/kg) | 0.072 ± 0.03 |
| 6 | 3.54 ± 0.01 | Ke (h−1) | 0.031 ± 0.0006 |
| 8 | 3.29 ± 0.02 | Vd (L/kg) | 1.28 ± 0.029 |
| 10 | 2.88 ± 0.01 | Ke (h−1) | 5.087 ± 0.21 |

Pharmacodynamic parameters

<table>
<thead>
<tr>
<th>Somatosensory thresholds at Cmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain detection threshold (randall) (g)</td>
</tr>
<tr>
<td>Cold detection threshold (sec)</td>
</tr>
<tr>
<td>Pressure pain threshold (von-Frey) (mN)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spontaneous pain</th>
<th>Cold allodynia</th>
<th>Mechanical hyperalgesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paw withdrawal duration (sec/paw withdrawal threshold (g)) at Cmax</td>
<td>23.75 ± 0.95</td>
<td>16 ± 1.73</td>
</tr>
<tr>
<td>Pain reversal (%) at Cmax</td>
<td>46.2 ± 1.5</td>
<td>35.3 ± 10.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PK-PD correlation</th>
<th>Intercept</th>
<th>Slope</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>−556.4</td>
<td>141.5</td>
<td>0.967</td>
</tr>
<tr>
<td>3</td>
<td>−358.6</td>
<td>93.5</td>
<td>0.92</td>
</tr>
<tr>
<td>4</td>
<td>−343.9</td>
<td>91.26</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Data represent mean ± SD includes n = 3 for pharmacokinetic studies and n = 6 for pharmacodynamic studies representing higher level of correlation on spontaneous pain then on cold allodynia and mechanical hyperalgesia.
Fig. 1 – Effect of Lamotrigine on a) spontaneous pain; b) dynamic allodynia; c) cold allodynia; d) mechanical hyperalgesia. Effect at different time points with significant effects showing level of significance 'P < 0.05, **P < 0.01, ***P < 0.001.
neutral temperature. Then foot lifting measurements were made. To quantify for dynamic allodynia, brisk foot withdrawal response to normally innocuous mechanical stimuli was measured by von-Frey filament (IITC Life sciences, CA, USA). In order to quantify cold sensitivity for cold allodynia, brisk foot withdrawal in response to acetone application was measured. To assess mechanical hyperalgesia, Randall device (IITC Life sciences, CA, USA) was used, with increasing amounts of pressure thus pain threshold of paw were assessed.7,8 Two Way ANOVA followed by Bonferroni post hoc multiple comparison test was performed to find the significance of pharmacodynamic studies. Statistical analysis was performed via Prism software (v. 5.0; GraphPad Software, Inc., San Diego, CA).

3. Results

3.1. Pharmacokinetics of Lamotrigine

Pharmacokinetic profile was obtained from three animals in each cohort. Using the pooled estimate of the total variance, the 95% confidence intervals were regarded as being statistically confirmed and shown in Table 1.

3.2. Pharmacodynamics of Lamotrigine on neuropathy induced rats and effect on behavioural studies (Fig. 1)

At 0 h, all the animals were observed for spontaneous behaviour of ipsilateral paw. The spontaneous behaviour of the ipsilateral paw was significantly observed compared to contralateral paw. Following treatment of LMT, spontaneous behaviour, threshold pressure, cold allodynic effect has been significantly altered at 2 h (P < 0.001) and maximum percent reversal of pain was found to be at 2 h (P < 0.001) post dose.

3.3. Pharmacokinetic and pharmacodynamic analysis

From the plasma concentration profile of the LMT, Cmax was found out to be 4.23 ± 0.63 μg/ml at 2 h, the pharmacodynamic data also showed a significant raise in paw withdrawal duration on spontaneous pain and paw withdrawal threshold on hyperalgesia at Cmax due to higher correlation coefficient with R² > 0.9 from Fig. 2 between the concentration of drug and the % pain reversal on mechanical hyperalgesia and spontaneous pain. Hence, it is clearly evident that there was a positive correlation. Further, the results of correlation (Table 1) proved that the pharmacokinetics of the drug are in greater correlation with the pharmacodynamic action.

4. Discussion

The data for Lamotrigine revealed that the maximum drug concentration obtained was found to be similar to that demonstrated by Jochen.9 From early trial phase 3 studies performed by Peck,10 the therapeutic anticonvulsant serum concentration was between 1 and 4 μg/ml and 3–14 μg/ml has proven to be quite safe. The extent of bioavailability (AUC0-24) was similar to the range reported by Jochen to be 69.75 μg/ml. The single dose of the drug was found to be sufficient to show the therapeutic efficacy as previously described by Jacques.11 From our findings, there was a significant effect on spontaneous pain and mechanical hyperalgesia by acting as a sodium channel blocker and an inhibitor for glutamate release. The present study, failed to produce significant anti-allodynic effects which can be comparable to the result obtained12 which did not result in overt behavioural side effects.

Most preclinical and clinical studies assess antinociceptive activity on neuropathic pain by drug efficacy on a dose-effect basis (i.e. reduction of pain). As pharmacodynamics is the relationship between drug concentration and the resulting effect, In this study, we have applied an integrated pharmacokinetic——
pharmacodynamic approach to characterize the concentration-effect relationship of the drug with anti-neuropathic pain properties. A simple graphical model along with correlation coefficient was applied. The results demonstrated the existence of a linear relationship between drug concentration in plasma and anti-neuropathic pain response. So, it could be possible that the plasma levels of Lamotrigine are good indicators of the concentration of the drug at its site of action.

Conflicts of interest

All authors have none to declare.

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