Biopharmaceutical distribution and pharmacodynamic evaluation of intra nasal in-situ gel of Lamotrigine for brain targeted drug delivery

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INTRODUCTION

The world health organization identifies epilepsy as the fourth most prevalent neurological condition. Seizure related injuries that are severe and life threatening regularly affect people with epilepsy. The mortality of epilepsy can be lowered by better seizure control. Many anti-epileptic drugs (AEDs) are available to treat the above condition. They work by selectively altering neuron excitability, which inhibits seizure-specific neuronal firing without impacting regular signals 1.

Among all the available [AEDs] Lamotrigine (LTG) is one of the best anti-epileptic drug widely used. It is used to treat myoclonic, generalized tonic-clonic, focal onset seizures 2. LTG acts by attaching to sodium channels, regulating presynaptic neuronal membranes and preventing glutamate release. It is mostly available in oral tablet dosage form consisting of both fast disintegrating and sustained release forms with t\textsubscript{1/2} up to 24 h 3. The usual oral daily dose of LTG is 25mg and belongs to BCS class-II with p\text{Hoe} 5.7 4. The available dosage forms are non-targeted drug delivery due to inability to cross BBB with effective therapeutic concentration. The decrease in oral bioavailability is shown due to hepatic metabolism and food drug interactions. More over the oral route delivery is not suitable for the unconscious patients. Due to these limitations, research was much focused on alternative route of delivery with minimising undesirable side effects associated with high dose. An LTG nanosuspension for intravenous(IV) delivery showed higher bioavailability and enhanced drug absorption at target site compared to oral route 5. But its application is constrained to emergencies due to the fact that is an invasive and requires a qualified practitioner for administration.

The intra nasal route is a feasible non-invasive method for delivering CNS drugs in effective concentration to the targeted site by bypassing the BBB 6. The pros of nasal delivery include ease of application, fast onset of action, avoiding first pass metabolism, more drug absorption due to large surface area, reaching the drug to CNS target site in effective concentration there by reducing the dose of drug and associated high dose side effects and patients’ compliance 7.

Smart natural polymers like gellan gum (GG) vary their consistency in response to physiological changes is used in the ion trigger in-situ gels. The clearance mechanisms due to nasal anatomy limit the nasal delivery. Particularly liquid formulations are more likely to seep out at the front of the nose or flow down the nasal cavity into the throat. To address the drawback, the viscosity of the formulation can be increased by employing the GG. The in-situ gels made with
this type of polymers first take the solution form later upon administration to nasal cavity, in response to heat stimulation, pH and ionic concentration converted to gel form (sol to gel). The first sol form makes the formulation easier to handle before and after administration. The next gel form lengthens nasal retention time and improves the drug absorption 8,9.

Wide research was done on GG, an in-situ gelling polymer, alinair, anionic exopolysaccharide generated by *Sphingomonas elodea*. It is also used in ocular, oral dosage forms 10. Locust bean gum (LBG) is natural polymer as a thickening agent in food and as a mucoadhesive agent in pharmaceutical industries. LBG is extracted from seeds of leguminous plant *Cassia Linn*. Belonging to Fabaceae family 11.

In an experimental model of epilepsy, no published research has yet assessed the therapeutic benefits of in-situ gel containing GG, LBG and PEG6000 when administered intra nasally. With a thorough examination of the literature review usage of biodegradable natural polymers GG and LBG selected for the formulation of in-situ gel. Due to practical challenges faced in administrating AED to an unconscious patient and patients needing immediate emergency medication, the intra nasal route was selected.

Previously, we conducted a research work on formulation and in-vitro and Ex vivo evaluation of intra nasal in-situ gel of LTG for brain targeting using GG as anionic in-situ gelling polymer, LBG as mucoadhesive polymer, PEG6000 as a permeation enhancer. A formulation with GG (0.45%w/v), LBG (3.0%w/v), PEG6000 (2%w/v) and benzalkonium chloride (0.05%w/v) was chosen as the optimized formula, having a viscosity of 124.45cps with in-vitro mucoadhesive strength as 2085.84dynes/cm² and drug release up to 12h was observed 12. Based on the aforementioned findings, the current study’s in-vivo pharmacokinetic, tissue distribution and pharmacodynamic tests examined the optimized formulation that was derived from the earlier work.

**MATERIALS AND METHODS**

Lamotrigine was a gift sample graciously provided by CTX life sciences Pvt. Ltd. Gujarat. Gelan gum was generously donated by Marine Hydrocolloids, Cochin, India. PEG 6000, benzalkonium chloride and LBG were bought from Merck life sciences Pvt. Ltd. Methanol, Propylene Glycol, Acetone, Acetic acid, Dimethyl formamide, Potassium dihydrogen phosphate, Sodium hydroxide were acquired from SD Fine chemical Ltd.

**Procurement and maintenance of animals**

In the in-vivo study, albino Wistar rats weighing between 150 and 200g had been used. They were kept inside polypropylene cages. The animals were acclimated to ordinary laboratory temperature (25±3°C) and kept on a light:dark =12:12 hours. Standard pellet and water diets were used to feed the animals. The institutional Animals Ethics committee (CPCSEA/1677/SPMVV/IAEC/1-03) approved the study, and all of the experiments were carried out in accordance with the CPCSEA protocol.

**In-vivo Pharmacokinetic and bio distribution studies**

**Animal handling and administration of nasal gel**

For the study, a total number of 36 rats were used, with 12 animals each in groups 1, 2 and 3. Where group 1 is control, group 2 is oral and group 3 is intra nasal (IN). For the animals in group 1, normal saline was supplied. For group 2 animals, an oral dose of pure drug suspension (LTG) of 4mg per kg of an animal was administered. The optimised formulation (derived from our past work 12) was given IN to group 3 animals in a dose equivalent to 4mg per kg of an animal. The dose was determined using a conversion of the surface area of the human body to that of an animal body 13. The rats were placed in a convenient position for IN administration and a polyurethane tube (24G x19mm) that was further linked to micro litre syringe was used to inject the in-situ gel into the nostril of the rats while they were lying on a convenient side for IN administration. To assure formulation delivery to the nasal cavity dome, the tube was placed into the nostril about 10mm deep. All of the animals underwent ether inhalation anaesthesia, and their body temperature was maintained by keeping them in a heated setting.

**Collection and analysis of drug in blood and tissues of vital organs of rats like brain, liver, kidney and heart**

Blood samples of about 1-2 ml have been collected by retro-orbital puncture at specific time intervals (0.5, 1, 2, 4, 8, 12 and 24 hours) and tissues from vital organs like the brain, liver, kidney, and heart were obtained through cervical dislocation at distinct time intervals (1,4,8 and 24 hours) and persevered at -20°C until further use. At each time point, n=3 animals from the same group were chosen for blood and tissue sample, allowing the rest period to be assemled for the subsequent sampling. The blood samples were centrifuged at 3000 rpm for 10 min at 4°C, supernatants were then collected and further diluted with methanol for protein precipitation. HPLC was used to determine the drug content of the diluted samples. For tissue sample preparation, whole tissue of different vital organs was removed and subjected for mincing. Later one gram of tissue was taken and was centrifuged at 10,000 rpm for 20 min at 4°C and supernatants were collected and the same process was followed as that of blood sampling to detect the drug concentration in tissues 14.

**Statistical analysis and treatment of data**

The analysis for final diluted samples of both blood and tissues was performed using a reverse phase HPLC system (Waters, USA) with a slight modification. Analysis was performed using C18 RP analytical column (250x4.6 mm, 5µm). Phosphate buffer pH 3.0: Acetonitrile (40:60%w/v) was used as a mobile phase at a flow rate of 1.0ml/min. The column temperature was maintained at 30°C. The LTG was detected at a wavelength of 270nm. The run time lasted was 8 minutes. Utilising the Kinetics 5.1SP1 software. (Thermo Fisher Scientific Inc. MA, USA), all potential pharmacokinetic parameters including elimination half-life (t1/2), elimination rate constant (Kc), the concentration of drug at 0 time (C0) and volume of distribution (Vd), were calculated. The Area under curve (AUC, AUC₀₋₅, AUC₀₋₁₀ and AUC₀₋₂₅) were calculated by applying the trapezoidal method. By visual evaluation of data, the maximum LTG concentration (Cmax) and the time required to reach the maximum concentration (Tmax) were directly recorded. The absolute bioavailability of LTG after IN administration was calculated using the equation, Absolute bioavailability (%) = \(\frac{\text{AUC(test)}}{\text{AUC(oral)}}\times100\). Where AUC(test) and AUC(oral) are the area under curves AUC₀₋₅ of the intranasal and oral administration respectively. The Dose(test) and Dose(oral) are the dose of the drug administered (mg/kg) through nasal and oral routes respectively. To accesses, the efficiency of the nasal route for drug administration, the drug targeting efficiency was calculated using the equation, %DTE=\(\frac{\text{AUC}_{\text{brain}}}{\text{AUC}_{\text{oral}}}\times100\). Where AUC (brain) and AUC (blood) are the area under the drug concentration – time curves for brain and plasma after IN and oral administration respectively. The %DTE higher than 100 indicates the route which was selected is more efficient than the oral route. The statistical comparisons were done between the nasal and oral routes of administration using unpaired t-test 15.
Pharmacodynamic studies

The pharmacodynamic activity was assessed for the developed in-situ nasal gel with pentyleneetrazol (PTZ) induced seizures. The protocol was approved by IAEC. The study was performed during a light cycle between 10.00 to 13.00h. The animals were divided into 3 groups (n=5) fasted overnight and transferred to the lab before one hour of the study. Group 1 is the diseased control group where PTZ is administered. Group 2 is the treatment 1 where Lamotrigine suspension was administered orally (4mg/kg of an animal). Group 3 is the treatment 2 where the formulation was administered IN. For group 3 the animals were anesthetized with ether before the administration of in-situ gel. For nasal doses, drug equivalent to 4mg/kg was administered. For group 2 and group 3, 0.5 and 1 hour respectively following the drug, PTZ is administered in a dose of 60mg/kg intraperitoneal (IP) route to induce onset of clonic convulsions in each group and was recorded 16.

- Oral group: PTZ was administered 1h after drug administration
- Nasal group: PTZ was administered 30m after formulation administration

RESULTS AND DISCUSSION

Calibration curve of LTG in rat plasma

The model HPLC chromatogram of LTG in rat plasma is shown in Figure 1. The LTG had an AUC of 927652 and was detected at 270nm with the retention time of 2.315 minutes. Figure 2 illustrates the calibration curve of LTG in rat plasma having correlation coefficient (r²) value of 0.999. As the r² value is close to one which indicates the high degree of correlation.

Pharmacokinetic study

Figure 3: Plasma conc - time profile plot of pure drug & optimized formula in plasma
(All the values expressed are mean ±SD, n=3).

Figure 4: Drug profile in brain tissue
(All the values expressed are mean ±SD, n=3)
Table 1. Pharmacokinetic parameters of optimized LTG in-situ gel formulation

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Concentration of drug in plasma</th>
<th>Concentration of drug in Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 2 Oral route</td>
<td>Group 2 Nasal route</td>
</tr>
<tr>
<td>t(_{1/2}) (h)</td>
<td>9.12</td>
<td>4.2</td>
</tr>
<tr>
<td>t(_{max}) (h)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C(_{max}) (ng/ml)</td>
<td>320±46.72</td>
<td>839.9±59.30</td>
</tr>
<tr>
<td>C(_{0}) (ng/ml)</td>
<td>251.02</td>
<td>539.09</td>
</tr>
<tr>
<td>K(<em>{e}) (T(</em>{-1}))</td>
<td>0.075</td>
<td>0.162</td>
</tr>
<tr>
<td>V(_{d}) (L)</td>
<td>3.90</td>
<td>1.81</td>
</tr>
<tr>
<td>AUC(_{0\to\alpha}) (ng h/ml)</td>
<td>2549.04</td>
<td>3959.763</td>
</tr>
</tbody>
</table>

Both group 2 and group 3 animal blood samples were evaluated for drug concentration in plasma. Drug suspension was given via the oral route for group 2 animals and optimised formulation was given via the nasal route for group 3 animals. Figure 3 and Figure 4 illustrates the drug concentration in brain tissue and plasma samples at distinct time intervals after administered orally and nasally respectively. Table 1 listed all predicted pharmacokinetic parameters for plasma and brain tissue. When compared to the oral route (C\(_{max}\)=320±46.72ng/ml), the nasal route’s C\(_{max}\)=839.9±59.30 ng/ml is found to be significantly higher P<0.05. Due to direct drug transport through the nasal route, the AUC\(_{0\to\alpha}\) of plasma for nasal route is more compared to oral route AUC\(_{0\to\alpha}\). Poonam Parashar et al. investigated the nasal in-situ gel of LTG consisting of sodium alginate and chitosan as polymers. Their formulation sustained the drug release up to 8 hours. Comparing in-situ gel to oral drug suspension (AUC\(_{last}\)=4.78±1.98 mcg h/ml, C\(_{max}\)=0.84±0.28 g/ml), an increased brain concentration of LTG was obtained (AUC\(_{last}\)=9.33±2.54 mcg h/ml, C\(_{max}\)=1.41±0.15 g/ml). The t\(_{1/2}\), K\(_{e}\), t\(_{max}\) and V\(_{d}\) of plasma where the drug is administered via oral route was found to be 9.12, 0.075 T\(_{-1}\), 2h and 3.90L respectively. The t\(_{1/2}\), K\(_{e}\), t\(_{max}\) and V\(_{d}\) of plasma where the drug is given through nasal route were 4.2h, 0.162 T\(_{-1}\), 1hand 1.81L respectively. The above-mentioned statement disclose that it takes longer time for a drug to show its action in the oral route than to the drug administered by intranasal route (IN). In the oral route, the delay in drug action is because of the hepatic metabolism of drug.

Tissue distribution study

The biodistribution of the drug in various tissues is clearly depicted in Figure 5. Similarly to how it is for plasma samples, in brain tissue also the C\(_{max}\) is significantly more for nasal route administration group animals (p<0.05). The C\(_{max}\) of drug in the brain tissue, administered through nasal route is almost 7folds more than for oral route. The AUC of drug in brain tissue for group 3 (nasal) is 6.5 folds higher than for group 2 (oral) animals. As indicated in table 1, group 3 brain tissue samples have longer t\(_{1/2}\), K\(_{e}\) and V\(_{d}\) than the group 2 where drug is administered through oral route. The absolute bioavailability was found to be 159.07%. Brain targeting was further evaluated for %DTE and was found to be 863.02. This finding demonstrated that the drug is directly transported to the brain via the olfactory region bypassing the BBB. The biodistribution study, which was carried out in other tissues such as liver, kidney and heart, further supports these conclusions. At one hour after oral administration, the drug’s concentration in liver tissue was found to be 310±7.91ng/ml;
however, when administered nasally, the concentration was almost zero ng/ml. At the end of the 24-hour period, neither the oral nor the IN routes had any drug detected in the liver, with the exception of 2±5.63 ng/ml at the end of the fourth hour. Both the oral and IN treatments took an hour or more to get the medication to the kidney. By the fourth hour, 60±7.54 and 4±6.65 ng/ml of drug, which was administered via oral and IN routes, respectively, were found in the kidney tissue. By the end of the following day, a negligible amounts 2±8.93 (via oral) and 0.1±8.56 (via IN) ng/ml had been identified in the kidney tissue. When given intra nasally, the drug had no effect on the heart, but when given orally, a tiny amount of the drug (3±7.17 ng/ml) was identified in heart tissue. The biopharmaceutical distribution of the drug in various mentioned organs transparently shows that the drug directly targets to the brain when administered through the nasal route and shows the fast onset of action later sustains its release up to 12 hours.

**Pharmacodynamic study**

![Pharmacodynamic study graph](image)

Figure 6: Effect of LTG suspension and LTG in-situ gel on PTZ induced onset of seizures in rats

*p <0.05 compared to diseased control, *p <0.05 compared to treatment 1 (pure drug 4 mg/kg/oral)

**Figure 7: Effect of LTG suspension and LTG in-situ gel on PTZ induced duration of seizures in rats**

As shown in (Figure 6&7) when compared to the diseased control group, the onset of action for seizures was significantly greater in the treatment groups (LTG+PTZ) at P<0.05. The nasal group’s showed delayed onset of action in comparison to the oral group which reflects its targeted action through nasal route. When compared to the diseased control group (saline), the treatment groups (LTG+PTZ) showed a significantly shorter duration of action for seizures (P<0.05). When compared to the oral group, the nasal group’s duration of action was shorter, indicating that it was acting at the intended site.

**CONCLUSION**

Thus, intra nasal delivery of in-situ gel of LTG showed promising in-vivo characteristics, pharmacokinetics and pharmacodynamics. Overall, in-situ gels by ion trigger system displayed superiority in targeted delivery of drugs devoid of hepatic metabolism, in the management of epilepsy disease in experimental animals. By using GG, LBG as a mucoadhesive polymer and PEG 6000 as a permeation enhancer, the current research offers a different mode of delivery for LTG with improved effective concentration of drug reaching the targeted area.

**Acknowledgments**

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**Conflict of interest**

Authors declare that they have no conflict of interest.

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