BIOEQUIVALENCE STUDY OF TOPIRAMATE

Ganesh Kumar Das*
*Research Scholar, Shri Jagdish Prasad Jhabarmal Tibrewala University, Jhunjhunu (Raj.)

ABSTRACT:
The study was performed to compare the bioavailability of Topiramate tablets 25 mg Test formulation with TOPAMAX® Topiramate tablet 25 mg from Ortho-McNeil Neurologics, INC., USA as reference formulation in 24 male human volunteers. The study was conducted as an open-label, randomized, crossover, 2 periods; 2 sequences, with a minimum washout period of 14 days, single dose study. Plasma samples were obtained over a 120 hours interval. The Topiramate was analyzed by LC/MS/MS technique, in the presence of Topiramate-D12 as an internal standard. The 90% confidence intervals in fasting conditions for $C_{\text{max}}$ were 96.04–117.5%, for $AUC_{0-t}$ were 98.99–114.5% and for $AUC_{0-\text{inf}}$ were 99.14 – 112.57%, respectively. The 90% confidence intervals in fed conditions for $C_{\text{max}}$ were 92.64–106.27%, for $AUC_{0-t}$ were 99.59 – 112.06% and for $AUC_{0-\text{inf}}$ were 95.21 – 107.83%, respectively. Since the 90% confidence intervals for $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\text{inf}}$ were within the 80–125% interval proposed by Food and Drug Administration. It was concluded that Topiramate tablets 25 mg was bioequivalent to TOPAMAX (Topiramate tablet 25 mg) from Ortho-McNeil Neurologics, INC., USA according to both the rate and extent of absorption in fasting and fed conditions.

Key Words: Topiramate; Bioequivalence; Bioavailability; Pharmacokinetics

INTRODUCTION:
Topiramate is a sulfamate-substituted monosaccharide. Topiramate is designated chemically as 2, 3,4, 5-Di-O-isopropylidene-ß-D-fructopyranose sulfamate. The precise mechanisms by which Topiramate exerts its anticonvulsant and migraine prophylaxis effects are unknown; however, preclinical studies have revealed four properties that may contribute to Topiramate's efficacy for epilepsy and migraine prophylaxis. Electrophysiological and biochemical evidence suggests that Topiramate, at pharmacologically relevant concentrations, blocks voltage-dependent sodium channels, augments the activity of the neurotransmitter gamma-aminobutyrate at some subtypes of the GABA-A receptor, antagonizes the AMPA/kainate subtype of the glutamate receptor, and inhibits the carbonic anhydrase enzyme, particularly isozymes II and IV. Topiramate is well known for its antiepileptic activity. Topiramate has potentially therapeutic effect on Genetically Determined Generalized Epilepsy [1, 2], Refractory Status Epilepticus in Children [3, 4, 5]. As well as antiepileptic activity, Topiramate...
has pharmacologically active therapeutic activity in migraine i.e. in treatment of Chronic Migraine [6], refractory migraine [7], migraine prophylaxis [8, 9], and prevention of pediatric migraine [10]. Other than antimigrain effects, Topiramate has its inherent capacity to inhibit the frequency dependent neuronal activity [11], and for the treatment of compulsive sexual behavior [12]. Various researchers have developed and evaluated the sensitive chromatographic methods in analytical aspects [13, 14, 15]. The objective of this study was to compare in healthy volunteers, the pharmacokinetics profiles and evaluate the bioequivalence of one test formulation of 25 mg tablet of Topiramate and the test formulation was compared with one commercial formulation “TOPAMAX”.

MATERIAL AND METHODS:

Study protocol: The study was performed in accordance with the Helsinki Declaration and Good Clinical Practice Guideline, and informed consent was obtained from participants prior to study commencement.

Subjects:
24 Volunteers aged from 18-45 years with a body mass index (BMI) of 18.67-23.85 kg/m² were enrolled according to the inclusion and exclusion criteria. They were assessed to be in healthy condition based on medical, systemic and physical examination including vital signs (blood pressure, pulse rate, oral temperature and respiratory rate) and normal laboratory tests results (haematology, biochemistry, urine analysis, 12-lead ECG and Chest X-ray (PA view)) including negative HIV-1 & 2, Hepatitis B, Hepatitis C. All the subjects provided written informed consent to participate after explaining the nature and purpose of the study. The study protocol was approved by the independent Ethics Committee.

Drug Products:
The test formulation used was Topiramate Tablets 25 mg and the reference formulation used was TOPAMAX® (Topiramate Tablets 25 mg) of Ortho-McNeil Neurologics, INC., USA. The study was conducted in an open randomized, balanced, two-treatment, two-period, two-sequence, single dose, two way crossover design with a wash out period of 14 days between the doses.

Study Design:
Balanced, Open label, randomized, two treatment, two period, two sequence, single dose, cross over comparative oral bioavailability study in healthy, adult, human male subjects under fasting or fed conditions.

Study Duration:
Duration of clinical phase was 17 days including a wash out period of at least 14 days.

Sample Size:
Sufficient number of subjects was enrolled to dose at least 24 subjects.

Screening:
Demographic data, medical and medication history, physical examination, 12 lead ECG, vital signs, hematology, Biochemistry, HIV I & II, Hepatitis B & C, urine analysis and Chest X-ray PA view. Drugs of abuse and alcohol were performed before check in and each ambulatory samples in each period.

Confinement:
In each period, the subjects were housed from at least 12 hours before drug administration to 36 hours after drug administration. Again visited the clinical facility for the ambulatory samples (48.0, 72.0, 96.0 and 120.0 hrs post dose) of each period.

Drug Administration Procedure:
In each period, after an overnight fast of at least 10 hours, subjects was received a single dose of Test (T) or Reference (R) product while in sitting posture with about 240 ml of water at ambient temperature according to the randomization schedule. But in case of fed study, high fat and high calorie breakfast was provided before dosing.

PK Sampling:
Total 18 samples collected from each subject per period at pre-dose (0.0) and 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 12.0, 24.0, 36.0 (within + 2 minute of scheduled time) and 48.0, 72.0, 96.0 and 120.0 hrs post dose ambulatory samples (within + 2 hrs of scheduled time).

Total Blood Loss:
Total 36 samples of 5 ml each (180 ml), 6.0 ml discarded blood, 14 ml blood for pre-study screening and 8 ml blood for post study test, amount to total blood loss of approximately 208.0 ml.

Sample Collection:
Samples were collected through an indwelling I.V. cannula (vein flow) inserted in the forearm vein of the subject. Also collected through a fresh vein puncture. 5ml of blood per sample was collected using syringe and transferred to the pre-labelled heparinized sample collection tubes kept in the ice box. Blood samples were collected after discarding the first 0.2 ml of heparinized blood from the venous cannula. Heparin in normal saline solution was used to keep the indwelling cannula free from the blockade.

Plasma Separation:
Blood samples was centrifuged to separate plasma as soon as possible (within one hour after last blood sample collection of respective time point) if required stored samples in the -20°C±2°C till centrifuge. The samples was centrifuged at 4000 rpm, between 8°C -10°C for 10 minutes. All plasma samples stored in interim storage at -20°C±2°C till all the samples were collected in each period. After completion of the study the plasma samples was transferred to the Bioanalytical department and stored upright at -70°C or colder.

Bioanalytical Procedure:
The concentration of Topiramate in plasma was quantified using LC-MS/MS method according to the regulatory guidelines and in-house procedures.

Pharmacokinetic Parameters:
Employing the estimated Plasma concentration time profile of Topiramate following Pharmacokinetic parameters was calculated Using SAS Statistical Software (9.1.3 or higher, SAS institute Inc., USA). Primary PK Parameters: C\text{max}, AUC\text{0-t} and AUC\text{0-∞} and Secondary PK Parameters: K\text{el}, T\text{max} and T\text{1/2}. Descriptive statistics like minimum, maximum, mean, geometric mean, median, standard deviation and coefficient of variation for all pharmacokinetic parameters was calculated.

Statistical Evaluation: Summary statistics, ANOVA, intra subject variability, 90% confidence intervals and power was calculated by non
compartmental method using SAS® statistical software (9.1.3 or higher, SAS institute Inc., USA). Bioequivalence between the Test and reference formulations was assayed by the calculation of the 90% confidence Interval of test / reference ratio (Least Square Mean) for Cmax, AUC0-t and AUC0-∞ based on Topiramate (after log transformation) for Topiramate.

**Bioequivalence Criteria:** To considered bioequivalent, T/R ratio & the 90% confidence interval of the primary parameters was fall within the interval 80.00% to 125.00%. The power of the ANOVA to detect a 20% difference (α=0.05) between formulations will be determined. 90% two one sided confidence interval for the difference of the means of the logarithmic transformed values of Cmax, AUC0-t and AUC0-∞ at 5% level of significance is between 80.00% and 125.00% for Topiramate.

**RESULTS AND DISCUSSION:**

**Topiramate under Fasting Condition:**
Twenty four (24) healthy male subjects were enrolled in the study. All 24 subjects were randomized to receive either of the sequence of administration of the investigational drug product. The plasma samples of 24 subjects who completed both the periods were analyzed for Topiramate plasma concentration and the data of the 24 subjects receiving reference and test product were used for pharmacokinetic and statistical analysis.

After oral administration, Cmax was attained for Topiramate within 0.50 to 12.0 hours for both reference product and test product. There was no pre-dose concentration detected for any subject in both the periods. The drug was detected in plasma for 120.0 hours post dose. Period effect, treatment effect and sequence effect are found to be statistically insignificant at 5% level of significance

**Pharmacokinetic and statistical analysis:**
Central and Dispersible Central and dispersion measures for all pharmacokinetic parameters for both formulations are shown in Tables presents the ratios and the respective confidence intervals for bioequivalence analysis.

**Table-1: Mean Pharmacokinetic Parameters under fasting condition**

<table>
<thead>
<tr>
<th></th>
<th>Reference Product</th>
<th>Test Product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arithmetic Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>267.0194</td>
<td>59.1700</td>
</tr>
<tr>
<td>AUC0-t (ng/mL).hr</td>
<td>9090.0111</td>
<td>2526.2432</td>
</tr>
<tr>
<td>AUC0-∞ (ng/mL).hr</td>
<td>10902.0024</td>
<td>2802.0755</td>
</tr>
<tr>
<td>Tmax (hrs)</td>
<td>1.9271</td>
<td>2.4688</td>
</tr>
<tr>
<td>Ke (hr⁻¹)</td>
<td>0.0170</td>
<td>0.0046</td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>44.1705</td>
<td>13.7006</td>
</tr>
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</table>
Table-2: Geometric mean Pharmacokinetic Parameters under fasting condition

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reference Product Geo LSM</th>
<th>Test Product Geo LSM</th>
<th>Ratio</th>
<th>Intra_CV</th>
<th>Power</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>260.2387</td>
<td>276.4605</td>
<td>106.23</td>
<td>20.55</td>
<td>95.08</td>
<td>96.04</td>
<td>117.5</td>
</tr>
<tr>
<td>AUC₀-ᵣ (ng/mL).hr</td>
<td>8779.5106</td>
<td>9346.7382</td>
<td>106.46</td>
<td>14.76</td>
<td>99.79</td>
<td>98.99</td>
<td>114.5</td>
</tr>
<tr>
<td>AUCᵢnf (ng/mL).hr</td>
<td>10577.9605</td>
<td>11174.4102</td>
<td>105.64</td>
<td>12.87</td>
<td>99.97</td>
<td>99.14</td>
<td>112.57</td>
</tr>
</tbody>
</table>

The number of volunteers must always ensure enough statistical power to ensure the reliability of the results of the bioequivalence study. The mean (± SD) plasma concentration time profile of the two formulations under fasting and fed were shown in figures.

Fig. 1: Mean graph for plasma concentration profile of Topiramate under fasting condition

Fig 2: Semi log graph for plasma concentration profile of Topiramate under fasting condition
Topiramate under Fed Condition:
Twenty four (24) healthy male subjects were enrolled in the study. All 24 subjects were randomized to receive either of the sequence of administration of the investigational drug product. The plasma samples of 24 subjects who completed both the periods were analyzed for Topiramate plasma concentration and the data of the 24 subjects receiving reference and test product were used for pharmacokinetic and statistical analysis. After oral administration, Cmax was attained for Topiramate within 1.5 to 36.0 hours for both reference product and test product. There was no pre-dose concentration detected for any subject in both the periods. The drug was detected in plasma for 120.0 hours post dose. Period effect, treatment effect and sequence effect are found to be statistically insignificant at 5% level of significance.

Pharmacokinetic and statistical analysis:
Central and Dispersible Central and dispersion measures for all pharmacokinetic parameters for both formulations are shown in Tables presents the ratios and the respective confidence intervals for bioequivalence analysis.

Table-3: Mean Pharmacokinetic Parameters under fed condition

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reference Product</th>
<th>Test Product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arithmetic Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>208.1577</td>
<td>49.0376</td>
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<tr>
<td>AUC0-t (ng/mL).hr</td>
<td>8828.3018</td>
<td>2367.7796</td>
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<tr>
<td>AUC0-∞ (ng/mL).hr</td>
<td>11497.9697</td>
<td>3180.1236</td>
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<tr>
<td>Tmax (hrs)</td>
<td>5.5625</td>
<td>6.8036</td>
</tr>
<tr>
<td>Ke (hr⁻¹)</td>
<td>0.0150</td>
<td>0.0050</td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>53.4595</td>
<td>24.2984</td>
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</table>

Table-4: Geometric mean Pharmacokinetic Parameters under fed condition

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reference Product</th>
<th>Test Product</th>
<th>Ratio</th>
<th>Intra_CV</th>
<th>Power</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>202.1952</td>
<td>200.6187</td>
<td>99.22</td>
<td>13.91</td>
<td>99.9</td>
<td>92.64</td>
<td>106.27</td>
</tr>
<tr>
<td>AUC0-t (ng/mL).hr</td>
<td>8523.7863</td>
<td>9004.4277</td>
<td>105.64</td>
<td>11.95</td>
<td>99.99</td>
<td>99.59</td>
<td>112.06</td>
</tr>
<tr>
<td>AUCinf (ng/mL).hr</td>
<td>11067.2836</td>
<td>11213.7399</td>
<td>101.32</td>
<td>12.6</td>
<td>99.98</td>
<td>95.21</td>
<td>107.83</td>
</tr>
</tbody>
</table>
The number of volunteers must always ensure enough statistical power to ensure the reliability of the results of the bioequivalence study. The mean (± SD) plasma concentration time profile of the two formulations under fasting and fed were shown in figures.

Figure 3: Mean graph for plasma concentration profile of Topiramate under fed condition

Figure 4: Semi log graph for plasma concentration profile of Topiramate under fed condition

Central and Dispersible Central and dispersion measures for all pharmacokinetic parameters for both formulations are shown in Tables presents the ratios and the respective confidence intervals for bioequivalence analysis.

The bioavailability of a pharmaceutical form refers to the extent and speed of absorption of the active principle in contained it. Two pharmaceutical forms are said bioequivalent when, to be administered to the same individual, in the
same experimental conditions and at the same dose, showed no significant differences in relation to bioavailability. In this study two formulations of Topiramate had been evaluated. The mean ratio of parameters $C_{\text{max}}$ and $AUC_{0-t}$ and 90% confidence intervals of correspondents were calculated to determine the bioequivalence. The statistical comparison of $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ clearly indicated no significant difference in the two formulations of Topiramate 25 mg tablet. 90% confidence intervals for the mean ratio (T/R) of $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were entirely within the US Food and Drug Administration acceptance range. Based on the pharmacokinetic and statistical results of this study, we can conclude that Topiramate 25 mg tablet, test formulation is bioequivalent to TOPAMAX® 25 mg tablet (Ortho-McNeil Neurologics, INC., USA), and that then the test product can be considered interchangeable in medical practice.

**Conclusion:**
The result of the study indicates that the test product Topiramate tablets 25 mg is bioequivalent with the reference product TOPAMAX 25 mg of Ortho-McNeil Neurologics, INC., USA with respect to the rate and extent of absorption under both fasting and fed condition. Both the study formulations were well tolerated by all the subjects in the study.

**References:**
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Chronic Migraine: A Randomized, Double-Blind, Placebo-Controlled Trial, American Headache Society, 47:170-180


