CHAPTER 7

PHARMACOKINETIC AND BIOAVAILABILITY EVALUATION OF LORNOXICAM AND CELECOXIB SOLID DISPERSIONS IN SUPERDISINTEGRANTS
PHARMACOKINETIC AND BIOAVAILABILITY EVALUATION OF LORNOXICAM AND CELECOXIB SOLID DISPERSIONS IN SUPERDISINTEGRANTS

Solid dispersions of lornoxicam and celecoxib in superdisintegrants exhibited markedly higher dissolution rates and dissolution efficiency values when compared to the corresponding pure drugs and were found suitable for formulation into compressed tablets by direct compression technique. Lornoxicam and celecoxib solid dispersions in HPMC-PGS were further subjected to in vivo pharmacokinetic and bioavailability assessment in rabbits. The following products were tested for in vivo performance.

1. Lornoxicam
2. Lornoxicam-HPMC-PGS 2:2:10 solid dispersion
3. Celecoxib
4. Celecoxib-HPMC-PGS 2:2:10 solid dispersion

Solid dispersions in HPMC-PGS were subjected to in vivo evaluation as these dispersions gave highest enhancement in the dissolution rate and efficiency.

IN VIVO STUDY PROTOCOL

Calculation of Animal Equivalent Dose from Human Dose

As per the Guidelines to industry for conducting clinical trials in humans the first step towards the dose fixing is NO OBSERVED ADVERSE EFFECT LEVEL DETERMINATION (NOAEL) from which the human dose is calculated. The NOAEL studies are generally calculated using suitable animals such as Rat, Mice, or rabbits. From the obtained animal dose the Human Equivalent Dose (HED) is calculated using following equation.

\[
HED = \frac{\text{Animal Dose (mg/kg)} \times \text{Animal Weight (kg)}}{\text{Human Weight (kg)}}
\]

\[0.33\]
To Calculate Animal Equivalent dose (AED) from Human Dose we can rewrite the above equation as follows:

\[
AED = \frac{\text{Human Dose (mg/kg)}}{\text{Animal weight (kg)} \times \text{Human Weight (kg)}}^{0.33}
\]

Using Above equation considering the average human weight as 70 kg, animal equivalent dose calculations were carried out.

Weight of rabbits in kg = 1.5 - 2.5

Human Dose of Drugs in mg: Celecoxib 50 mg and Lornoxicam 8 mg.

Calculated Animal Equivalent Dose (AED):

Celecoxib = 1.15mg/kg

Lornaxicam = 0.8mg/kg

Healthy rabbits of either sex (weighing 1.5 – 2.5 kg) were fasted overnight. Celecoxib and its dispersions were administered at dose equivalent to 1.15mg/kg of celecoxib. Lornoxicam and its dispersions were administered at a dose equivalent to 0.8 mg/kg of lornoxicam. Each product was repeated 4 times (n = 4). The *in vivo* experiments were conducted as a crossover study as follows.

A group of 4 rabbits were given celecoxib initially and after a washout period of one month, they were given with celecoxib solid dispersion. Another group of 4 rabbits were given lornoxicam initially and after a washout period of one month, they were given lornoxicam solid dispersion.

After collecting the zero hour blood sample (blank), the product in the study was administered orally in a capsule shell with 10 ml water. Blood samples (0.5ml) were collected from marginal ear vein at 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 and 12.0 h after administration. The blood samples were allowed to clot and centrifuged at 5000 rpm and the serum separated was collected into
dry tubes. All the samples were stored under refrigerated conditions prior to assay. Serum concentration of the drug (celecoxib or lornoxicam) was determined by the HPLC methods.

The serum concentration data following the administration of various products are given in Tables 7.1 and shown in Figs. 7.2.

From the time versus serum concentration data various pharmacokinetic parameters such as peak concentration (C_{max}), time at which peak occurred (T_{max}), area under the curve (AUC), elimination rate constant (K_{el}), biological half life (t_{1/2}), percent absorbed to various times and absorption rate constant (K_a) were calculated in each case. The results are given in Table 7.2.

**Estimation of Celecoxib Serum Samples**

Celecoxib in serum samples was estimated according to High Performance Liquid Chromatographic (HPLC) method of Diwan et al.

**Materials**

1. Celecoxib (Gift sample from Ipca Laboratories, Mumbai)
2. Ketoprofen (Gift sample from Dr.Reddy’s Laboratories, Hyderabad)
3. Acetonitrile (HPLC grade, Qualigens)
4. Methanol (HPLC grade, Qualigens)
5. Glacial acetic acid (Excelar, Qualigens)
6. Distilled water (Tripel glass distilled)

**Chromatographic Conditions**

Instrument: A gradient high pressure liquid chromatograph (Shimadzu)

Column: C-18 RP (ODS-A) 250 x 4.6 mm I.D; Particle size: 5 µm

Mobile Phase: Acetonitrile : water (55:45 v/v), pH adjusted to 2.95 with glacial acetic acid

Flow Rate: 1.5 ml/min

Injection volume: 20 µl
Detector: UV-VIS Spectrophotometric detector at 254 nm

Extraction Procedure

To 50 µl of internal standard (ketoprofen, 5 µg/ml in methanol) and 4.45 ml of methanol was added and the tubes were vortex-mixed for 15 min and centrifuged at 5000 rpm for 10 min. The supernatant was transferred to clean tubes and evaporated to dryness. The residue was reconstituted with 0.5 ml mobile phase and 20 µl of the solution was injected into the HPLC system after filtering through 0.2 µ nylon membrane filter.

Calibration Curve

Standard solutions containing 0.5, 1.0, 2.0, 3.0 and 4.0 µg/ml of Celecoxib were prepared in methanol. To each 50 µl of standard solution, 50 µl of internal standard (ketoprofen, 5 µg/ml in methanol), 500 µl of blank serum and 4.4 ml of methanol were added and the tubes processed as above. Standard curve was obtained by plotting peak area ratio of Celecoxib to internal standard vs. concentration. The results are given in Table 7.1 and Fig. 7.1.

Calibration Curve for the Estimation of Celecoxib in Serum by HPLC

Table 7.1: Calibration Curve for the Estimation of Celecoxib in Plasma by HPLC

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Amount of (ng) celecoxib added to 1.0 ml serum</th>
<th>Mean (n = 5) ratio of peak area of Celecoxib to peak area of internal standard</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0.420</td>
<td>1.10</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0.845</td>
<td>1.42</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>1.679</td>
<td>0.99</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>2.514</td>
<td>1.87</td>
</tr>
<tr>
<td>5</td>
<td>400</td>
<td>3.487</td>
<td>1.88</td>
</tr>
</tbody>
</table>
The serum concentration data following the administration of various products are given in Table 7.2. From the time versus serum concentration data various pharmacokinetic parameters such as peak concentration ($C_{\text{max}}$), time at which peak occurred ($T_{\text{max}}$), area under the curve AUC, elimination rate constant $K_{\text{el}}$, biological half-life ($t_{\frac{1}{2}}$), percent absorbed to various times and absorption rate constant ($K_{\text{a}}$) were calculated in each case. The results are given in Table 7.3.

**DETERMINATION OF VARIOUS PHARMACOKINETIC PARAMETERS**

**Determination of $C_{\text{max}}$ and $T_{\text{max}}$**

From the time versus serum concentration curves, peak serum concentration ($C_{\text{max}}$) and time at which peak occurred ($T_{\text{max}}$) were recorded.

**Determination of Elimination Rate Constant ($K_{\text{el}}$) and Biological Half-Life ($t_{\frac{1}{2}}$)**

Time versus serum concentration data was plotted on a semi logarithmic graph paper as shown in Fig. 7.2. The elimination rate constant ($K_{\text{el}}$) was calculated from the
slope of the linear line in the elimination phase (the best fit linear regression line for the points in the elimination phase was drawn by the method of least squares). The corresponding biological half-life was calculated using the equation \( t_{1/2} = 0.693/K_{el} \).

**Determination of Percentage Absorbed to Various Times and Absorption Rate Constant (K_a)**

Percentage absorbed to various times and absorption rate constant (K_a) were calculated from serum concentration data by the method described by the Wagner and Nelson. The equation developed for the determination of absorption rate from blood data is

\[
dA/dt = V_d \cdot dC_b/dt + K_{el} \cdot C_b
\]

Where \( dA/dt \) = Absorption rate

\( V_d \) = Apparent volume of distribution

\( dC_b/dt \) = Rate of change of blood concentration (C_b) at time t and

\( K_{el} \) = Elimination rate constant.

The equation may be integrated between the limits of t = 0 and t = T and divided by V_d to give,

\[
\frac{A_t}{V_d} = C_T + K_{el} \cdot \int C_b dt
\]

Where \( A_t \) = Amount of drug absorbed to time t.

\( C_T \) = blood Concentration at time t and the quantity under the integral sign in the area under the blood concentration versus time curve between the indicated limits. When the successive values of \( A_t/V_d \) are calculated, a maximum or asymptotic value \([A_T/V_d]_\infty\) is obtained. The maximum asymptotic value is divided into successive values of AT/Vd to yield percentage absorbed data i.e.

\[
\frac{(A_T/V_d) / [A_T/V_d]_\infty \times 100}{\text{as a function of time.}}
\]

The sequences of calculations are shown in Table 7.2. When a semi logarithmic plot of percentage unabsorbed versus time was drawn. Straight line (the
The best fit linear regression line was drawn by the method of least squares) was obtained, the slope of which was equal to \(-K_a/2.303\). The absorption rate constant (\(K_a\)) was calculated from the slope of this line. The results are given in Table 7.3.

**Estimation of Area Under the Curve [AUC]**

The area under the time versus serum concentration by applying trapezoidal rule. The remaining area from 12 hours to \(\infty\) time was calculated using the following equation,

\[
[AUC]_{12-\infty} = \text{Concentration at 12}\text{th hour} / K_\text{el}
\]

Then \([AUC]_{0-\infty} = [AUC]_{0-12 hr} + [AUC]_{12-\infty hr}\)
Table 7.2.
Serum Concentration of Celecoxib and Celecoxib Solid dispersion Following their Oral Administration in Rabbits (n = 4)

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Serum Concentration (ng/ml) of Celecoxib (x ± s.d.,)</th>
<th>Celecoxib Solid Dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Celecoxib</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>2.80±1.12</td>
<td>64.48±2.31</td>
</tr>
<tr>
<td>0.50</td>
<td>4.70±1.25</td>
<td>73.98±2.65</td>
</tr>
<tr>
<td>1.0</td>
<td>6.68±2.35</td>
<td>75.59±3.02</td>
</tr>
<tr>
<td>2.0</td>
<td>8.30±3.02</td>
<td>71.90±3.54</td>
</tr>
<tr>
<td>3.0</td>
<td>10.19±3.54</td>
<td>66.86±2.89</td>
</tr>
<tr>
<td>4.0</td>
<td>12.43±2.89</td>
<td>61.69±3.54</td>
</tr>
<tr>
<td>6.0</td>
<td>9.49±2.84</td>
<td>49.95±2.87</td>
</tr>
<tr>
<td>8.0</td>
<td>7.09±1.76</td>
<td>37.59±2.69</td>
</tr>
<tr>
<td>12.0</td>
<td>3.68±2.38</td>
<td>19.24±2.57</td>
</tr>
</tbody>
</table>
Fig 7.2.

Serum Concentration of Celecoxib and Celecoxib Solid dispersion Following their Oral Administration in Rabbits (n = 4)
Table 7.3.
Summary of Pharmacokinetic Parameters estimated following Oral Administration of Celecoxib and its Products

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Pharmacokinetic Parameter</th>
<th>Celecoxib Pure Drug</th>
<th>Celecoxib Solid Dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>12.09</td>
<td>76.21</td>
</tr>
<tr>
<td>2</td>
<td>$T_{\text{max}}$ (hr)</td>
<td>4.29</td>
<td>1.02</td>
</tr>
<tr>
<td>3</td>
<td>$K_{\text{el}}$ (hr⁻¹)</td>
<td>0.159</td>
<td>0.158</td>
</tr>
<tr>
<td>4</td>
<td>$T_{\frac{1}{2}}$ (hrs)</td>
<td>2.485</td>
<td>4.39</td>
</tr>
<tr>
<td>5</td>
<td>$(\text{AUC})_{0\rightarrow12}$ ng-hr/ml</td>
<td>91.55</td>
<td>588.31</td>
</tr>
<tr>
<td>6</td>
<td>$(\text{AUC})_{0\rightarrow\infty}$ ng-hr/ml</td>
<td>114.33</td>
<td>709.31</td>
</tr>
<tr>
<td>7</td>
<td>$K_{\alpha}$ (hr⁻¹)</td>
<td>0.4135</td>
<td>3.560</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Pharmacokinetic parameters estimated following the oral administration of celecoxib and its solid dispersion are given in Table 7.3. The elimination rate constant ($K_{el}$) for Celecoxib was found to be 0.1620 hr$^{-1}$ and the corresponding biological half life ($t \frac{1}{2}$) was found to be 4.27 hrs after the oral administration of celecoxib. The $t\frac{1}{2}$ values of celecoxib obtained in the present study is in good agreement with earlier reported value of 4.33 hr$^{-1}$.

The absorption rate constant $K_a$ was found to be 0.411 hr$^{-1}$ following the oral administration of celecoxib. Celecoxib was found to be absorbed slowly when given orally and a peak serum concentration ($C_{max}$) of 12.44 ng/ml was observed at 4.0 hr following oral administration.

All the pharmacokinetic parameters of absorption (Table 7.3) namely $K_a$, $C_{max}$, $T_{max}$, % absorbed to various times and AUC indicated rapid absorption and higher bioavailability of celecoxib when administered as solid dispersion in HPMC-PGS. Higher $C_{max}$ and shorter $T_{max}$ values were observed with celecoxib solid dispersion when compared to those of celecoxib as such. The absorption rate constant ($K_a$) was found to be 3.543 hr$^{-1}$ in the case of celecoxib-HPMC-PGS solid dispersions. Whereas in the case of celecoxib, $K_a$ was only 0.412 hr$^{-1}$. An increase of 8.59 fold in $K_a$ was observed with celecoxib-HPMC-PGS solid dispersion. AUC (extent of absorption) was also much higher in the case of celecoxib solid dispersion when compared to celecoxib. AUC was increased from 91.55 ng-hr/ml for celecoxib to 588.16 ng-hr/ml for celecoxib solid dispersion.
STUDIES ON LORNOXICAM PRODUCTS

Estimation of Lornoxicam in Serum Samples

Lornoxicam in serum samples was estimated according to High Performance Liquid Chromatographic (HPLC) method.

Materials

1. Lornoxicam (Gift sample from Hetero Drugs Ltd., Hyderabad)
2. Acetonitrile (LobaChemie)
3. Water HPLC grade (LobaChemie)
4. Orthophosphoric acid (LobaChemie)

Chromatographic Conditions

Instrument: High Performance Liquid chromatography equipped with auto sampler and DAD or UV detector.

Column: Agilent Zorbax (4.6 x 250 mm, 5 µm)

Flow rate: 1.0 mL per min

Wavelength: 274 nm

Injection volume: 20 µl

Temperature: Ambient

Run Time: 8.0 min

Preparation of Phosphate buffer:

Weigh 3.48 grams of dibasic potassium phosphate into 1000 ml beaker, dissolve and diluted to 1000 ml with HPLC water. Adjusted the pH to 4.5 with ortho phosphoric acid.
Preparation of mobile phase:

Mix a mixture of above buffer 600 ml (60%) and 400 ml of acetonitrile HPLC (40%) and degas in ultrasonic water batch for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Preparation of the Lornoxicam sample solution:

Accurately weigh and transfer 10 mg of lornoxicam working standard into a 10 ml, volumetric flask and about 7 ml of diluents and sonicate to dissolve it completely and make up volume to the mark with the same solvent (stock solution). Further pipette 1.0 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents. Mix well and filter through 0.45 µ filter.

Sample Solution Preparation:

Pipette 20 µl of plasma sample, add 50µl of acetonitrile mix for 5 minutes and centrifuge for 10 mins. Filter the solution and inject 20 µl into HPLC system.

Table 7.4. : Calibration Curve for the Estimation of Lornoxicam in Plasma by HPLC

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Linearity Level</th>
<th>Concentration(nɡ/ml.)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>100</td>
<td>3209</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>200</td>
<td>6418</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>300</td>
<td>9629</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>400</td>
<td>12836</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>500</td>
<td>16045</td>
</tr>
</tbody>
</table>

Correlation Coefficient 0.9999
Fig. 7.3. Calibration Curve for the Estimation of Lornoxicam in Plasma by HPLC

Table 7.5: Serum Concentration of lornoxicam and lornoxicam Solid dispersion Following their Oral Administration in Rabbits (n = 4)

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Serum Concentration (ng/ml) of lornoxicam (x ± s.d.,)</th>
<th>lornoxicam</th>
<th>lornoxicam solid dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td>91.98±1.35</td>
<td>285.90±1.56</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td>109.98±1.98</td>
<td>368.29±1.34</td>
</tr>
<tr>
<td>1.5</td>
<td></td>
<td>123.87±1.78</td>
<td>487.24±1.59</td>
</tr>
<tr>
<td>2.0</td>
<td></td>
<td>121.59±1.63</td>
<td>451.35±1.83</td>
</tr>
<tr>
<td>3.0</td>
<td></td>
<td>107.35±1.75</td>
<td>420.98±1.30</td>
</tr>
<tr>
<td>4.0</td>
<td></td>
<td>100.51±1.58</td>
<td>324.59±1.50</td>
</tr>
<tr>
<td>6.0</td>
<td></td>
<td>89.57±1.69</td>
<td>184.79±1.67</td>
</tr>
<tr>
<td>8.0</td>
<td></td>
<td>68.95±1.38</td>
<td>91.02±1.33</td>
</tr>
<tr>
<td>12.0</td>
<td></td>
<td>20.87±1.66</td>
<td>34.67±1.20</td>
</tr>
</tbody>
</table>
Fig. 7.4. Serum Concentration of Lornoxicam and Lornoxicam Solid dispersion Following their Oral Administration in Rabbits (n = 4)

Table 7.6: Summary of Pharmacokinetic Parameters Estimated following Oral Administration of Lornoxicam and its Products

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Pharmacokinetic Parameter</th>
<th>Lornoxicam Pure Drug</th>
<th>Lornoxicam Solid Dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>$123.87 \pm 3.9$</td>
<td>$484.5 \pm 4.8$</td>
</tr>
<tr>
<td>2</td>
<td>$T_{\text{max}}$ (hr)</td>
<td>$1.50 \pm 5.4$</td>
<td>$0.9 \pm 0.9$</td>
</tr>
<tr>
<td>3</td>
<td>$K_{\text{el}}$ (hr$^{-1}$)</td>
<td>$0.098$</td>
<td>$0.354$</td>
</tr>
<tr>
<td>4</td>
<td>$T_{\frac{1}{2}}$ (hrs)</td>
<td>$2.30$</td>
<td>$0.910$</td>
</tr>
<tr>
<td>5</td>
<td>$(AUC)_{0\rightarrow12}$ ng-hr/ml</td>
<td>$237$</td>
<td>$311$</td>
</tr>
<tr>
<td>6</td>
<td>$(AUC)_{0\rightarrow\infty}$ ng-hr/ml</td>
<td>$383$</td>
<td>$491.9$</td>
</tr>
<tr>
<td>7</td>
<td>$K_{\alpha}$ (hr$^{-1}$)</td>
<td>$0.298$</td>
<td>$0.758$</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Pharmacokinetics of lornoxicam and L-HPMS-PGS solid dispersions

Pharmacokinetic parameters estimated following the oral administration of lornoxicam and its solid dispersion are given in Table 7.6. The elimination rate constant ($K_{el}$) for lornoxicam was found to be $0.098 \text{ hr}^{-1}$ and the corresponding biological half life ($t_{1/2}$) was found to be $2.30 \text{ hrs.}$ after the oral administration of lornoxicam. The $t_{1/2}$ values of lornoxicam obtained in the present study is in good agreement with earlier reported value of $2.35\text{hr}^{-1}$.

The absorption rate constant $K_a$ was found to be $0.298\text{hr}^{-1}$ following the oral administration of lornoxicam. Lornoxicam was found to be absorbed slowly when given orally and a peak serum concentration ($C_{max}$) of $123.87\text{ng/ml}$ was observed at $4.0 \text{ hr}$ following oral administration.

All the pharmacokinetic parameters of absorption (Table 7.6) namely $K_a$, $C_{max}$, $T_{max}$, % absorbed to various times and AUC indicated rapid absorption and higher bioavailability of celecoxib when administered as solid dispersion in HPMC-PGS. Higher $C_{max}$ and shorter $T_{max}$ values were observed with celecoxib solid dispersion when compared to those of lornoxicam as such. The absorption rate constant ($K_a$) was found to be $0.758\text{hr}^{-1}$ in the case of lornoxicam -HPMC-PGS solid dispersions. Whereas in the case of lornoxicam, $K_a$ was only $0.298 \text{ hr}^{-1}$. An increase of $2.54$ fold in $K_a$ was observed with lornoxicam -HPMC-PGS solid dispersion. AUC (extent of absorption) was also much higher in the case of lornoxicam solid dispersion when compared to lornoxicam. $(AUC)_{0\rightarrow\infty}$ was increased from $383\text{ng-hr/ml}$ for lornoxicam to $491.9\text{ng-hr/ml}$ for lornoxicam solid dispersion.