CHAPTER VIII

PHARMACOKINETIC EVALUATION OF

EFAVIRENZ- βCD- SOLUTOL HS15 COMPLEXES

Drug-CD complexes with and without Solutol HS15 gave markedly higher dissolution rates of efavirenz and ritonavir from solid complex systems as well as from their tablet formulations. Pharmacokinetic evaluation was done on efavirenz - βCD and efavirenz- βCD-Solutol HS15 complexes in comparison to pure drug with a view to evaluate their in vivo performance.

Pharmacokinetic evaluation of the following efavirenz products was done in healthy Newzeland white rabbits weighing 1.5 – 2.5 kg (n=6) of either sex in a cross over study at a dose equivalent to 10 mg/kg of drug.

The following products were tested.

Efavirenz

Efavirenz - βCD (1:2)

Efavirenz – βCD- Solutol HS15 (1:2:0.05)

In vivo study protocols were approved by the Institutional Animal Ethics Committee (Regd.No 516/01/a/CPCSEA/125). A wash out period of one month was given between testing of two products.

After collecting the zero hour blood sample (blank), the product in the study was administered orally in a capsule shell with 10 ml of water. No food or liquid other than water was permitted until 4 hours following the administration of the product. Blood samples (3 ml) were collected from marginal ear vein at 0.5, 1, 2, 3, 4, 6, 8 and 12 hours after administration. The blood samples were collected in heparinized tubes and were centrifuged at 10000 rpm for 10 min and the plasma separated was collected into dry tubes. All the samples were stored under refrigerated conditions prior to assay.
on the same day. Plasma concentrations of efavirenz were determined by a known HPLC method described in Chapter V.

As the CD systems evaluated for in-vivo performance are fast dissolving and rapidly absorbing systems the in-vivo study is limited to 12 hours only to understand the absorption characteristics. And also as the plasma concentration reached elimination phase in about 8-10 hours the $t_{1/2}$ could be assessed from 12 hours data. For rapidly absorbing and eliminating systems a 12 hours study is adequate.

From the time versus plasma 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 ($t_{1/2}$), percent absorbed to various times and absorption rate constant ($K_{\text{a}}$), were calculated in each case as per known standard methods (Wagner and Nelson, 1963, 1964).

**DETERMINATION OF PHARMACOKINETIC PARAMETERS**

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

From the time versus plasma concentration curves, peak plasma 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 ($t_{1/2}$):**

Time versus plasma concentration data was plotted on a semi logarithmic graph paper. 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,

$$t_{1/2} = 0.693 / K_{\text{el}}$$
Determination of Percentages Absorbed to Various Times and Absorption Rate Constant ($K_a$):

Percentages absorbed to various times and absorption rate constant ($K_a$) were calculated from plasma concentration data by the method described by Wagner and Nelson\textsuperscript{1,2}. The equation developed for the determination of absorption rate from blood data is

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

Where, \(\frac{dA}{dt}\) = absorption rate, \(V_d\) = apparent volume of distribution \(\frac{dC_b}{dt}\) = rate of change of blood concentration ($C_b$) with respect to 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,

\[
\frac{A_T}{V_d} = C_T + K_{el} \cdot \int_{t=0}^{T} C_b dt
\]

\[
\frac{A_T}{V_d} = C_T + K_{el} \cdot \left[ \text{AUC}_{0\to T} \right]
\]

Where $A_T$ = amount of drug absorbed to time $T$, $C_T$ = blood concentration at time $T$ and the quantity under the integral sign is 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 $A_T/V_d$ to yield percentage absorbed data i.e.,

\[
\frac{A_T/V_d}{[A_T/V_d]_\infty} \times 100 \text{ as a function of time}
\]
A graph of log percent unabsorbed Vs time is a linear plot, the slope of which is equal to \(- \frac{K_a}{2.303}\) from which \(K_a\) was calculated.

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

The area under the time versus plasma concentration curve (AUC) for 12 hour period was estimated, from an arithmetic plot of time versus plasma concentration by applying trapezoidal rule. The remaining area from 12 hours to \(\infty\) time was calculated using the following equation,

\[
[AUC]_{12}^\infty = \frac{\text{concentration at 12th hour}}{K_{el}}
\]

Then, \([AUC]_0^\infty = [AUC]_0^{12} + [AUC]_{12}^\infty\)
RESULTS

Table-8.1

Plasma Concentrations of Efavirenz Following the Oral Administration of Efavirenz and its CD Complexes in Rabbits.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Plasma Concentrations of (µg/ml)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Efavirenz</td>
<td>Efavirenz-βCD (1:2)</td>
</tr>
<tr>
<td>0.5</td>
<td>3.62 ± 0.6</td>
<td>18.35 ± 1.6</td>
</tr>
<tr>
<td>1</td>
<td>5.73 ± 1.2</td>
<td>21.86 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>7.24 ± 0.8</td>
<td>22.82 ± 0.8</td>
</tr>
<tr>
<td>3</td>
<td>9.46 ± 1.1</td>
<td>19.28 ± 1.4</td>
</tr>
<tr>
<td>4</td>
<td>11.35 ± 0.7</td>
<td>14.76 ± 1.6</td>
</tr>
<tr>
<td>6</td>
<td>11.35 ± 1.2</td>
<td>12.45 ± 0.8</td>
</tr>
<tr>
<td>8</td>
<td>6.37 ± 0.9</td>
<td>8.86 ± 1.1</td>
</tr>
<tr>
<td>12</td>
<td>3.58 ± 0.8</td>
<td>4.32 ± 1.5</td>
</tr>
</tbody>
</table>

Fig. 8.1 Plasma Concentrations of Efavirenz Following the Oral Administration of Efavirenz and its CD Complexes in Rabbits.
Fig. 8.2 Semi logarithmic plot of plasma concentration Vs Time following oral administration of Efavirenz during elimination phase to determine $t_{\frac{1}{2}}$.

\[ y = -0.063x + 1.3097 \]
\[ R^2 = 1 \]

Fig. 8.3 Semi-logarithmic plot of Log percent unabsorbed Vs Time following oral administration of Efavirenz and its CD complexes.

\[ y_a = -0.211x + 2.0065 \]
\[ R^2 = 0.9679 \]

\[ y_b = -0.7635x + 1.9759 \]
\[ R^2 = 0.9882 \]

\[ y_c = -1.0386x + 1.9721 \]
\[ R^2 = 0.9914 \]
Table 8.2
Summary of Pharmacokinetic Parameters Estimated Following The Oral Administration of Efavirenz Products

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Efavirenz</th>
<th>Efavirenz-βCD (1:2) Complex</th>
<th>Efavirenz-βCD-Solutol HS15 (1:2:0.5) Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>11.35±0.7</td>
<td>22.82±0.8</td>
<td>24.52±1.4</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>4.0</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>$K_{\text{el}}$ (h⁻¹)</td>
<td>0.1450</td>
<td>0.1768</td>
<td>0.1513</td>
</tr>
<tr>
<td>$t_{\frac{1}{2}}$ (h)</td>
<td>4.77</td>
<td>3.91</td>
<td>4.58</td>
</tr>
<tr>
<td>$(AUC)_{0-12h}$</td>
<td>86.82</td>
<td>149.92</td>
<td>168.61</td>
</tr>
<tr>
<td>$(AUC)_{0-\alpha}$</td>
<td>111.50</td>
<td>174.35</td>
<td>207.27</td>
</tr>
<tr>
<td>BA (%)</td>
<td>100</td>
<td>156.36</td>
<td>185.89</td>
</tr>
<tr>
<td>$K_a$ (h⁻¹)</td>
<td>0.4859</td>
<td>1.7583</td>
<td>4.920</td>
</tr>
<tr>
<td>Percent Absorbed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 h</td>
<td>23.43</td>
<td>64.84</td>
<td>75.05</td>
</tr>
<tr>
<td>1.0 h</td>
<td>38.68</td>
<td>82.76</td>
<td>90.85</td>
</tr>
<tr>
<td>2.0 h</td>
<td>54.06</td>
<td>99.39</td>
<td>100.0</td>
</tr>
</tbody>
</table>
DISCUSSION OF RESULTS

Drug-CD complexes with and without Solutol HS15 gave markedly higher dissolution rates of efavirenz and ritonavir from solid complex systems as well as from their tablet formulations. Pharmacokinetic evaluation was done on efavirenz - βCD and efavirenz- βCD-Solutol HS15 complexes in comparison to pure drug with a view to evaluate their in vivo performance.

As the CD systems evaluated for in-vivo performance are fast dissolving and rapidly absorbing systems the in-vivo study is limited to 12 hours only to understand the absorption characteristics. And also as the plasma concentration reached elimination phase in about 8-10 hours the t₁/₂ could be assessed from 12 hours data. For rapidly absorbing and eliminating systems a 12 hours study is adequate.

Plasma concentrations of efavirenz following the oral administration of efavirenz and its CD complexes are given in Table 8.1 and shown in Fig.8.1. Pharmacokinetic parameters estimated are summarized in Table 8.2.

The biological half-life (t₁/₂) estimated from the elimination phase of the plasma level curves (Fig.8.2-8.3) was found to be 4.77, 3.91 and 4.58 h respectively following the oral administration of efavirenz, and its CD complexes, efavirenz - βCD (1:2) and efavirenz – βCD- Solutol HS15 (1:2:0.05). The close agreement of the t₁/₂ values in the three cases indicated that the elimination characteristics of efavirenz have not changed when it was administered as CD complexes.

Efavirenz was found to be absorbed slowly when given orally and a peak plasma concentration (Cₚₐₓ) of 11.35±0.7µg/ml was observed at 4.0 h after administration. The absorption rate constant (Kₐ) was found to be 0.4859 h⁻¹.

All the pharmacokinetic parameters (Table 8.2) namely Cₚₐₓ, Tₚₐₓ, Kₐ and (AUC)₀ⁿ indicated rapid and higher absorption and bioavailability of efavirenz when
administered as CD complexes. Higher $C_{\text{max}}$ values and lower $T_{\text{max}}$ values were observed with the CD complexes when compared to those of efavirenz as such. The absorption rate constant ($K_a$) was found to be $1.7583 \, \text{h}^{-1}$, $2.3918 \, \text{h}^{-1}$ respectively with efavirenz - βCD (1:2) and efavirenz – βCD- Solutol HS15 (1:2:0.05) complexes, whereas in the case of efavirenz $K_a$ was only $0.4859 \, \text{h}^{-1}$. A 3.62, and 4.92 fold increase in the $K_a$ was observed respectively with efavirenz - βCD (1:2) and efavirenz – βCD-Solutol HS15 (1:2:0.05) complexes when compared to efavirenz pure drug.

$(AUC)_0^{\infty}$ (extent of absorption) was also much higher in the case of CD complexes when compared to efavirenz pure drug. $(AUC)_0^{\infty}$ was increased from $111.50 \mu\text{g.h}/\text{ml}$ for efavirenz pure drug to $174.35$ and $207.27 \mu\text{g.h}/\text{ml}$ for efavirenz - βCD (1:2) and efavirenz – βCD- Solutol HS15 (1:2:0.05) complexes respectively. A 1.56 and 1.85 fold increase in $(AUC)_0^{\infty}$ was observed respectively with Efavirenz - βCD (1:2) and efavirenz – βCD- Solutol HS15 (1:2:0.05) complexes when compared to efavirenz pure drug.

Thus βCD has markedly enhanced both the rate ($K_a$) and extent $(AUC)$ of absorption (i.e. bioavailability) of efavirenz. Addition of Solutol HS15 has further enhanced both the rate of absorption and extent of absorption of efavirenz from efavirenz – βCD- Solutol HS15 (1:2:0.05) complex.