CHAPTER – XII

PHARMACOKINETIC EVALUATION OF PREDISOLONE MATRIX TABLETS FORMULATED EMPLOYING TAMARIND KERNEL GUM
Prednisolone is a corticosteroid anti-inflammatory, analgesic agent used in treatment of inflammatory diseases\(^1\). It is indicated for the treatment of primary or secondary adrenocortical insufficiency such as congenital adrenal hyperplasia and thyroiditis\(^2\). Prednisolone is a poorly water soluble drug. There are several reports \(^3\)-\(^6\) on the development of oral sustained release formulations of prednisolone.

The design of sustained release formulations of water insoluble drugs is always difficult because in the absence of rapid dissolution of the drug, the release of the drug from the formulated product such as a matrix tablet is controlled more by the dissolution of the drug than by the nature of the polymer employed. It is common with oral sustained release formulations of poorly water soluble drugs that they show incomplete release from hydrophilic polymeric matrix systems.

In this present study, the developed matrix tablet employing solid dispersion of prednisolone and tamarind kernel gum as controlled release polymer was subjected to *in-vivo* pharmacokinetic evaluation in rabbits in comparison with that of pure drug employed as reference standard.
MATERIAL AND METHODS

Materials:
Prednisolone pure drug was obtained as a gift sample from M/s. Cipla Ltd., Mumbai; Ethyl acetate, Isoamyl alcohol, Hexane, Perchloric acid, Tetrahydrofuran and Water - all the chemicals are of HPLC grade.

The pharmacokinetic evaluation was done on the following products.

❖ Prednisolone pure drug

❖ Prednisolone CR formulation containing the more dissolving dispersion (Pr: PVP at 1:3 ratio)

The in-vivo experiments were carried out in healthy rabbits as per the following experimental design and protocol. The protocol was approved by the IAEC (Regd no. 993/a/06/CPCSEA)

EXPERIMENTAL WORK

A crossover randomized block design (RBD) in which four rabbits each weighing 3.5 - 4.0 kg received one treatment (product) each every 15 days such that all the product are tested in all the four rabbits during the study. Thus, each treatment is replicated four times.
**In-vivo study protocol**

Rabbits weighing between 3.5-4.0 kg of either sex were used. They were fed on uniform diet. In the experiments, 18 hours fasted rabbits were used. The rabbits were not given food during the experiment; however they had free access to water.

After collecting the zero hour blood sample (blank), the product involved in the study was administered orally employing Ryle's tube (intubation tube) at a dose equivalent to 2.0 mg of prednisolone. After administration 2 ml blood samples were collected from the marginal ear vein at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 18.0, and 24.0 hrs after administration of the product. The blood samples were centrifuged at 5,000 rpm and the serum separated was collected into dry tubes and stored under refrigerated conditions till assayed. Prednisolone content of the serum samples was determined by a known HPLC reported previously. The serum prednisolone concentrations were calculated from a standard calibration graph. From the time versus serum concentrations data, various pharmacokinetic parameters such as peak concentration ($C_{\text{max}}$), time at which peak concentration resulted ($T_{\text{max}}$), area under curve (AUC), elimination rate constant ($K_{\text{el}}$), biological half life ($t_{1/2}$), percent absorbed to various times, the absorption rate constant ($K_{a}$), and mean residence time (MRT) were calculated in each case.
Estimation of Prednisolone in serum samples Chromatographic Conditions

The chromatographic system consisted of a Model Agilent 1120 compact LC; samples were chromatographed at room temperature on a reversed phase C18 column (Qualisil BDS CB, 150 X 4.6 mm, 5 um). The mobile phase consisting of (20: 80 v/v) tetrahydrofuran and water was used at a flow rate of 1.0 ml / min and the pressure was approximately 240 kg / sq.cm. Ultraviolet absorption was measured at 250 nm using Agilent variable wavelength detector.

Procedure

Prednisolone was isolated from 1.0 ml of rabbit serum via precipitation with perchloric acid (60%), followed by liquid - liquid extraction with 5.0 ml of ethyl acetate: hexane : isoamyl alcohol (80:19:1 v/v). The samples were vortexed for 60 seconds and then centrifuged at 10,000 rpm for 10 minutes at room temperature. The supernatant is then transferred to 16 mm x 150 mm test tube and mixed with 5.0 ml of ethyl acetate: hexane : isoamyl alcohol(80:19:1 v/v). After shaking samples for 45 to 60 seconds and centrifuging at 3200 rpm for 10 minutes, the organic layer was transferred to a clean 13 m x 100 mm test tube and evaporated to dryness.
Fig 12.1: Serum Concentrations of prednisolone following its oral administration CR formulation and as pure drug.

At 40 °C. The samples were then reconstituted with the mobile phase and 20 μl was injected into the HPLC column.

**Statistical Analysis of Data:**

In vivo pharmacokinetic data was analysed by unpaired student ‘t’-test. *p<0.05 (less significant), **p<0.01 (average significant), ***p<0.001 (more significant).
RESULTS AND DISCUSSION

Table 12.1: Summary of Pharmacokinetic Parameters of Prednisolone after administration CR formulation and as pure drug

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Pure drug</th>
<th>Controlled release tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ ($\mu$g/ml)</td>
<td>$0.45\pm0.0288$</td>
<td>$1.56\pm0.010^{***}$</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>$4\pm0.1197$</td>
<td>$2\pm0.2295^{**}$</td>
</tr>
<tr>
<td>$K_{\text{el}}$ (h$^{-1}$)</td>
<td>$0.4870$</td>
<td>--</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>$1.42$</td>
<td>--</td>
</tr>
<tr>
<td>$(\text{AUC})_0^{24}$ ($\mu$g.h/ml)</td>
<td>$3.016\pm0.1542$</td>
<td>$7.23\pm0.4023^{***}$</td>
</tr>
<tr>
<td>$(\text{AUC})_0^{\infty}$ ($\mu$g.h/ml)</td>
<td>$3.083\pm0.132$</td>
<td>$7.27\pm0.4019^{**}$</td>
</tr>
<tr>
<td>$K_a$ (h$^{-1}$)</td>
<td>$0.667\pm0.009$</td>
<td>$0.553\pm0.0146^{***}$</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>$3.06\pm0.1351$</td>
<td>$4.01\pm0.3462^{*}$</td>
</tr>
<tr>
<td>BA (%)</td>
<td>$100.0$</td>
<td>$236$</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01, ***P<0.001 Vs Pure drug at respective time interval. Statistical analysis was carried out using One way ANOVA followed by Tukey's test.

The elimination rate constant $K_{\text{el}}$ for prednisolone was found to be $0.48$ hr$^{-1}$ and the corresponding biological half life $t_{1/2}$ was found to be $1.42$ hrs. Following the administration of prednisolone as a solution, the $t_{1/2}$ value obtained in the present work is in good agreement with the earlier reported
value of 1-4 hours. The plasma concentration vs time profile obtained with different prednisolone products administered is shown in Fig. 12.1 and the summary of the pharmacokinetic parameters is given in Table 12.1.

Application of Wagner - Nelson method to the serum concentrations data indicated a very slow absorption of Prednisolone given orally in CR form. The absorption rate constant $K_a$ is found to be $0.553 \pm 0.327 \, \text{hr}^{-1}$. Peak serum concentration of $1.56 \pm 0.253 \, \mu\text{g/ml}$ was observed.

Whereas when Prednisolone was administered as pure drug there is found to be very low rate of absorption as evidenced by the low $K_a$ value $(0.667 \pm 0.148 \, \text{hr}^{-1})$. Application of Wagner - Nelson method to the serum concentration data indicated slow and significant ($P<0.05$) absorption of Prednisolone from the CR products compared to that from the pure drug.

Additionally it is also evident that the Prednisolone concentrations were stabilized and maintained over a narrow range and for longer periods of time in the case SR products. The mean residence time (MRT) was found to increase significantly ($P<0.05$) from $3.06 \pm 0.345$ hrs for Prednisolone to $4.011 \pm 0.455$ hrs with the CR formulation. CR formulation showed a relative bioavailability of 236% when compared to Prednisolone taken as 100%.
CONCLUSION

Thus the results of the pharmacokinetic studies carried out on the CR formulation of prednisolone developed by employing the solid dispersions of prednisolone indicated the drug was released and absorbed over longer periods of time which in turn maintained the serum concentrations within a narrow range for extended periods of time. Thus the present in vivo study corroborates the usefulness of the approach of employing solid dispersions of prednisolone in designing formulations (as reported in our previous communications) for controlled release of prednisolone.
References


