8.1. OBJECTIVE

Pharmacokinetic studies of optimized and characterized AD-SLN formulation

8.2. BACKGROUND

Solid lipid nanoparticles (SLNs) have emerged as important tools to modify the release profile for a large number of herbal drugs used for cancer treatment. Due to their versatility in loading both lipophilic and hydrophilic molecules in the solid lipid matrix, SLNs depict the ability to prolong, extend or sustain the release profile of the loaded molecules, therefore reducing the repeated administration, and increasing the therapeutic value of a certain treatment. Additional advantages include reduction of drug toxicity and increase of drug bioavailability. To develop SLN formulations for drug targeting and delivery, a basic pharmacokinetic understanding of drug distribution is of major relevance, as well as the biopharmaceutical aspects of the administration route (Souto & Doktorovová, 2009).

Pharmacokinetic parameters were calculated by non-compartmental analysis also called as Model independent analysis. Following oral drug administration, the maximum observed concentration in the concentration-time profile ($C_{\text{max}}$) and the time to reach that concentration ($t_{\text{max}}$) are important descriptors of the extent and nature of drug exposure. $C_{\text{max}}$, an indicator of maximum drug exposure, may sometimes relate better to pharmacological or toxicological effects than other measures of exposure.

When blood, plasma (most commonly obtained), or serum drug concentrations are plotted versus time, the area under the curve (AUC) is the primary measure of overall exposure following oral administration of a drug. AUC is most commonly determined using the linear trapezoidal method and calculated as:

$$AUC_{0-\infty} = AUC_{0-t_{\text{last}}} + AUC_{t_{\text{last}}-\infty}$$

Bioavailability is the fraction of extravasally-administered dose that reaches the systemic circulation. Absolute bioavailability is determined by calculating the ratio of dose-normalized AUCs following iv and extravascular administration. Relative bioavailability is calculated between two dose routes, forms or formulations as:

$$F_{\text{rel}} = \frac{AUC_{\text{test}}}{AUC_{\text{ref}}}$$
For an orally administered drug, numerous factors can influence bioavailability. Dissolution, solubility, and membrane permeability (influenced by polarity and thus ionization state) affect the fraction of drug absorbed.

Mean residence time ($MRT_{0-t}$) is the arithmetic mean of the duration that each drug molecule resides in the body. It is equivalent to the ratio of $AUMC/AUC$, where $AUMC$ is the area under the first moment curve. It is computed as:

$$MRT_{0-t} = \frac{AUMC_{0-t}}{AUC_{0-t}}$$

The in vivo study was performed to quantify AD after oral administration of AD-SLNs. The plasma profiles in Wistar rats, following oral administration of the SLN formulation and drug suspension of AD, were compared. For pharmacokinetic studies AD-SLNs prepared using cetyl alcohol as well as gelucire 50/13 were taken into consideration.

8.3. ANIMAL HOUSING AND HANDLING

Approval to carry out in vivo study was obtained from Jamia Hamdard, Institutional Animal Ethics Committee, (Registration No. 173/CPCSEA, project no. 878 dated 22.10.2012) New Delhi, and their guidelines were followed. The animals used for in vivo experiments were adult female albino wistar rats obtained from the Central Animal House of Hamdard University, New Delhi, India. The animals were kept under standard laboratory conditions, temperature at $25 \pm 2^\circ C$ and relative humidity ($55 \pm 5\%$), and housed in polypropylene cages, six per cage, with free access to standard laboratory diet (Lipton feed, Mumbai, India) and water ad libitum.

Animals used for the study: Rats
- **Species:** Albino wistar rats
- **Age/wt:** 10-12 Weeks (150-200 gm)
- **Gender:** Female
- **No. of animals in each group:** Six per group
- **Total time of study and no. of blood samples for each animal:**
  The blood sampling was carried out for around Seventy two hours and 8 to 10 samples of blood were taken from each animal in the group.
8.4. METHODOLOGY

8.4.1. Determination of pharmacokinetic parameters

The AD-SLNs and standard AD suspension (AD equivalent to 10 mg Kg⁻¹ body weight) were given orally (Table 25), using the oral feeding needle. Standard AD suspension was prepared in 10% w/v gum acacia with particle size $2.453 \pm 0.074 \mu m$ and distribution $0.32 + 0.0027$ (polydispersity index). The rats were anesthetized with ether, and blood samples (0.5 ml) were withdrawn from the tail vein of rat at 0 (pre-dose), 0.25, 0.5, 1, 2.5, 4, 6, 12, 18 and 24 h in microcentrifuge tubes in which 8.0 mg of EDTA was added as an anticoagulant. The blood collected was mixed with the anticoagulant properly and centrifuged at 5000 rpm for 20 min. The plasma was separated and stored at -21°C until drug analysis was carried out using the developed UPLC/Q-TOF/MS/MS method.

Table 25: Different formulations with their doses given to different group of animals

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulations</th>
<th>Formula</th>
<th>Dose (mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AD suspension</td>
<td>AD suspension in 10% w/v gum acacia</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>A-SLNs CA</td>
<td>AD-SLNs using cetyl alcohol and tween 80</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>AD-SLNs GL</td>
<td>AD-SLNs using gelucire 50/13 and tween 80</td>
<td>10</td>
</tr>
</tbody>
</table>

The plasma samples were prepared by spiking 5% aqueous analytes in plasma sample (50 µL aqueous aliquots to 950 µL plasma sample). Samples (200 µL each) were taken into a borosilicate glass tube and 20 µL of IS (100 ng mL⁻¹) was added in it. Further, 80 µL of 2% (w/v) formic acid was added and vortexed for five min at 300×g speed, then 1.2 mL of ethyl acetate was also added to the solution and again vortexed at same speed and time, kept on an ice bath for five min. The supernatant was taken and evaporated to dryness at 40°C under a stream of nitrogen (30 psi; five min). The residue was re-dispersed in the 200 µL of mobile phase (acetonitrile: formic acid; 10: 90, v/v) and vortexed for 10 s at 300×g. The solution was transferred into the clean auto-sampler vials and 10 µL was injected into UPLC/QTOF-MS/MS system for analysis.

The pharmacokinetic parameters were calculated based on a non-compartmental model. Peak concentration ($C_{max}$) and time of peak concentration ($T_{max}$) were obtained directly.
from the individual plasma concentration-time profiles. The area under the concentration-time curve from time zero to time t (AUC$_{0→t}$) was calculated using the trapezoidal method.

### 8.4.2. Statistical analysis

Statistical evaluation of pharmacokinetic data was performed using one-way analysis of variance (ANOVA). Data were expressed as mean and standard deviation of separate experiments ($n = 6$). Statistically significant differences were assumed when p<0.05. All values are expressed as their mean ± SD.

### 8.5. RESULTS AND DISCUSSION

One of the most useful techniques for isolating desired components from a mixture is liquid-liquid extraction (LLE). LLE is a method used for the separation of a mixture using two immiscible solvents. In most LLEs, one of the phases is aqueous and the other is an immiscible organic solvent. The concept “like dissolves like” works well in LLE. The drug concentration in plasma after oral administration of AD and AD-SLNs to albino wistar rats was determined by the in-house developed and validated UPLC/QTOF-MS/MS method. The chromatogram of blank plasma and AD-SLNs is given in **Figures 82 & 83**.

![UPLC chromatogram of blank plasma](image)

**Figure 82: UPLC chromatogram of blank plasma**
An *in vivo* study was performed to quantify AD after oral administration of AD formulations. The plasma profiles of drug in adult female albino wistar rats, following oral administration of the AD-SLN formulation and drug suspension of AD, were compared. *Figure 84* demonstrates that the plasma concentration profile of AD-SLN represents greater improvement of drug absorption than the simple drug suspension. Pharmacokinetic parameters were calculated by non-compartmental analysis also known as Model independent analysis. All the pharmacokinetic parameters (\(t_{\text{max}}, C_{\text{max}}, AUC_{0,t}\)) were calculated individually for each subject in the group, and the values were expressed as mean ± SD (n=6).

Peak concentration \(C_{\text{max}}\) and the time of peak concentration \(T_{\text{max}}\) were obtained from the *Figure 84*. The area under the concentration-time curve from time zero to time \(t\) \((AUC_{0,t})\) was calculated and given in *Table 26*. The area under the curve \((AUC)\) determines the bioavailability of the drug for the same given dose in the formulation. The relative bioavailability was calculated as:

\[
\%F_{\text{rel}} = \left( \frac{AUC_{\text{AD-SLN}}}{AUC_{\text{AD}}} \right) \times 100
\]
The oral pharmacokinetic parameters were listed in Table 26. The $C_{\text{max}}$ of AD in cetyl alcohol- and gelucire 50/13-derived SLNs 3.272 ± 0.7 and 2.97 ± 0.8 µg ml$^{-1}$, respectively) was higher ($p < 0.01$) than that obtained with the standard AD suspension (0.833 ± 0.3 µg ml$^{-1}$). AUC of AD-SLNs (with cetyl alcohol and gelucire 50/13) and AD suspension were found to be 37.044 ± 9.15, 35.377 ± 11.23 and 10.863 ± 2.29 µg h mL$^{-1}$, respectively. The AUC of cetyl alcohol- and gelucire 50/13-derived SLNs after oral administration were 3.41-fold and 3.25-fold higher than the AUC of drug suspension of AD, respectively. The $C_{\text{max}}$ of cetyl alcohol- and gelucire 50/13-derived SLNs were 3.92-fold and 3.56-fold greater than the $C_{\text{max}}$ of drug suspension of AD, respectively. The $t_{\text{max}}$ was increased than that of AD suspension, indicating the sustained/controlled release pattern of AD-SLNs. The higher value of AUC and $C_{\text{max}}$ of AD-SLNs ensured higher drug availability at the site of action over a prolonged period of time. Statistically, the difference in $t_{\text{max}}$ of AD-SLN formulation was significant, when compared with $t_{\text{max}}$ of AD suspension ($p < 0.01$). The higher value of AUC and $C_{\text{max}}$ of AD-SLNs ensured higher drug availability at the site of action over a prolonged period of time.

**Figure 84:** Plasma concentration profile of AD after oral administration of different formulation to adult albino wistar rats. Data is expressed as mean ± SD (n=6).
Lack of suitable drug-delivery systems, that can produce adequate therapeutic levels, accounts for the limited utility of AD; AD-SLNs were prepared and found better than the previously reported delivery systems. Chellampillai et al., (2011) reported a 2.2-fold increase in bioavailability of AD when encapsulated in polymeric nanoparticles, whereas AD-SLNs showed a 4.6-fold increase in the relative bioavailability. Maiti et al., (2010) prepared herbosomes of AD and found almost 1.4-fold increase in \( C_{\text{max}} \), but AD-SLNs showed a 4.4-fold increase in the \( C_{\text{max}} \) when compared with AD.

The AUC and \( C_{\text{max}} \) of cetyl alcohol-derived SLNs were 1.04-fold and 1.10-fold increase than the AUC and \( C_{\text{max}} \) gelucire 50/13-derived SLNs, respectively. But the \( t_{\text{max}} \) was almost similar for both types of SLNs.

The results showing incorporation into SLNs resulted in increased absorption of AD by oral administration. As shown in Figure 84, the AD plasma concentrations were significantly higher for rats treated with AD-SLNs than for those treated with AD suspension at all-time points. The improved bioavailability resulted by the SLNs formulation may be attributed to increased permeability by surfactants, direct uptake of nanoparticles through the GI tract, and decreased degradation and clearance. The uptake of drug from SLNs could be uptaken through the GI tract, where the particle size of the drug is one of the major parameters that affect absorption of drug from the GI tract. As the SLNs exhibited the nano-size, it produced the highest \( C_{\text{max}} \) and AUC with decreased \( T_{\text{max}} \). The mechanisms involved diffusion of particles through mucus and their accessibility to enterocyte surface, epithelial interaction and cellular trafficking, and exocytosis and systemic dissemination. The size of SLNs in the range of 20-500 nm allows the efficient uptake in intestine, particularly in the lymphoid sections of this organ; therefore bypass the liver first-pass metabolism (Duchene and Ponchel, 1997).
Table 26: Pharmacokinetic parameters for AD and AD-SLNs after oral administration to albino wistar rats

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$C_{\text{max}}$ (µg mL$^{-1}$)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>AUC$_{0-\infty}$ (µg h mL$^{-1}$)</th>
<th>Relative bioavailability (%)</th>
<th>AUMC$_{0-\infty}$ (µg h$^2$ mL$^{-1}$)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>0.833 $\pm$ 0.3</td>
<td>0.5 $\pm$ 0.04</td>
<td>10.863 $\pm$ 2.29</td>
<td>-</td>
<td>12.665 $\pm$ 2.51</td>
<td>1.165 $\pm$ 0.78</td>
</tr>
<tr>
<td>AD-SLNs CA</td>
<td>3.272 $\pm$ 0.7</td>
<td>2.5 $\pm$ 0.07</td>
<td>37.044 $\pm$ 9.15</td>
<td>340.99</td>
<td>74.139 $\pm$ 4.36</td>
<td>2.001 $\pm$ 0.92</td>
</tr>
<tr>
<td>AD-SLNs GL</td>
<td>2.97 $\pm$ 0.8</td>
<td>2.5 $\pm$ 0.12</td>
<td>35.377 $\pm$ 11.23</td>
<td>325.66</td>
<td>70.387 $\pm$ 9.08</td>
<td>1.98 $\pm$ 0.37</td>
</tr>
</tbody>
</table>

CA-Cetyl alcohol
GL-Gelucire 50/13

$C_{\text{max}}$: Maximum observed concentration in the concentration-time profile
$T_{\text{max}}$: Time to reach that concentration
AUC: Area under the curve
AUMC: Area under first moment curve
MRT: Mean residence time
CHAPTER - 8  

PHARMACOKINETICS OF AD-SLNs

The surfactant, like tween-80, has contributed to an increase in the permeability of the intestinal membrane or improved the affinity between lipid particles and the intestinal membrane, and may exhibit bioadhesion to the GI tract wall. By incorporation into nanoparticles, AD can be embedded into a solid lipid matrix, thus not only reducing its exposure to bacterium as well as enzymatic degradation during absorption process, but also offering a long time contact with the wall of intestine \textit{in vivo} due to the nice adhesiveness of SLNs to the mucosal surface of intestinum tenue (Luo et al., 2006). In addition, AD-SLNs could provide AD with a long circulation effect \textit{in vivo} with sustained-release property, which prolonged the drug-residence time in systematic circulation and resulted in better bioavailability.

As a result, SLNs appear to be an effective approach for increased absorption after oral administration of AD in comparison to earlier reported results.

\textbf{8.6. CONCLUSION}

The bio-analytical method was developed and validated in accordance with FDA criteria. The assay achieved good sensitivity and specificity for the determination of AD in rat plasma after oral administration of AD nanoparticles. No significant interferences caused by endogenous compounds were observed. The plasma profiles of AD in adult female albino wistar rats following oral administration of the SLN formulation was compared with plasma profile obtained following administration of drug suspension. It was found that the plasma concentration profile of AD-SLNs represent greater improvement of drug absorption than the AD suspension.

The AUC and C\textsubscript{max} of cetyl alcohol-derived SLNs were 1.04-fold and 1.10-fold increase than the AUC and C\textsubscript{max} gelucire 50/13-derived SLNs, respectively. But the t\textsubscript{max} was almost similar for both types of SLNs. The percent relative bioavailability of cetyl alcohol-derived SLNs was 340.99\% and 325.66\% for gelucire 50/13-derived SLNs.

The AUC and C\textsubscript{max} of cetyl alcohol-derived SLNs were little higher than that of gelucire 50/13-derived SLNs. This may be due to smaller particle size and high entrapment efficiency of AD-SLNs prepared by cetyl alcohol.
The AD-SLN formulation prepared by using cetyl alcohol was selected for further studies on the basis of smaller particle size (154.1 nm), uniform size distribution (PDI- 0.172), higher entrapment efficiency (91.4%), sustained release pattern from in vitro dissolution study (77.89% release in 36 h), higher AUC (37.044 ± 9.15 µg h mL⁻¹) and Cₘₐₓ (3.272 ± 0.7) and higher percent relative bioavailability (340.99%). Of course, non-availability of large number of Balb/c mice for antitumor activity was also the main reason for selecting only one formulation i.e. cetyl alcohol derived AD-SLN for further studies i.e. antitumor activity.