CHAPTER III

PHARMACOKINETICS OF CURCUMIN LOADED SOLID LIPID NANOPARTICLES IN RAT PLASMA
1.0 INTRODUCTION

Formulating curcumin for clinical efficacy has presented many challenges due to its poor physicochemical properties. In spite of the numerous formulation challenges, several strategies like nanoparticles, liposomes, complexation with phospholipids and cyclodextrins and solid dispersions have been developed to improve the bioavailability of curcumin (Bisht et al., 2007; Maiti et al., 2007; Tiyaboonchai et al., 2007). However, in vivo evaluation establishing the superiority of most of the above developed systems is still lacking.

Single dose pharmacokinetic studies of the developed and characterized curcumin loaded solid lipid nanoparticles (C-SLNs) was performed. In order to determine the concentration of curcumin in rat plasma four doses of C-SLNs {very high (VH), 50; high (H), 25; medium (M), 12.5; and small (S), 1 mg/kg}, and free curcumin {C-S (50 mg/kg)}, were administered orally to rats. Further, a highly sensitive and validated LC–MS/MS method (as established in chapter II) was used for determining the concentration of curcumin at various time points during pharmacokinetic profiling.

2.0 MATERIALS AND METHODS

2.1 Study design for in vivo pharmacokinetic studies

For in vivo pharmacokinetic studies, male Wistar rats weighing 250–300 g were used. The protocol was duly approved by the Institutional Animal Ethics Committee of Panjab University, Chandigarh, India. The animals were divided into five groups (n = 3). Group 1 (VH) was administered 50 mg/kg body weight (bw) of C-SLNs; Group 2 (H; 25 mg/kg bw C-SLNs), Group 3 (M; 12.5 mg/kg bw C-SLNs); Group 4 (S; 1.0 mg/kg bw C-SLNs) and Group 5 was administered 50 mg/kg bw free curcumin (C-S; solution of curcumin in 25% tween 80) per orally using an oral dosing cannula. The blood samples (0.5 ml) were withdrawn from sinus under clavicle and, collected into heparinized microcentrifuge tubes (containing 20 μl of 1000 IU heparin/ml of blood) at different time intervals. After each sampling, 1 ml of dextrose–normal saline was orally administered to animals to prevent changes in the central compartment volume and electrolyte concentration. Plasma was separated by centrifuging the
blood samples at 4000 rpm for 10 min at 4°C. After centrifugation, the plasma obtained was stored at -20°C until analysis.

2.2 Quantification of curcumin in plasma

The LC–MS/MS analysis was performed using API 4000 mass spectrometer, Applied Biosystems, Sciex Toronto, Canada with an automatic liquid chromatographic sampler and an autoinjection system hyphenated to a Micromass Quattro Ultima tandem quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray ionization (ESI) source. The separation was achieved using a Chromolith rod™-C18 column (150×4.6 mm I.D.; 5 μm, Agilent, Palo Alto, CA, USA). The system delivered a constant flow of 30 μl/min and the mobile phase consisted of 80% acetonitrile and 20% of 10 mM ammonium acetate (pH:3.5). The volume of injection was 10 μl. For the operation in MS/MS mode, a mass spectrometer with an orthogonal Z-spray electrospray interface was used. During analyses, the ESI parameters were set as follows: capillary voltage, 4.5 kV for negative mode; source temperature, 40°C; desolvation temperature, 300°C; and desolvation gas flow, 50 l/h. The cone voltage of m/z 367 was adjusted to maximize the intensity of the deprotonated molecular ion (precursor) as 65 V and the collision voltage was also adjusted to optimize the product ion signals as 16 eV for curcumin analysis.

The MRM used to monitor the transition of the deprotonated molecule m/z 367 [M-H]− to the product ion 217 for curcumin analysis and m/z 307/229 for internal standard analysis.

3.0 DATA ANALYSIS

The pharmacokinetic parameters were calculated based on a non-compartmental model. The area under the concentration–time curve from time zero to time t (AUC₀⁻ᵗ) was calculated using the trapezoidal method. Peak concentration (Cₘₐₓ) and time of peak concentration (Tₘₐₓ) were obtained directly from the individual plasma concentration–time profiles. The area under the total plasma concentration–time curve from time zero to infinity was calculated by: AUC₀→∞=AUC₀⁻ᵗ+Cₜ/Ke, where Cₜ is
the curcumin concentration observed at last time, and $K_e$ is the apparent eli-
rate constant obtained from the terminal slope of the individual concentration–time curves after logarithmic transformation of the concentration values and application of linear regression. The data obtain-
pharmacokinetic parameters were analyzed statistically using Win-Nonlin. St-
Statistically significant differences were assumed at $p<0.05$. All values are ex-
as their mean ± S.D.

4.0 RESULTS

4.1 In vivo pharmacokinetic study

Plasma levels after oral administration of different concentrations of C-SLNs 12.5 and 1 mg/kg) were compared with 50 mg/kg free curcumin (C-S; usin-
amounts of tween 80, corresponding to that used in the final SLN disper-
illustrated in Figure 1a and 1b. The mean curcumin concentrations in the after oral administration of C-SLNs and C-S after single dose in Wistar rats determined using the earlier developed LC/MS/MS method.

Figure 1 (a). The mean plasma concentration-time area curve of curc-
rat after single oral dose of C-SLNs at VH (50 mg/kg) mg/kg); M (12.5 mg/kg) and S (1 mg/kg)
Figure 1 (b). The mean plasma concentration-time curve of curcumin in rats after single oral dose of C-SLNs at VH (50 mg/kg) and C-S (5 mg/kg)

The relevant pharmacokinetic parameters including $C_{\text{max}}$, $T_{\text{max}}$, $V_d$, $C_l$ and $\text{AUC}_{0-\infty}$ are listed in the Table 1.

Table 1. Various pharmacokinetic parameters obtained from plasma concentration-time data for orally administered C-S and C-SLNs at varying doses

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Dose (mg/kg)</th>
<th>$\text{AUC}_{0-\infty}$ (h*pg/ml)</th>
<th>$C_{\text{max}}$ (pg/ml)</th>
<th>$V_d$ (l/kg)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>$C_l$ (l/hr/kg)</th>
</tr>
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<tbody>
<tr>
<td>C-S</td>
<td>50</td>
<td>1.075±0.12 (^a)</td>
<td>0.292±0.06 (^a)</td>
<td>41.17 ± 0.352 (^a)</td>
<td>0.25±0.0</td>
<td>46.50±0.21 (^a)</td>
</tr>
<tr>
<td>VH</td>
<td>50</td>
<td>41.990±6.18 (^b)</td>
<td>14.29±0.15 (^b)</td>
<td>7.72±0.43 (^b)</td>
<td>0.5±0.02</td>
<td>1.19±0.05 (^b)</td>
</tr>
<tr>
<td>H</td>
<td>25</td>
<td>17.156±3.24 (^b)</td>
<td>8.00±1.87 (^b)</td>
<td>8.742±1.26 (^b)</td>
<td>0.25±0.04</td>
<td>1.46±0.95 (^b)</td>
</tr>
<tr>
<td>M</td>
<td>12.5</td>
<td>15.789±2.29 (^b)</td>
<td>7.87±3.02 (^b)</td>
<td>5.686±0.42 (^b)</td>
<td>0.25±0.05</td>
<td>0.79±0.38 (^b)</td>
</tr>
<tr>
<td>S</td>
<td>1</td>
<td>3.343±1.17 (^b)</td>
<td>1.00±0.01 (^b)</td>
<td>2.535±0.18 (^b)</td>
<td>0.5±0.01</td>
<td>0.30±0.12 (^b)</td>
</tr>
</tbody>
</table>

\(^b\) All the values are significantly different from \(^a\) at $p \leq 0.05$

Note: For C-SLNs {very high (VH); high (H); medium (M); and small (S), and free curcumin (C-S)}
The studies revealed a significant improvement ($p<0.05$) in relative bioavailability (39 times at 50 mg/kg; 155 times at 1 mg/kg and, 59 and 32 times at 12.5 and 25 mg/kg respectively) after administration of C-SLNs at all the doses w.r.t. C-S.

5.0 DISCUSSION

Curcumin targets multiple chemotherapeutic and inflammatory pathways and has demonstrated safety and tolerability in humans; however, the clinical literature lacks conclusive evidence supporting its use as a therapeutic agent due to its low bioavailability. The pluripharmacology of curcumin, compels researchers to play with its limiting biopharmaceutical properties and develop it as a shotgun for treatment of various ailments. *Biologists, are enamored with this wonder molecule which has a history of more than 100 years while the pharmaceutical scientist is dissuaded by its compromised pharmacokinetics and puts a question mark on its use as a drug.*

Numerous pharmacokinetic studies in humans and rats report, very low serum and plasma concentrations, irrespective of the route of administration due to its poor absorption, extensive intestinal and hepatic metabolism and rapid elimination thus restraining the BA of curcumin (Anand et al., 2007; Pan et al., 1999; Sharma et al., 2007).

In a very recent study in healthy volunteers, a 650 mg/kg dose of solid lipid curcumin particles showed plasma levels of 22.43 ng/ml, while free curcumin (95% curcuminoïds extract) at the same dose was undetectable (Gota et al., 2010). To what degree the enhanced bioavailability is a result of increased absorption or due to reduced conversion of free curcumin to conjugates is however not clear in the study because they did not treat the samples with glucuronidase. A 2-3-fold increase in curcumin absorption has been reported by simply dissolving or mixing curcumin in different types of lipids (Liu et al., 2006; Maiti et al., 2007).

In our study, oral administration of C-S resulted in a sharp $C_{\text{max}}$ of 0.292 µg/ml within 15 min after which the plasma concentration declined rapidly, indicating a rapid metabolism of curcumin. Whereas, relatively slow increase and sustained plasma
concentration of curcumin for a longer time was observed after administration of C-SLNs. Results show a very low volume of distribution (7.72±0.43 l/kg) and a significantly (p<0.05) high $C_{\text{max}}$ of 14.293 µg/ml at 0.5 h for the VH dose (5.3 times lower than C-S) which was still detectable at 24 h (0.012 µg/ml), suggesting the sustained effect of the solid lipid nanoparticles. There was a marked difference in the AUC$_{0-\infty}$ between C-S, and C-SLNs at all the doses. The AUC$_{0-\infty}$ for C-SLNs was appreciably higher (39 times) at 50 mg/kg dose of C-SLNs vis-a-vis C-S when administered orally to rats (Table 2). Liu et al. reported (Liu et al., 2006) similar $C_{\text{max}}$ values (0.266 µg/ml), however these levels were achieved at 100 mg/kg dose which is double the dose used by us (50 mg/kg: $C_{\text{max}}$-0.292 µg/ml). Yang et al. (Yang et al., 2007) used a 10 times higher dose (500 mg/kg: $C_{\text{max}}$-0.060 µg/ml) and Pan et al. (Pan et al., 1999) used a 20 times higher dose (1 g/kg:$C_{\text{max}}$-0.220 µg/ml); while the $C_{\text{max}}$ values recorded are either same or lower. It may be concluded that either the method used in the present study is more accurate and sensitive, or curcumin does not follow dose dependent kinetics. Further the use of tween 80 for solubilising curcumin (C-S), may also exert a penetration enhancing effect. We wanted to confirm that any BA enhancement observed with C-SLNs is not solely attributable to the use of surfactants (in their preparation) that is why we used C-S as the control for comparison.

Tsai et al. (2011) reported the pharmacokinetics of curcumin loaded PLGA nanoparticles in rat plasma, and could achieve a $C_{\text{max}}$ of 44 ng/ml, post administration of curcumin nanoparticles at a dose of 50 mg/kg, while free curcumin administered at a dose of 1 g/kg could achieve a $C_{\text{max}}$ of only 22 ng/ml (Tsai et al., 2011). Although the authors report an enhancement in bioavailability of 22 times, nevertheless it was almost 318 times lower than the $C_{\text{max}}$ (14,023 ng/ml) achieved by us after administration of C-SLNs at a similar dose. In another study, 2.5 mg/kg of curcumin PLGA nanoparticles administered by i.v route could achieve a concentration of 450 ng/ml of plasma while we could attain a 2.2 times higher concentration than that achieved by them after administration of 1 mg/kg dose of C-SLNs after per oral administration (Anand et al., 2010).
Liu et al. (2006) achieved a $C_{\text{max}}$ of 0.6 $\mu$g/ml with the prepared phospholipid complexes of curcumin. Yang et al. (2007) showed that 10 mg/kg of curcumin given i.v. in rats gave a maximum serum curcumin level of 0.36±0.05 $\mu$g/mL. Multiplying the dose with a factor of 5 (to have an arbitrary value for a 50 mg/kg dose) would mathematically result in a $C_{\text{max}}$ of 1.8 $\mu$g/mL. Envisaging the above, our results are highly appreciable, as oral administration of C-SLNs at 50 mg/kg could achieve a $C_{\text{max}}$ of 14.20 $\mu$g/mL which has not been achieved even with a similar dose (as discussed above) when given by i.v route. Furthermore, even a 40 times higher oral dose of 2 g could achieve a $C_{\text{max}}$ of only 1.35±0.23 $\mu$g/mL in an earlier study (Shoba et al., 1998).

Encouraged by the promising results achieved with the 50 mg/kg dose of C-SLNs, we performed single dose pharmacokinetics with subsequent subordinate doses of C-SLNs (25, 12.5 and 1 mg/kg). To our astonishment the results were highly encouraging with an increase of 155 times in BA with 1 mg/kg; 59 times with 12.5 and 32 times with 25 mg/kg doses of C-SLNs. This pattern of BA enhancement could possibly be attributed to the fact that the amount of curcumin absorbed (60–66% of the given dose) remained constant regardless of the dose indicating that administration of more curcumin does not result in higher absorption. Similar observation, that is, there is a dose-dependent limitation to bioavailability in rats has been reported earlier (Ravindranath and Chandrasekhara, 1981). Nevertheless, several observations in volunteers and patients also suggest that curcumin might possess biological activity even at low oral dose (Sharma et al., 2007).

BA of the developed SLNs is inveterate by the $C_{\text{max}}$ values as illustrated in the Table 2. However, the superiority of the developed nanoparticles was also established from the obtained values of volume of distribution ($V_d$) and clearance (Cl). As it is indicated that higher the $V_d$, more is the drug distributed to other extravascular (tissues) compartment. The $V_d$ at 50 mg/kg of C-SLNs was 53 times lower and Cl about 39 times lower; while at 1 mg/kg the $V_d$ was 162 times lower and Cl about 155 times lower w.r.t C-S. The above results are directly conclusive of prolonged circulation times of the developed SLNs with significantly lower clearance and volume of distribution values.
As the average particle size of nanoparticles was maintained below 200 nm, it helps bypassing the liver first pass metabolism which has been reported to be the major site of curcumin degradation. In addition, use of surfactants such as tween 80 and lecithin, in the preparation of SLNs may contribute towards an increase in the permeability of the intestinal membrane or affinity between lipid particles and intestinal membrane, and also may exhibit bioadhesion to the GI tract wall. Also, by incorporation into SLNs, curcumin is now embedded into a solid lipid matrix which not only reduces the exposure of curcumin to enzymatic degradation during the process of absorption, but also offers a long contact time in vivo. Lastly, C-SLNs could provide curcumin with long circulation times, which reduced the clearance from systemic circulation and resulted in its better bioavailability.

Curcumin with its ability to treat a variety of diseases is an interesting molecule of research today. With increasing literature evidence suggesting a link between multiple disease conditions in patients, multi-modulatory activity of curcumin can play an important role in curing them. Several clinical trials have determined the potential of curcumin in treating numerous disorders.

6.0 CONCLUSION

This highly bioavailable and stable solid lipid nanoparticulate formulation of curcumin may help establish the clinical efficacy of curcumin, reinventing its role from a preventative dietary supplement to a therapeutic agent. To best of our knowledge this is the first ever reported data on a novel drug system achieving such a high $C_{\text{max}}$ value at an extremely low dose of 1 mg/kg.