Pharmacokinetics of Different Pharmaceutical Nano Curcumin Products by Oral Administration

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Received September 9, 2017; accepted October 10, 2017

ABSTRACT
Curcumin has shown several potential pharmacological activities but it is limited on clinical application due to its poor bioavailability. Nano curcumin products have been investigated with purpose to increase curcumin’s bioavailability. Recently, for the first time, nanocurcumin was formulated in effervescent tablet (commercially available as SCurma Fizzy™). The aim of this study was to compare the pharmacokinetic parameters of this novel formulation of nanocurcumin and traditional formulation. In this study, the pharmacokinetic parameters of nanocurcumin in effervescent tablet, hard capsule and soft capsule in rats with a dose of 150 mg curcumin/kg body weight were investigated. The method to determine curcumin’s concentration was validated and the concentration of curcumin in rat’s plasma was examined at pre-dose (0 min), 30, 60, 120, 150, 180 min after administration of curcumin. This data showed that the SCurma Fizzy tablet significantly improved the curcumin’s concentration in plasma as compared with others products studied. In conclusion, the results suggested that effervescent tablet for nanoparticulate curcumin is recommended formulation to improve its bioavailability and therefore pharmacological activities.

KEYWORDS: Nanocurcumin; Oral absorption; Pharmacokinetics; Effervescent; Bioavailability.

Introduction
Turmeric (Curcuma longa Linn), is used in traditional medicine and food in many Asian countries (Hatcher, Planalp et al. 2008). Curcumin, a main polyphenol found in the rhizome of Curcuma longa, possesses many pharmacological activities. It has been showed to have anti-oxidative, anti-inflammatory and anti-carcinogenic activities etc. Curcumin has a molecular weight of 368.38 Da, and the melting point of 183 ºC. Curcumin has low solubility in water, so that it is not stable at neutral and basic pH medium. Many studies confirmed that curcumin have not any toxicity even at quite high doses (Lao, Ruffin et al. 2006). However, curcumin is limited in clinical treatment due to its very low aqueous solubility and rapid metabolism, therefore oral systemic bioavailability is very low. Oral administration of curcumin showed very few amounts presenting in the blood, and most of them being elimined in the feces (Hassaninasab, Hashimoto et al. 2011) in form of curcumin conjugated with glucuronides and sulfates. To improve the bioavailability of curcumin there are different methods which have been developed such as adjuvant with piperine, solid dispersions with polyvinylpyrrolidone, phytosome, liposome, nanoparticle. Oral drug administration is very common and convenient used of drug. Oral drug absorption is depended by drug's phytochemical properties and physiology of the gastrointestinal tract. The important factors determine drug absorption include drug dissolution velocity, the permeation through the membrane, and the first-pass organs by the liver and intestine (Pang 2003). Saipin Setthacheewakul et al., have developed the self-microemulsifying drug delivery systems to increase solubility, and bioavailability curcumin (Setthacheewakul, Mahattanadul et al. 2010). They have demonstrated that plasma concentration–time profiles of curcumin in rats dosed with self-microemulsifying drug delivery systems to increase solubility, and bioavailability curcumin (Setthacheewakul, Mahattanadul et al. 2010). They have demonstrated that plasma concentration–time profiles of curcumin in rats dosed with self-microemulsifying drug delivery systems increased absorption of curcumin. Ravichandran et al., have prepared nanoparticulate solid oral formulation of curcumin and formulated in a capsule. The authors showed that nanoparticulate curcumin significantly enhanced the curcumin concentration in kidney and liver (Ravichandran 2013). Other study revealed that
nanoparticles curcumin increased 9-folds bioavailability when compared to curcumin administered together with piperine (Shaikh, Ankola et al. 2009). Effervescent tablets are tablets which are dissolved in water before its administration. First of all, that's the best way to check out the quality of the nanocurcumin material. In addition, when in solution, nano curcumin will ensure its smallest size, and can be distributed throughout the digestive tract and reduce irritation of the gastric mucosa. In effervescent tablets, the excipients are swellable polymers in combination with effervescent components to generate carbon dioxide upon contact with gastric fluids (Singh and Kim 2000). Therefore, the effervescent tablets show high bioavailability.

This study aimed to evaluate the bioavailability of effervescent tablet curcumin (Commercially available as Scurma Fizzy™) as compared with nanocurcumin hard capsule and soft capsule and (curcumin + piperine) capsule and studied the pharmaco-kinetic parameters after oral administration.

Materials and Methods

Chemicals and Reagents

Effervescent tablet curcumin (Scurma Fizzy™), nanocurcumin hard capsule and nanocurcumin soft capsule and (curcumin + piperine) capsule were prepared ourselves. Citric acid, sodium hydroxide and acetonitrile were from Xilong Chemical, China. Organic solvents meet requirement of HPLC grade and all other chemicals were reagent grade.

Animal Experimental

Male Wistar albino rats were maintained under a twelve-hour light/dark cycle on polycarbonate cage in a colony room. Rats were fasted overnight and given SCurma Fizzy™ effervescent tablet curcumin, commercial nano curcumin hard capsule and soft capsule and (curcumin + piperine) capsule at 150 mg/kg body weight by oral administration. At pre-dose (0 min), 30, 60, 120, 150, 180 min, rats were exsanguinated under anaesthesia. Each time point had 10 rats. The blood was collected by cardiac puncture to heparinized tubes, immediately centrifuged at 7000 × g for 15 min, the supernatant was carefully removed to another vial and stored at −80 °C until analysis.

Sample Preparation

Added 100 μL of plasma into a vial contained 200 μL of acetonitrile, then vortexed for 1 minute, then centrifuged at 15,000g for 5 minute. Take 100 μL of supernatant into new vials. This solution was injected directly into the column.

Prepared Stock and Working Standard Solutions

Prepared stock solutions of curcumin in methanol (1 μg/mL). These solutions were stored at -20°C. Working solutions of curcumin were prepared by diluting the stock solution with methanol. Standards curcumin solutions in blank plasma in different concentrations, including 3.91; 7.8125; 15.625; 31.25, 62.5 and 125 ng/mL also were prepared.

HPLC Analysis of Curcumin

Curcumin was determined by high-performance liquid chromatography method with UV-VIS detection which was described by Ma et al. (Ma, Shayeganpour et al. 2007) with minor modifications. Ratio 1% citric buffer and acetonitrile as mobile phase was 70:30.

Other conditions of HPLC methods were:

- Detector: UV –VIS, 425 nm.
- Flow rate: 1.0 mL/ min
- Injection volume: 10 μL
- Column: Agilent ZORBAX Eclipse Plus 95Å C18, 4.6 × 100 mm, diameter 3.5 μm.

Precision

Precision of intra-day assay (n = 6) was examined by analyzing three different concentrations, including 15.63; 31.25 and 62.5 ng/mL. Precision of inter-day was examined for three independent experimental assays of the three replicates at concentrations of 15.63; 31.25 and 62.5 ng/mL.

Precision was assessed by coefficient of variation, which was calculated as (Ma, Shayeganpour et al. 2007):

\[
CV\% \text{(intra – day)} = \frac{SD}{\text{Mean measured concentration}} \times 100
\]

\[
CV\% \text{(inter – day)} = \frac{CV\% \text{ day }1 + CV\% \text{ day }2 + CV\% \text{ day }3}{3} \times 100
\]

Pharmacokinetic Analysis

Pharmacokinetic analysis were performed on each animal using the PK Solver software by non-compartmental method (Zhang, Huo et al. 2010). The data were expressed as mean ± SEM. Statistical analysis were analyzed by t-test to compare different groups. Different significance was set at p < 0.05.

Results and Discussion

Chromatography Spectrum

HPLC spectrum of analyzing blank plasma sample did not show any interfering components. The typical chromatogram of a curcumin in plasma sample is showed in Fig. 1. The retention time of curcumin in plasma were 16.797 min.

Extraction Efficiency

The extraction efficiency of curcumin from plasma was determined by comparing the peak area obtained from extracts of curcumin plasma sample with peak area obtained from the direct injection of known concentration of standard solutions of curcumin. Assessed at concentration of 15.63, 31.25 and 62.5 ng/mL (three replicates) the recovery was 95.25, 93.57 and 92.23 %, respectively (Table 1). The data showed this extraction method to analyze curcumin in plasma samples was suitable.
TABLE 1
Extraction efficiency of curcumin from rat plasma (n = 3).

<table>
<thead>
<tr>
<th>Curcumin (ng/mL)</th>
<th>Extraction efficiency (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.63</td>
<td>95.25 ± 2.64</td>
</tr>
<tr>
<td>31.25</td>
<td>93.57 ± 2.18</td>
</tr>
<tr>
<td>62.5</td>
<td>92.23 ± 3.22</td>
</tr>
</tbody>
</table>

Linearity and Limit of Detection

The linearity was performed by plotting the peak-area of curcumin vs. the concentration of curcumin. The calibration curve was showed linearity with value $r^2$ of 0.9998 in the range of 3.91–125 ng/ml for curcumin extracted from plasma sample (Fig. 2).

TABLE 2
The obtained peak-area of each concentration of curcumin in standard solution.

<table>
<thead>
<tr>
<th>Concentration of curcumin (ng/ml)</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.91</td>
<td>7.66</td>
</tr>
<tr>
<td>7.81</td>
<td>14.93</td>
</tr>
<tr>
<td>15.63</td>
<td>29.43</td>
</tr>
<tr>
<td>31.25</td>
<td>59.32</td>
</tr>
<tr>
<td>62.5</td>
<td>103.23</td>
</tr>
<tr>
<td>125</td>
<td>200.47</td>
</tr>
</tbody>
</table>

Fig. 2. Graph of calibration curve for curcumin.

Limit of detection of curcumin in plasma sample was 3.91 ng/mL.

Precision

Table 3 presents the summary of intra- and inter-day precision for curcumin in plasma sample. The intra-day and inter-day accuracy of curcumin with precision (CV) $<12\%$. Precision study showed that the method is accurate and reproducible; and therefore the method is suitable for pharmacokinetic studies of curcumin in rats.

TABLE 3
The precision of intra-day (n = 6) and inter-day (n = 3).

<table>
<thead>
<tr>
<th>Known concentration</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>CV%</td>
</tr>
<tr>
<td>15.63</td>
<td>15.65 ± 1.7</td>
<td>11.02</td>
</tr>
<tr>
<td>31.25</td>
<td>32.17 ± 3.1</td>
<td>10.66</td>
</tr>
<tr>
<td>62.5</td>
<td>63.21 ± 5.2</td>
<td>9.37</td>
</tr>
</tbody>
</table>

Pharmacokinetic Analysis

In this present investigation, the pharmacokinetics of nanocurcumin formulated in effervescent tablet, hard capsule and soft capsule and (curcumin + bioperine) capsule in rats was investigated. Figure 3 showed the mean plasma curcumin concentration versus time profiles of four formulations studied at dose of 150 mg of curcumin/kg b.w. mice.

TABLE 4
Pharmacokinetic parameters.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\text{AUC}_{0-2.5h}$ (ng min/mL)</th>
<th>$C_{\text{max}}$ (ng/mL plasma)</th>
<th>$T_{\text{max}}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCurma Fizzy tablet</td>
<td>447.975 ± 1.76</td>
<td>226 ± 14.8</td>
<td>1.25</td>
</tr>
<tr>
<td>Hard capsule</td>
<td>377.125 ± 14.8</td>
<td>203 ± 14.8</td>
<td>1.50</td>
</tr>
<tr>
<td>Soft capsule</td>
<td>345.625 ± 16.2</td>
<td>189.5 ± 16.2</td>
<td>1.75</td>
</tr>
<tr>
<td>Curcumin + piperine</td>
<td>75.272 ± 5.9</td>
<td>48.7 ± 5.9</td>
<td>2.0</td>
</tr>
</tbody>
</table>

AUC: area under the curve of the plasma concentration vs time curve; $C_{\text{max}}$: peak concentration; and $T_{\text{max}}$: time to reach $C_{\text{max}}$. 

Fig. 3. Curcumin’s concentration plasma in rat after a single oral administration of: ScurmaFizzy™ Effervescent tablets; Hard capsules; Soft capsules; and (curcumin + bioperine) capsule (150 mg curcumin/kg body weight). Significant difference was set at $p < 0.05$ (vs (curcumin + piperine) capsule group). Values are represented as means ± SEM (n = 10).
The peak concentration and time of peak concentration were obtained directly from each rat plasma curcumin concentration vs. time profiles. The area under the curve from 0 to 2.5h (AUC_{0-2.5}) was calculated using the trapezoidal method (Bayomi, Al-Angary et al. 1998, Ravichandran 2013). The AUC represents for the oral bioavailability of the formulation. The pharmacokinetic parameters of studied formulations are presented in Table 4. Figure 3 showed the effervescent tablet has significantly higher plasma curcumin concentrations in rats as compared with others formulations. The C_{max} of curcumin in the effervescent tablet group (226.7 ± 1.7 6 ng/mL) was much greater than that obtained with marketed hard capsules (203.4 ± 14.8 μg/L), soft capsules (189.5 ± 16.2 ng/mL) and (curcumin + piperine) capsule (48.7 ± 5.9 ng/mL), (Table 4). The AUC_{0-2.5h} value of curcumin after oral administration of effervescent tablet was 447.875 μg min/L, which was higher than AUC of marketed hard and soft capsules and was 5.95 fold greater than that after administration of (curcumin + piperine) capsule.

Many researchers have shown that nanoparticles could enhance drug absorption and bioavailability (Maiti, Mukherjee et al. 2007). Our data also showed that (curcumin + piperine) capsule has lowest the absorption and others formulations of curcumin nanoparticle have greater absorption. That mean the nanoparticle curcumin has improved its absorption. The increased bioavailability of nanoparticles might be related to uptake directly of nanoparticles through the gastrointestinal tract, increasing permeability, and decreasing degradation and clearance of curcumin. First, the uptake of nanoparticle curcumin may be occurred through the gastrointestinal tract, where particle size plays an important role in absorption rate (Hussain, Jaitley et al. 2001). The mechanisms involved in this process maybe included the diffusion of nanoparticles through mucus and enterocyte surface, and exocytosis. Nanoparticle drug size arounds 200 nm allows efficiency through mucus and enterocyte surface, and exocytosis. The surfactants involved in the formulations may improve the permeability and solubility of drugs then across the membrane of the gastrointestinal tract. Third, by nanoparticle curcumin can be installed into the phospholipid bilayer. This will reduce its exposure to enzymatic system degradation during the absorption process. This will allow for prolonged contact with intestinal wall due to the adhesive property. Then, nanoparticle exhibited toward the epithelial mucosal surface of intestine (Lim, Lee et al. 2004). Among three formulations, the effervescent tablet showed highest bioavailability. The pharmacokinetic parameters (AUC and C_{max}) obtained in this study with the effervescent tablet and two capsules were significantly different (p<0.05). The effervescent tablet permits a faster availability of therapeutically significant plasma concentrations of curcumin, than the hard and soft capsule formulation, assuring the systemic disposition of higher total quantity of curcumin. This characteristic may be important in anti-inflammation treatment. Curcumin has been used for treatment of inflammatory bowel disease. It is postulated that for inflammatory bowel disease, nanocurcumin is required to be homogeneously distributed in the gastrointestinal tract to achieve maximum effect. Therefore, the effervescent tablet for oral delivery of nanocurcumin may improve the pharmacological activity of curcumin due to its higher solubility and bioavailability.

**Conclusion**

Our study has showed that nanocurcumin had significantly higher solubility and bioavailability than curcumin. The pharmacokinetic studies showed the effervescent tablet has higher curcumin's bioavailability as compared with (curcumin + piperine) capsule and a hard capsule and soft capsule at the same dose (150 mg/kg b.wt). Our results illustrated the SCurma Fizzy tablet for oral delivery of curcumin will be more appropriate to improve its therapeutics, such as for gastritis and peptic ulcer disease. The effervescent formulation helps in improving compliance, being preferable in subjects with difficulty in swallowing. That’s also the best way to check out the quality of the nano curcumin material simply by observing the dispensation of nano particles in water when dissolving the effervescent tablet and ensure its smallest size of nano particle. Nano curcumin in solution can be distributed throughout the digestive tract and reduce irritation of the gastric mucosa. It’s known that size of nano curcumin can be affected by pH in gastrointestinal tract, so administration in solution can perfectly resolve this issue. For all reasons, in our opinion, SCurma Fizzy tablets are recommended way to use nano curcumin.

**Significance statement:** This study showed that the nanocurcumin SCurma Fizzy tablets significantly improved the curcumin concentration in plasma as compared with others products. This study suggests that effervescent tablet for nano curcumin is recommended formulation to improve its bioavailability and therefore pharmacological activities.

**Author Contribution**

Bui Thanh Tung and Nguyen Thanh Hai conceived and designed the study. Duong Thi Duyen, Hoang Thi Thuy and Ho Thi Hang performed the experiments. Bui Thanh Tung and Nguyen Thi Thanh Binh, Karel Díezuez Santana, Maykel Cruz Monteaegudo and Teresa Garrigues analyzed data. Bui Thanh Tung and Nguyen Thanh Hai wrote the manuscript.

**Conflict of interest:** The authors have declared that there is no conflict of interest.
References


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