Evaluation of an Oral Carrier System in Rats: Bioavailability and Antioxidant Properties of Liposome-Encapsulated Curcumin

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To enhance the curcumin absorption by oral administration, liposome-encapsulated curcumin (LEC) was prepared from commercially available lecithins (SLP-WHITE and SLP-PC70) and examined for its interfacial and biochemical properties. A LEC prepared from 5 wt % of SLP-PC70 and 2.5 wt % of curcumin gave a good dispersibility with 68.0% encapsulation efficiency for curcumin, while those from SLP-WHITE did not. Moreover, the resulting LEC using SLP-PC70 was confirmed to be composed of small unilamellar vesicles with a diameter of approximately 263 nm. The resulting LEC was then examined for its effect on bioavailability in Sprague–Dawley (SD) rats. Three forms of curcumin [curcumin, a mixture of curcumin and SLP-PC70 (lecithin), and LEC] were then administered orally to SD rats at a dose of 100 mg curcumin/kg body weight. The pharmacokinetic parameters following curcumin administration were determined in each form. Pharmacokinetic parameters after oral administration of LEC were compared to those of curcumin and a mixture of curcumin and lecithin. High bioavailability of curcumin was evident in the case of oral LEC; a faster rate and better absorption of curcumin were observed as compared to the other forms. Oral LEC gave higher Cmax and shorter Tmax values, as well as a higher value for the area under the blood concentration–time curve, at all time points. These results indicated that curcumin enhanced the gastrointestinal absorption by liposomes encapsulation. Interestingly, the plasma antioxidant activity following oral LEC was significantly higher than that of the other treatments. In addition, the plasma curcumin concentration was significantly correlated to plasma antioxidant activities, and enhanced curcumin plasma concentrations might exert a stronger influence on food functionality of curcumin. The available information strongly suggests that liposome encapsulation of ingredients such as curcumin may be used as a novel nutrient delivery system.

KEYWORDS: Liposome; curcumin; oral administration; pharmacokinetics; antioxidant activity

INTRODUCTION

Curcuma longa L. (Ukon) rhizomes have been widely used for centuries as an indigenous medicine in India and other Asian countries (1). The medicinal properties have been attributed to the main component present in the rhizome, curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-hepadiene-3,5-dion], which exhibits anti-HIV (2), antitumor (3, 4), antioxidant (5, 6), and anti-inflammatory activities (7–9). To date, a number of studies have tried to elucidate the pharmacokinetics of curcumin, as it is poorly absorbed from the gastrointestinal (GI) tract after oral administration due to its low water solubility (10) and low stability against GI fluids and/or alkali/higher pH conditions (11). These characteristics lead to an unacceptably low oral bioavailability.

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To enhance the bioavailability and food functionality of curcumin, we focused our attention on the feasibility of using liposomes as an efficient carrier of functional food materials. Liposomes are nanoparticles prepared from phospholipids (12). There have been several reports on the employment of liposomes to protect pharmaceuticals such as insulin (13), peptides (14), calcitonin (15), and cyclosporin (16) from enzymatic degradation and to efficiently deliver them into the bloodstream. Liposome-encapsulated Ukon extract (LUE), which contains curcumin, has previously been developed, and its biochemical characteristics have been evaluated in our laboratory (17). It was confirmed to be composed of small unilamellar vesicles (SUVs) with a diameter of approximately 100 nm. On treatment with simulated gastric and intestinal fluids, the LUE showed a 2-fold higher residual rate of curcumin as compared to the unencapsulated extract. The bioactivity of the LUE was further examined for its suppressive effect on carbon tetrachloride (CCl4)-induced
liver injury in mice. Interestingly, oral administration of LUE at a dose of 10 mg Ukon extract/kg body weight showed a much higher suppressive effect on serum aspartate aminotransferase and alanine aminotransferase levels as compared to the uncapsulated extract at a dose of 33 mg/kg. To demonstrate the high functionality of LUE in more detail, the increased curcumin absorption due to encapsulation required investigation.

Thus, the purpose of this study was to provide evidence of liposomal enhancement of the oral delivery of curcumin. We prepared liposome-encapsulated curcumin (LEC) from two kinds of lecithins and curcumin using the mechanochemical method with a microfluidizer (18, 19) and investigated particle size by dynamic light scattering (DLS) measurements and electron microscopy. We also evaluated the plasma pharmacokinetics of curcumin concentrations from the most stable LEC formulation after oral administration in rats. The plasma antioxidant activity of rats orally administered with LEC was also examined using the trolox equivalent antioxidant capacity (TEAC) assay (20). To our knowledge, this is the first report on the pharmacokinetics and biochemical characterization of LEC.

**MATERIALS AND METHODS**

**Materials.** Curcumin (purity, >98%) was purchased from Wako Pure Chemical Co. (Osaka, Japan). Two kinds of soybean lecithins, SLP-WHITE and SLP-PC70, were purchased from Tsui Oil Mill Co. Ltd. (Mic, Japan) and were the only sources of lecithin used. The average compositions of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid (PA), as provided by the supplier, are summarized in Table 1. Sulfatase type H-5 (β-glucuronidase/sulfatase) and 2,2′-azobisiso-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma Ltd. (Tokyo, Japan). Manganese dioxide and trolox (6-hydroxy-2,5,7,8-tetramethoxychroman-2-carboxylic acid) were purchased from Kanto Chemical Co. (Tokyo, Japan). Manganese dioxide and trolox (6-hydroxy-2,5,7,8-tetramethoxy-2-carboxylic acid) were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). All other chemicals and reagents were purchased from Wako Pure Chemical Co.

**Preparation of LEC.** Two types of LEC were prepared from SLP-WHITE and SLP-PC70 as follows. Curcumin (2.5 g) was added into deionized water (0.5 L) and then forced dispersed by a homogenizer (TK 100 MPa and at 1 pass for 5 min, unless otherwise indicated. The mixture was vortexed for 3 min, and 4 mL of hexane was added. The mixture was shaken vigorously and centrifuged at 1000 g for 5 min, unless otherwise indicated. To strengthen the replica. The replicate was placed on a 400 mesh copper grid after being washed with water, methanol, and chloroform. It was then examined and photographed using a JEM-1011 (JEOL) transmission electron microscope.

**Measurement of Purified LEC Particle Size.** The particle size of purified LEC was estimated by DLS detector at 24 °C using a FPAR-1000 (Otsuka Electronics Co. Ltd., Osaka, Japan). The light source was a diode-pumped solid-state laser with a wavelength of 658 nm and a scattering angle of 90°. The diffusivity of the liposomal suspension (D) was obtained with the above measurement, and the average LEC diameter (dav) was calculated as follows:

\[
d_{av} = kT/3\pi\eta D
\]

where \( k \) is the Boltzmann constant, \( T \) is the absolute temperature, \( \pi \) is the circular constant, and \( \eta \) is the viscosity.

**Observation of Purified LEC by Freeze-Fracture Electron Microscopy (FFEM).** FFEM was used to determine the structure of purified LEC. The sample was frozen with liquid nitrogen at −189 °C. The fracture process was performed with a JFD-9010 (JEOL, Tokyo, Japan) at −130 °C, and the fractured surface was then replicated by evaporating platinum at an angle of 60°, followed by carbon at an angle of 90° to strengthen the replica. The replicate was placed on a 400 mesh copper grid after being washed with water, methanol, and chloroform. It was then examined and photographed using a JEM-1011 (JEOL) transmission electron microscope.

**Experimental Design for Oral Administration.** Male Sprague–Dawley rats (age, 7 weeks; Japan SLC Inc., Shizuoka, Japan), weighing 230–260 g, were housed in a temperature- (23 ± 1 °C) and light- (12 h light/dark cycle) controlled room. They were allowed ad libitum access to distilled water and commercial feed ( Orienta Yeast Co. Ltd., Tokyo, Japan) for 7 days. Rats were randomly divided into three groups. The first group comprised the control and was given curcumin only (curcumin group). The second and third groups were given a curcumin and lecithin mixture (mixture group) and LEC (LEC group), respectively. The curcumin dose in all samples was fixed at 100 mg curcumin/kg body weight. The mixture group received a lecithin (SLP-PC70) dose that was roughly equivalent to the lecithin component of LEC. The samples were orally administered by direct stomach intubation. Prior to and 30, 60, and 120 min after administration, blood was harvested from the abdominal great vein of rats under light anesthesia (diethyl ether) and placed into heparinized tubes. Plasma was immediately prepared by centrifugation at 1000g for 15 min at 4 °C and stored at −80 °C until use. This study was conducted in accordance with the Guidelines for Animal Experimentation of Kanehige Bio Co. Ltd.

**LC-MS Analysis.** LC-MS analysis was performed by a Mariner mass spectrometer (JNM-400, Jasco, Tokyo, Japan) using a puresil 5 mm ODS column (150 mm × 4.6 mm) kept at 40 °C. The mobile phase was a mixture of acetonitrile/water (48:52, v/v) with 10 mM trifluoroacetic acid, and the flow rate was 1 mL/min. The electro spray ionization mode (positive) was used.

**Enzymatic Hydrolysis of Conjugated Curcumin.** Enzymatic hydrolysis was performed prior to HPLC analysis to detect the conjugated

**Table 1. Composition of Edible Lecithins Used in This Study**

<table>
<thead>
<tr>
<th>type of lecithin</th>
<th>PC</th>
<th>PE</th>
<th>PI</th>
<th>PA</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLP-WHITE (soybean, unhydrogenated)</td>
<td>35.5</td>
<td>29.5</td>
<td>19.0</td>
<td>16.0</td>
<td>100.0</td>
</tr>
<tr>
<td>SLP-PC70 (soybean, unhydrogenated)</td>
<td>80.1</td>
<td>15.4</td>
<td>2.5</td>
<td>2.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>
curcumin. For conjugates with both glucuronide and sulfate, plasma (200 μL) was mixed with 200 μL of sulfatase type H-5 (glucuronidase/sulfatase) solution in 0.1 M sodium acetate buffer (pH 5.0) and incubated at 37 °C for 60 min. This sulfatase type H-5 preparation was found to have substantial β-glucuronidase activity, being equivalent to 100 U of sulfatase and 2000 U of β-glucuronidase.

**Plasma TEAC Assay.** The TEAC assay was performed according to Miller et al. (20). ABTS radical cations were prepared by adding solid manganese dioxide to a 5 mM aqueous stock solution of ABTS. The ABTS radical cations were passed through a Whatman #1 filter paper and a PVDF syringe filter. The concentration was adjusted with 75 mM Na/K phosphate buffer (pH 7.0). A total of 200 μL of ABTS radical cation solution was mixed with 20 μL of diluted plasma in 96-well plates, and the absorbance was read after 75 min. Samples were analyzed in triplicate. A fresh trolox was used as an antioxidant standard; a trolox standard curve was prepared for each batch of plasma analyses, and micromolar trolox equivalents (TEs) were calculated.

**Statistical Analysis.** Results are expressed as means ± standard errors (SEs). Data were analyzed by one-way analysis of variance, and differences among the means of groups were analyzed by the Bonferroni test. Differences were considered significant at \( P < 0.05 \).

**RESULTS AND DISCUSSION**

**Preparation of LEC.** We have previously developed a new mechanochemical method for the large-scale preparation of liposomes from commercially available soybean lecithins (17, 21). LEC was obtained by this method using homogenization and microfluidization under optimal conditions.

**Figure 1.** Visual observation of LEC solutions prepared from two different lecithins by the mechanochemical method. (A) SLP-PC70 and (B) SLP-WHITE. Liposomal solutions, composed of 5 wt % of either lecithin A or B and 2.5 wt % of curcumin, were subjected to microfluidization treatment (100 MPa, 1 pass) after a homogenization treatment. The arrow indicates precipitation.

**Figure 2.** Particle size distribution of purified LEC prepared by the mechanochemical method. The hydrodynamic diameter was determined to be 263 ± 86.0 nm (mean ± SD).

**Figure 3.** Freeze fracture electron micrograph of purified LEC prepared by the mechanochemical method. The liposome solution was composed of 5 wt % SLP-PC70 and 2.5 wt % curcumin treated by ultrapressure homogenization (100 MPa, 1 pass) after homogenization. The scale is 100 nm. The particle size of LEC was approximately 220 nm.

**Plasma Pharmacokinetics of Curcumin Preparations.** On the basis of the previous studies, LEC prepared from SLP-PC70 and curcumin was chosen for the pharmacokinetics studies. **Figure 4** shows the mean plasma curcumin concentration versus time profiles before and after oral administration of curcumin, a mixture of curcumin and lecithin, and LEC, at a dose of 100 mg of curcumin/kg body weight for each treatment group. The peak concentration (\( C_{\text{max}} \)) and time of peak concentration (\( T_{\text{max}} \)) were obtained directly from the individual plasma curcumin concentration versus time profiles. The area under the concentration–time
Figure 4. Concentration of curcumin in rat plasma after a single oral administration of: curcumin, a mixture of curcumin and lecithin, and LEC (100 mg curcumin/kg body weight). Curcumin group (●), mixture group (○), and LEC group (×). The asterisks represent a significant difference at P < 0.01 (vs curcumin group). Values are represented as means ± SEMs (n = 7).

Table 2. Pharmacokinetic Parameters Derived from Rat Plasma Curcumin Levels vs Time Profiles

<table>
<thead>
<tr>
<th>Sample</th>
<th>AUC_0–120min (µg min/mL)</th>
<th>C_max (µg/mL plasma)</th>
<th>T_max (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin group</td>
<td>5342.6</td>
<td>64.6 ± 10.7</td>
<td>120</td>
</tr>
<tr>
<td>Mixture group</td>
<td>7132.6</td>
<td>78.3 ± 17.9</td>
<td>120</td>
</tr>
<tr>
<td>LEC group</td>
<td>26502.8</td>
<td>319.2 ± 70.4</td>
<td>30</td>
</tr>
</tbody>
</table>

* AUC, area under the blood concentration vs time curve; C_max, maximum concentration; and T_max, time to reach C_max.

Table 3. ABTS Radical Cation Scavenging Activity of Rat Plasma before and after Oral Administration of Curcumin, a Mixture of Curcumin and Lecithin, and LEC

<table>
<thead>
<tr>
<th>Sample Plasma</th>
<th>ABTS radical cation scavenging activity (µmol TE/20 µL plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Curcumin group</td>
<td>1.99 ± 0.91</td>
</tr>
<tr>
<td>Mixture group</td>
<td>1.99 ± 0.91</td>
</tr>
<tr>
<td>LEC group</td>
<td>1.99 ± 0.91</td>
</tr>
</tbody>
</table>

* The asterisks represent significant differences at P < 0.05 (vs curcumin group). Values are represented as means ± SDs (n = 5).

Plasma Antioxidant Activity after LEC Administration. We investigated whether plasma antioxidant activity is affected by the enhanced bioavailability of curcumin after LEC administration. The TEAC assay was used to measure plasma antioxidant activity. Table 3 shows ABTS radical cation scavenging activity of plasma before and after oral administration of curcumin, a mixture of curcumin and lecithin, and LEC. Plasma antioxidant activities increased in all treatment groups after oral administration. There was no significant difference in plasma antioxidant activity between the curcumin and the mixture groups, whereas the plasma antioxidant activity of the LEC group was significantly higher than either of the other treatment groups at all time points. In addition, the plasma antioxidant activity and plasma curcumin concentration at 30 min after oral administration were significantly correlated in all groups (Figure 5). It appears that the plasma antioxidant activity was positively correlated to plasma curcumin concentrations. Therefore, it is thought that the curcumin absorption has direct influence on the blood antioxidant.

The goal of our study was to provide evidence of the relationship between the results of our previous study (17), which revealed a substantial improvement in hepatoprotective activity by LUE, and the increased curcumin absorption due to liposome encapsulation. One of the mechanisms involved in mouse liver damage, induced by the CCL_4 model, is considered to be the biotransformation of CCL_4 to the toxic trichloromethyl radical by the cytochrome P450 enzyme system, resulting in lipid peroxidation and liver injury (31–33). In the present study, plasma scavenged the ABTS radical cations in a curcumin concentration-dependent manner. Thus, the curcumin-associated radical scavenging action of plasma may contribute to its hepatoprotective action in CCL_4-induced liver injury. The trichloromethyl radicals or lipid peroxides generated by CCL_4 treatment may have been scavenged by plasma curcumin in the Ukon extract. Accordingly, the encapsulation of curcumin in the Ukon extract might effect a great improvement in hepatoprotective activity.

Future study should attempt to reveal the mechanisms involved in liposome-associated curcumin absorption and subsequent transcytosis and to correlate in vitro observations to this in solubility is enhanced when they are emulsified to increase surface area (28). Thus, the surfactants involved in the formulations could affect the permeability and solubility of drugs across the membrane of the GI tract. Third, by incorporation into liposomes, curcumin can be embedded into the phospholipid bilayer. This reduces its exposure to bacteria as well as enzymatic degradation during the absorption process. Encapsulation also allows for prolonged contact with the intestinal wall due to the adhesive property that liposomes exhibit toward the epithelial mucosal surface of the small intestine (29). Accordingly, it seems that encapsulation of curcumin is highly advantageous for optimizing food functionality. Recent studies have found that curcumin has an antioxidant activity in vitro (30). We evaluated the antioxidant activity of the resulting curcumin-containing plasma in the subsequent experiments.
Figure 5. Correlation between concentration of curcumin and ABTS radical cation scavenging activity in rat plasma 30 min after oral administration of (A) curcumin, (B) a mixture of curcumin and lecithin, and (C) LEC. Statistical analysis was done with linear regression analysis.

vivo performance. These liposomal formulations can enable enhanced curcumin food functionalization.

ABBREVIATIONS USED

ABTS, 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; AUC, area under the concentration–time curve; DLS, dynamic light scattering detector; FFEM, freeze-fracture electron microscopy; GI, gastrointestinal; LEC, liposome-encapsulated curcumin; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SUVs, small unilamellar vesicles; TEAC, trolox equivalent antioxidant capacity.

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LITERATURE CITED


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