Biliary Excretion of Curcumin Is Mediated by Multidrug Resistance-Associated Protein 2

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Received September 29, 2011; accepted January 25, 2012; published online February 10, 2012

Curcumin has a wide spectrum of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. Recently, its potential as effective chemoprevention against cholangiocarcinoma, a highly malignant tumor of the bile duct with limited therapeutic options, was reported. The purpose of the present study was to investigate the contribution of multidrug resistance-associated protein 2 (Mrp2) to the biliary excretion of curcumin using Sprague-Dawley rats (SDR) and Eisai hyperbilirubinemic rats (EHBRR). After intravenous administration of curcumin with a loading dose of 4.5 mg/kg, followed by a constant infusion of 18 mg/kg/h to the SDR and EHBRR, the pharmacokinetic parameters of curcumin were estimated. In EHBRR, the total area under the bile concentration–time curve from 0 to 80 min following curcumin administration was dramatically decreased (0.094%) compared to that in SDR. In addition, the plasma-to-biliary and liver-to-biliary clearances were both significantly decreased compared to SDR. These results provide the first evidence that Mrp2 mediates the biliary excretion of curcumin and thus may be a major factor in the control of exposure of curcumin to the bile duct. This study may be helpful to the potential use of curcumin as a treatment for bile duct cancer, and to understanding the genetic polymorphism of Mrp2 for clinical trials of curcumin.

Key words curcumin; cholangiocarcinoma; Eisai hyperbilirubinemic rat; multidrug resistance-associated protein 2; biliary excretion

Curcumin [(E,E)-1,7-bis(4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3,5-ione], a yellow hydrophobic phenolic pigment derived from the rhizome of the herb Curcuma longa, has been used as a dietary spice and coloring agent in foods. It has a wide spectrum of biological and pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, as well as negligible toxic side effects in rodents and humans (when administered at doses of up to 10 g/d).5

Recently, growing attention has focused on the potential of curcumin as an anticancer agent. Its anticancer property comes from its activity to suppress proliferation of various cancer cells and to down-regulate transcription factors, chemokines, cell surface adhesion molecules, and growth factor receptors, as well as to inhibit the activity of c-Jun N-terminal kinase, protein tyrosine kinases, and protein serine/threonine kinases.4 Specifically, curcumin suppresses proliferation and induces apoptosis in biliary cancer cells, and thus could be developed into effective chemoprevention against cancers of the epithelial cells of the bile ducts, such as cholangiocarcinoma (CCA).6–8 CCA is the second most common primary hepatic tumor and accounts for an estimated 15% of primary liver cancer worldwide.9 Its prevalence is geographically heterogeneous, with the mortality rates being highest in Japan and Chile, followed by East Asia and India.8,9 Several epidemiological studies have shown that the incidence and mortality rates of CCA are increasing worldwide.10–12 Biliary tract tumors remain a challenge to treat and manage due to their poor sensitivity to conventional therapies and discouraging treatment options.13 In fact, radical surgery, the only effective treatment, is applicable in a minority of patients due to the common late clinical presentation and diagnosis of this tumor.14–16

Curcumin is reported to be excreted into bile, which may explain its behavior in CCA. Previous studies have shown that after intraperitoneal administration of 0.6 mg [3H]curcumin to rats, 72.9% of the dosage was excreted in the feces, mostly within the first 24 h.17 Additionally, after intravenous administration of [3H]curcumin, 85.1% of the radioactivity was detected in the bile from cannulated rats after 6 h.17 However, the exact mechanism or transporters involved in the biliary excretion of curcumin remain unknown. Considering its potential to treat bile duct cancer, assessing how much curcumin is exposed to the biliary tract and elucidating the transport mechanism related to the excretion of curcumin via the bile may be clinically meaningful.

Canalicular transport proteins are responsible for the hepatic excretion of drugs and metabolites, and belong to the ATP-binding cassette (ABC) family of transport proteins, which mediate the ATP-dependent transfer of solutes. Multidrug resistance-associated protein 2 (Mrp2, Abcc2) is a major xenobiotic efflux pump on the canalicular membrane, and plays a key role in the biliary excretion of numerous anionic drugs as well as anionic endogenous compounds such as bilirubin. Although there are no data examining whether curcumin is a substrate of Mrp2, curcumin is known to interact with Mrp2 as an inhibitor.18,19 Thus, we hypothesized that Mrp2 could be involved in the hepatobiliary excretion of curcumin.

In the present study, we investigated the contribution of Mrp2 to the biliary exposure of curcumin using Eisai hyperbilirubinemic rats (EHBRR), which have distinct mutations in the Abcc2 gene.20,21

MATERIALS AND METHODS

Materials and Animals Curcumin (from C. longa) was purchased from Sigma Aldrich (St. Louis, MO, U.S.A.). All other chemicals were of analytic grade, and solvents were of
high-performance liquid chromatography (HPLC) grade.

Male Sprague-Dawley rats (SDR; the wild-type form of EHBR; weighing 230–260 g) and EHBR (weighing 210–250 g) were purchased from Orient (Seoul, South Korea) and Central Laboratory Animals, Inc. (Seoul, South Korea), respectively. The rats, each weighing 200–250 g, were maintained at a 25°C and a 12 h/12 h light/dark cycle with free access to water and feed in a housing facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (Animal Center for Pharmaceutical Research, College of Pharmacy, Kyung Hee University, Seoul, South Korea). All experiments were conducted according to the guidelines of the Committee on Care and Use of Laboratory Animals of the Kyung Hee University.

**Pharmacokinetics of Curcumin in EHBR** The jugular vein (for drug administration), carotid artery (for blood sampling), and bile duct of each rat were cannulated with polyethylene tubing (Natsume, Tokyo, Japan) under anesthesia at doses of 25 mg/kg and 25 mg/kg for tiletamine and zolazepam, respectively. Cannulae were filled with heparinized saline (20 IU/mL) to prevent blood clotting.

The vehicles for dissolving curcumin were solutions A (dimethylacetamide:polyethylene glycol 400:5% dextrose=15:45:40) and B (1 N NaOH:0.1 N HCl:distilled water=40:52:8). Curcumin dissolved in solution (A:B=2:1) was intravenously administered to rats at an infusion rate of 18 mg/kg/h over 80 min to SDR (n=3) and EHBR (n=3). Just before the infusion, 4.5 mg/kg of curcumin dissolved in solution (A:B=1:1) was administered as a loading dose. Plasma and bile samples were collected at 0, 20, 40, 60, and 80 min after starting the infusion and stored at −80°C for subsequent use in the HPLC analysis. At the end of the infusion, the brain, liver, kidney, and small intestine of each rat were removed, homogenized in 2× phosphate-buffered saline using a T10 Basic ULTRA-TURRAX® Homogenizer (IKA® Works, Inc., SP, Brazil), and stored at −80°C until analysis.

**HPLC Analysis of Curcumin** Concentrations of curcumin in the plasma, bile, and tissue samples were determined using a slight modification of a reported HPLC method. Briefly, a 200-µL aliquot of acetonitrile was added to a 100-µL aliquot of biological sample for deproteinization. After vortex-centrifugation (12000 rpm, 5 min), 100 µL of the supernatant were injected directly into the HPLC system. A YMC-Pack Pro C18 column (YMC Co., Ltd., Kyoto, Japan; 250×4.6 mm; particle size, 5 μm) was used and the mobile phase, 55% acetonitrile and 45% citric buffer (1% w/v citric acid solution, adjusted to pH 3.0 using concentrated sodium hydroxide solution) was run at a flow rate of 1.0 mL/min. The column effluent was monitored with a UV detector at 428 nm.

The intra-day precision and accuracy of the replicate assays were tested using 3 different concentrations of the drug solutions, and the inter-day precision and accuracy were determined with 3 independent experimental assays of the aforementioned replicates.

**Data Analysis** Pharmacokinetic parameters were calculated using the non-compartmental pharmacokinetic analysis method, where the total area under the plasma concentration–time curve from time 0 to 80 min (AUC0–80 min) and the total area under the bile concentration–time curve from time 0 to 80 min (AUC0–80 min, bile) were determined. The total body clearance (CL) was calculated by dividing the infusion rate by steady-state plasma concentration. The plasma-to-bile and liver to bile clearances were calculated by dividing biliary excretion rate of unchanged curcumin by steady-state plasma concentration and liver concentration at 80 min, respectively. The tissue-to-plasma concentration ratio (Kt) was determined for the brain, liver, kidney, and small intestine. A p value of less than 0.05 was considered statistically significant using an unpaired Student’s t-test. All data are expressed as mean±standard deviation (S.D.).

**RESULTS AND DISCUSSION**

No impurities or interfering peaks from endogenous substances were observed at any elution time for curcumin. The HPLC method was linear (r2>0.99) over the curcumin concentration ranges of 0.1–10 µg/mL for plasma and tissue samples and 1–50 µg/mL for bile samples when evaluated by the least-squares linear regression. The accuracy (measured by relative % error) and intra-day precision (evaluated as the relative standard deviation of the mean expressed as a percent; coefficient of variation) of this method indicated that all relative errors and coefficients of variation at each concentration level were below 15%.

The mean arterial plasma concentration–time profiles and bile concentration–time profiles of curcumin after intravenous infusion to SDR and EHBR are shown in Figs. 1A and B, respectively, and the relevant pharmacokinetic parameters are

![Figure 1](image-url)
9.40% of that in SDR (rats.26) In rats, the predominant clearance pathway for probenecid is via metabolism, and biliary elimination of the parent molecule accounts for only <10% of the total clearance.27 Therefore, the metabolism of curcumin may mainly affect the elimination route and the parent drug was a substrate of oxido-reductases,28) and thus, the increase of renal Mrp3 that is expressed in the basolateral membrane of the proximal tubule cells was 245% (p=0.001) higher in EHBR compared to that in SDR.29) In addition, the reduced biliary pravastatin excretion in Mrp2-deficient TR rats was partly compensated by increased urinary excretion of pravastatin.30 However, this appears to be unlikely because the percentage of the dose of curcumin excreted during 80 min via urine was negligible (data not shown) and there is a possibility that the renal excretion of curcumin via renal Mrp2 could decrease in EHBR. The higher Kp value of curcumin in the kidney (Table 2) complicates interpretation further. More studies for the change in the renal excretion of curcumin in EHBR would be necessary.

In EHBR, despite the dramatically lower biliary excretion of curcumin, the Kp value of curcumin in the liver was comparable with that of SDR. According to the “Well-stirred model,” the Kp value in the liver is affected by the sum of the metabolic and biliary clearances. When metabolic clearance is larger than biliary clearance, the Kp value in the liver would be constant even if biliary clearance is decreased. As mentioned above, the metabolic clearance of curcumin appears to be considerably greater than its biliary clearance. Interestingly, the bile flow rate was slower in EHBR than in SDR (Table 1). This phenomenon is also reported in many EHBR studies.26,30 Whether the biliary clearance of curcumin is bile flow-dependent or -independent is unclear, but this would not impact the conclusions of the present study because the decrease in the liver-to-bile clearance of curcumin is

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SDR</th>
<th>EHBR</th>
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<tbody>
<tr>
<td>$AUC_{0-80\text{min}}$ ($\mu$g min/mL)</td>
<td>72.7±18.1</td>
<td>93.2±15.5</td>
</tr>
<tr>
<td>Total body clearance (mL/min/kg)</td>
<td>342.7±99</td>
<td>264±43</td>
</tr>
<tr>
<td>$AUC_{0-80\text{min}}, bile$ ($\mu$g min/mL)</td>
<td>368.55±3</td>
<td>34.5±20.3***</td>
</tr>
<tr>
<td>Mean bile flow rate ($\mu$L/min)</td>
<td>17.7±5.2</td>
<td>7.43±1.63***</td>
</tr>
<tr>
<td>Liver concentration ($\mu$g/mL)</td>
<td>0.154±0.042</td>
<td>0.111±0.027</td>
</tr>
<tr>
<td>Biliary excretion rate ($\mu$g/min/kg)</td>
<td>0.314±0.083</td>
<td>0.0138±0.0089**</td>
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</tbody>
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Data are shown as mean±S.D. *p<0.05 vs. SDR (unpaired Student’s t-test). **p=0.001 and ***p<0.001 vs. SDR (unpaired Student’s t-test).
much greater than the decrease in the bile flow rate (91.3% vs. 58.0% decrease) in EHB.

In conclusion, the bile exposure, and the plasma-to-bile and liver-to-bile clearances of curcumin were significantly reduced in EHB than in SDR due to decreased function of Mrp2. Our results provide the first evidence that Mrp2 mediates the biliary excretion of curcumin. As rat Mrp2 and human MRp2 are orthologs, and their amino acid sequence identity is 78%, this finding is meaningful for understanding the mechanism of biliary excretion of curcumin in humans as well as for using curcumin effectively as a treatment for bile duct cancer. Furthermore, determining the genetic polymorphism of Mrp2 may be helpful for clinical trials of curcumin.

Acknowledgements
This work was supported by a Grant from Kyung Hee University in 2011 (KHU-20110094) and National Research Foundation of Korea (NRF) Grant provided by the Korean government (MEST) (No. 2009-0092562).

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