The increased cellular uptake and biliary excretion of curcumin by quercetin: a possible role of albumin binding interaction

Han Gyul Kim, Joo Hyun Lee, Seung Jae Lee, Ju-Hee Oh, Eunji Shin, Young Pyo Jang, and Young-Joo Lee

Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul, South Korea (H.G.K., J.O., Y.P.J., Y.L.), College of Pharmacy, Kyung Hee University, Seoul, South Korea (J.H.L., S.J.L., E.S.)
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Corresponding author:

Prof. Young-Joo Lee.
Division of Biopharmaceutics, College of Pharmacy, Kyung Hee University
1 Hoegi-dong Dongdaemun, Seoul, 130-701, South Korea
Tel 82-2-961-9256/Fax 82-2-966-3885
e-mail: yj_lee@khu.ac.kr

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List of nonstandard abbreviations:

AUC, Area under the plasma concentration time curve; Vd<sub>ss</sub>, Volume of distribution at steady-state; RSA, rat serum albumin; HPLC, high-performance liquid chromatography; CSS, steady-state concentration of curcumin in plasma
ABSTRACT

Curcumin and quercetin are natural compounds with a wide spectrum of activities, including antioxidant and anticancer activities. In this study, the combined effect of the 2 compounds was investigated, with special emphasis on the pharmacokinetics of curcumin by the quercetin-induced changes in the albumin-binding of curcumin. We evaluated the effect of quercetin on the binding of curcumin to albumin and on the uptake of curcumin into the cells of the human colon carcinoma cell line WiDr. Additionally, we also investigated the changes in the in vivo pharmacokinetics of curcumin and curcumin sulfate, major metabolite of curcumin by co-administered with quercetin. We found that quercetin inhibited the binding of curcumin to albumin and increased the uptake of curcumin into WiDr cells, the human colon carcinoma cell. The quercetin-induced increased uptake (1.6-fold) of curcumin into WiDr cells was also confirmed by an ex vivo study. The in vivo pharmacokinetics of curcumin showed obvious changes when it was co-administered with quercetin, with the significantly lower plasma concentration and greater biliary excretion of curcumin and curcumin sulfate. The present study suggests that quercetin could enhance the cellular uptake of curcumin and modulate in vivo pharmacokinetics of curcumin and it could be related with albumin binding interaction.
INTRODUCTION

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, is commonly used as a dietary spice and flavoring and coloring agent in food. It has a wide spectrum of biological and pharmacological activities, including antioxidant, antiinflammatory, antimicrobial, and anticancer activities (Mahady et al., 2002; Sharma et al., 2005; Aggarwal et al., 2007). In addition, curcumin is effective in the treatment and prevention of various diseases, especially colorectal cancer (Hsu and Cheng, 2007; Shehzad et al., 2010; Watson et al., 2010).

Serum albumin is a plasma protein affecting the transport, distribution, and metabolism of many exogenous ligands (Barik et al., 2003). Curcumin has high affinity for binding with albumin via both hydrophilic and hydrophobic interactions, and this binding mainly occurs in the subdomain IIA (site I), where tryptophan 214 is located (Pulla Reddy et al., 1999; Mandeville et al., 2009).

Quercetin is a flavonoid antioxidant found in a wide variety of plant products (Boots et al., 2008). It is one of the most potent bioflavonoids studied and possesses diverse pharmacological activities, including antiinflammatory, antineoplastic, cardioprotective, and anticancer activities (Wang, 2000). Because curcumin and quercetin have similar pharmacological activities, their combination has been tested for use as a therapeutic agent or health supplement (Cruz-Correa et al., 2006).

Interestingly, quercetin also has a high affinity for serum albumins, and quercetin binds to albumin at the same site as curcumin, i.e., the IIA domain (Boulton et al., 1998). Because changes in the free drug concentration due to displacement of protein binding may affect drug efficacy in cases of intravenously administered high extraction ratio drugs (Rolan, 1994; Benet and Hoener, 2002), the objective of this study was to study the effect of quercetin on the pharmacokinetics of curcumin; to this end, we examined the effects of quercetin on the transport of curcumin in \textit{in vitro}, \textit{ex vivo}, and \textit{in vivo} studies. We evaluated the effect of quercetin on the binding of curcumin to albumin and on the uptake of curcumin into the cells of the human colon carcinoma cell line WiDr (Noguchi et al., 1979; Cunderlikova et al., 2005). Additionally, we also investigated the changes in the \textit{in vivo} pharmacokinetics of curcumin including biliary excretion when the latter was co-administered with quercetin.
MATERIALS AND METHODS

Materials. Curcumin and quercetin dihydrate were purchased from Sigma Aldrich (St. Louis, MO). Dulbecco’s modified Eagle’s medium, trypsin-ethylenediaminetetraacetic acid, penicillin-streptomycin, and fetal bovine serum were purchased from Cellgro (Mediatech, Inc., Manassas, VA). Curcumin sulfate was synthesized as described (Ireson et al., 2001) and identified using an AccuTOF-TLC single-reflection time-of-flight mass spectrometer (JEOL Ltd., Tokyo, Japan) equipped with a direct analysis in real time ion source (IonSense, Saugus, MA). All chemicals and biochemicals used in this study were of analytical grade, and the solvents used were of high-performance liquid chromatography (HPLC) grade.

Animals. Adult Sprague-Dawley male rats (weight: 270 ± 30 g) were purchased from Taconic Farms Inc. (Samtako Bio Korea, O-San, Korea). All rats were housed in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Kyung Hee University, Seoul, South Korea) maintained at a temperature between 20°C and 23°C, relative humidity of 50% ± 5%, and a 12-h light and dark cycle. The protocol of this study was approved by the Animal Care and Use Committee of College of Pharmacy of Kyung Hee University.

Albumin-binding affinity of curcumin. To measure the binding affinity of curcumin to rat albumin, we used the spectroscopy method with rat serum albumin (RSA) by performing a slightly modified version of a previously described procedure (Pulla Reddy et al., 1999). A stock solution (500 μg/mL) of curcumin was prepared in absolute ethyl alcohol. Curcumin solutions with or without quercetin were mixed with 5.745 μg/mL RSA, and the fluorescence intensities of these solutions were measured using a spectrofluorometer (FP-750; Jasco Corporation, Tokyo, Japan) at an excitation wavelength of 280 nm and an emission wavelength of 330 nm. The degree of binding between curcumin and albumin was calculated as the percentage of fluorescence quenching in the curcumin-containing solutions to that in the RSA solutions not containing curcumin.

In vitro cellular uptake study. We quantified the uptake of curcumin into WiDr cells by using a previously reported method (Zhang et al., 2007). WiDr cells were seeded onto 12-well plates at a concentration of 10^5 cells/cm^2 and cultured overnight. The culture medium was then removed, and each well was washed twice with 2 mL of phosphate-buffered saline at 37°C; then, 1 mL of complete medium containing curcumin (15 μM with 0.1% DMSO) and 10% fetal bovine serum were added to each well, and
batches of the cells were incubated for 10, 20, or 30 min. Quercetin was added to evaluate whether the curcumin uptake by WiDr cells was increased via quercetin-mediated inhibition of the binding between curcumin and albumin. At the end of the incubation period, the medium was removed by suction, and the wells were washed twice with 2 mL of ice-cold phosphate-buffered saline (4°C). The cells were subsequently lysed in 1% Triton X-100. The concentration of curcumin in the cells was determined by HPLC.

**Ex vivo study of the effect of quercetin on the uptake of curcumin into WiDr cells.** The plasma samples collected from the quercetin-treated rats were used in an *ex vivo* study. Plasma containing quercetin and its metabolites was collected 80 min after the intravenous infusion of quercetin (16.5 mg/kg/h) was started. The plasma samples obtained from the vehicle-treated rats were used as the control. The plasma (1 ml) was pretreated with acetonitrile (2 ml) to remove proteins, evaporated, and reconstituted in 1 ml of the complete medium; then, curcumin was added to obtain a concentration of 15 μM. The procedure employed in the uptake experiment was the same as that employed in the experiment on cellular uptake.

**In vivo pharmacokinetic study.** Under anesthesia, the left femoral vein (for drug administration) and artery (for blood sampling) and the bile duct of each rat were cannulated using a polyethylene tube (Natsume, Tokyo, Japan) (Oh et al., 2009). Curcumin dissolved in dimethylacetamide (15%), polyethylene glycol 400 (PEG400; 45%), and 5% dextrose (40%) was administered to the rats as an intravenous infusion at the rate of 18 mg/kg/h was administered intravenously just before the infusion was started (Ma et al., 2007). Quercetin was coin infused intravenously at the rate of 16.5 mg/kg/h for quercetin-treated rats immediately after intravenous administration of quercetin at a dose of 56 mg/kg. Plasma and bile samples were collected at 0, 20, 40, and 80 min after starting the infusion and stored in a freezer (-80°C) for subsequent use in HPLC analysis.

**Analysis of curcumin and quercetin.** The HPLC method was used to determine the curcumin concentration in the plasma and bile (Ma et al., 2007) and the quercetin concentration in the plasma (Morand et al., 1998). Quantification of curcumin sulfate by HPLC based on previous report by Ireson *et al.* (Ireson et al., 2001). The intra-day precision and accuracy of the replicate assay were tested by using 3 different concentrations of the drug solutions, and the inter-day precision and accuracy were determined.
for 3 independent experimental assays of the aforementioned replicates.

**Data analysis.** Pharmacokinetic parameters were calculated using the non-compartmental pharmacokinetic analysis method (Gibaldi and Perrier, 1982). Biliary clearance was calculated by dividing the excreted amount of curcumin into bile by AUC for 80 minutes. A $P$ value of <0.05 was considered to be statistically significant using a unpaired t-test. Data were expressed in terms of mean ± standard deviation values.

**RESULTS AND DISCUSSION**

The analysis of plasma concentration of curcumin and quercetin was validated using the standard procedure (Wilson, 1990). The calibration curves of curcumin and quercetin showed good linearity over the tested concentrations. The precision and accuracy of the tests were within the standard range.

Figure 1A represents the quercetin-mediated inhibition of the albumin-binding of curcumin. The IC$_{50}$ of quercetin required to inhibit the albumin-binding of curcumin was approximately 1 μM. Interestingly, almost similar results were obtained in the curcumin-uptake study (Fig. 1B): quercetin treatment resulted in a dose-dependent increase in curcumin uptake by WiDr cells, and the EC$_{50}$ of quercetin in this case was approximately 0.3 μM. Thus, the dose-dependent effect of quercetin on the albumin-binding was similar to that on the curcumin uptake into WiDr cells. It is common knowledge that drugs binding with albumin cannot permeate the biological membrane, and the quercetin-mediated inhibition of curcumin–albumin binding is likely to result in an increased uptake of curcumin. Curcumin has a high affinity for binding with albumin, and the binding site for curcumin is the same as that for quercetin (subdomain IIA). Thus, quercetin can effectively block the binding of albumin with curcumin, resulting in an increase in the fraction of unbound curcumin in the medium and thereby increasing the uptake of curcumin by WiDr cells. Our findings are also supported by those of recent *in vitro* studies, which showed that quercetin and warfarin compete for albumin binding (Di Bari et al., 2010). The possibility that the increase in the curcumin uptake was caused by quercetin-induced transporter inhibition (Anuchapreeda et al., 2002; Zhou et al., 2004; Li and Choi, 2009) can be excluded by considering the findings of studies on the effect of probenecid and cyclosporine A—multi-specific active drug transport inhibitors (Shitara et al., 2005); these
chemical inhibitors did not lead to any increase in the uptake of curcumin into WiDr cells.

Additionally, the quercetin-induced increase in the uptake of curcumin into WiDr cells was confirmed in the *ex vivo* study. Constituents of the plasma obtained from the quercetin-treated rats increased the uptake of curcumin into WiDr cells; the curcumin uptake in the media containing constituents of plasma obtained from the quercetin-treated rats was significantly higher than that in the control media (1.6-fold at 20 min, Fig. 2). This finding suggests that the co-administration of curcumin and quercetin may enhance the *in vivo* delivery of curcumin into tumor cells. Since albumin is present in tumor tissue (Luo et al., 2005), the availability of unbound curcumin to tumor cells would enhance the overall pharmacological effect of curcumin.

To evaluate the effect of quercetin on the *in vivo* pharmacokinetics of curcumin, we assessed the pharmacokinetic parameters at a point when the concentration of curcumin reached a steady state during continuous infusion of curcumin. Rats were administered an intravenous infusion of curcumin (18 mg/kg/h), with or without co-infusion of quercetin (16.5 mg/kg/h), and the changes in their mean arterial plasma concentration were determined (Fig. 3A); the relevant pharmacokinetic parameters assessed are listed in Table 1. The plasma concentration of curcumin was significantly lower and the total curcumin clearance in the quercetin-treated group was significantly higher than the corresponding values in the curcumin-treated group (*P* < 0.05, Fig. 3A, Table 1). However, the biliary excreted amount of curcumin was considerably high in the quercetin-treated group (3.0-fold, *P* < 0.001, Fig. 3B). Because curcumin suppresses proliferation and induces apoptosis in biliary cancer cells, and thus could be developed into effective chemoprevention against for cancers such as cholangiocarcinoma (Prakash et al., 2009; Suphim et al., 2010; Prakobwong et al., 2011), this finding may be meaningful for using curcumin effectively as a treatment for bile duct cancer.

Since albumin is a ubiquitous protein synthesized by hepatocytes, we can infer that quercetin can effectively inhibit the albumin-binding of curcumin in the plasma and liver; this, in turn, would make available a large amount of unbound curcumin in the plasma and liver tissue, thereby resulting in an increased transport of curcumin through the sinusoidal and canalicular membranes of the liver. Although the plasma concentration of quercetin in the rats was low (6.56 μM) compared to albumin concentration, the total concentration of quercetin and its metabolites may be much higher, considering extensive
metabolic cascade of quercetin in the liver (Jones et al., 2004). Because metabolites of quercetin also bind with albumin (Janisch et al., 2004), quercetin and its metabolites may contribute simultaneously to block curcumin–albumin binding.

Even if changes in albumin binding have an important influence on clinical effects or toxicity in limited cases, it should be taken into account when high extraction ratio drugs are administered intravenously like as in this study (Sansom and Evans, 1995; Benet and Hoener, 2002; Gambacorti-Passerini et al., 2003; Smith et al., 2010). Our results clearly showed that metabolite formation increased by quercetin; AUC of curcumin sulfate was decreased by 2.5-fold and its biliary excretion was increased by 1.7-fold in the quercetin-treated group compared to control (Fig. 3B).

All these results indicated that quercetin could modify the pharmacokinetics of curcumin in vivo and it may be related with the interaction of quercetin with the albumin-binding of curcumin. On the basis of this finding, we consider that the combined use of quercetin and curcumin enhances the pharmacological effects of curcumin and that this enhancement results from the physical interaction of quercetin with plasma and tissue albumin. Further studies, including those on the anticancer effect of the curcumin and quercetin combination in tumor-bearing mice, are currently underway in our laboratory.

In conclusion, the present study suggests that quercetin could enhance the in vitro cellular uptake of curcumin and in vivo biliary exposure of curcumin and it could be related with albumin binding interaction. The combined use of curcumin and quercetin could be used to enhance the pharmacological effect of curcumin by increasing its biliary exposure to biliary cancer cells.
Authorship Contributions

Participated in research design: Kim, Jang, and Y-J. Lee.


Contributed new reagents or analytic tools: S.J. Lee and Y. P. Jang.

Performed data analysis: Kim, J.H. Lee, and Y-J. Lee.

Wrote or contributed to the writing of the manuscript: Kim, J.H. Lee, Oh and Y-J. Lee.
References


sensitivity of chronic lymphocytic leukemia cells to ABT-737 and ABT-263 due


Footnotes

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FIGURE CAPTIONS

**Fig. 1.** The inhibition of the albumin-binding of curcumin by quercetin (A) and the effect of quercetin on the uptake of curcumin into WiDr cells. The IC$_{50}$ of quercetin required to inhibit the albumin-binding of curcumin and the EC$_{50}$ required to increase the uptake of curcumin into WiDr cells were 1 μM and 0.3 μM, respectively. Bars represent SD.

**Fig. 2.** *Ex vivo* effect of constituents of plasma obtained from the quercetin-treated rats on the uptake of curcumin into WiDr cells. As a control, constituents of plasma obtained from vehicle-treated rats were used. Bars represent SD (*p < 0.05 and ***p < 0.001 vs. control).

**Fig. 3.** Time profiles for plasma concentration (A) and biliary excretion (B) of curcumin and curcumin sulfate during continuous intravenous infusion with (●:curcumin, ▼:curcumin sulfate) or without (○:curcumin, ▽:curcumin sulfate) quercetin. Bars represent SD (*p < 0.05, **p < 0.01, and ***p < 0.001 vs. control).
Table 1. Pharmacokinetic parameters of curcumin after intravenous infusion of curcumin (18 mg/kg/h) with or without co-infusion of quercetin (16.5 mg/kg/h) in rats. Data are shown as mean ± SD (*p < 0.05 and ***p < 0.001 vs. control).

<table>
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<tr>
<th></th>
<th>Curcumin</th>
<th>Curcumin + Quercetin</th>
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<tbody>
<tr>
<td></td>
<td>(n = 13)</td>
<td>(n = 8)</td>
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<tr>
<td>AUC (µg/mL·min)</td>
<td>300 ± 68.0</td>
<td>240 ± 43.7</td>
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<tr>
<td>Total body clearance (mL/min·kg)</td>
<td>93.1 ± 19.2</td>
<td>114 ± 20.2*</td>
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<tr>
<td>Vdss (L/kg)</td>
<td>7.44 ± 1.53</td>
<td>9.14 ± 1.62*</td>
</tr>
<tr>
<td>CLp-b (mL/min/kg)</td>
<td>0.584 ± 0.199</td>
<td>2.29 ± 0.776***</td>
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AUC: Area under the plasma concentration time curve  
Vdss: Volume of distribution at steady-state  
CLp-b: Plasma-to-bile clearance
Fig 2

The diagram shows the uptake of curcumin into the WtDr in plasma of control rats and plasma of quercetin-treated rats at different time points: 10 min, 20 min, and 30 min. The bars indicate the percentage uptake with error bars. The comparison shows a significant increase in uptake in the quercetin-treated group at 20 min compared to control, indicated by the *** symbol. Additionally, there is a significant increase at 30 min, indicated by the * symbol.