International Journal of Pharmaceutics 358 (2008) 224–229

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Curcuma drugs and curcumin regulate the expression and function of P-gp in Caco-2 cells in completely opposite ways

Xiao-Long Hou[a,1], Kyoko Takahashi[b], Ken Tanaka[c], Katsuhiko Tougou[a], Feng Qiu[d], Katsuko Komatsu[c], Koichi Takahashi[e], Junichi Azuma[a,*]

[a] *Department of Clinical Pharmacology and Pharmacogenomics, Graduate School of Pharmaceutical Science, Osaka University, 1-6 Yamadaoka, Suita City, Osaka, Japan*
[b] *Department of Medicinal Resources, Graduate School of Pharmaceutical Science, Osaka University, 1-6 Yamadaoka, Suita City, Osaka, Japan*
[c] *Division of Pharmacognosy, Department of Medicinal Resources, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama, Japan*
[d] *Department of Natural Product Chemistry, Shenyang Pharmaceutical University, 103 Road Wenhua, Shenyang, China*
[e] *Department of Pharmaceutics, School of Pharmaceutical Sciences, Mukogawa Women's University, 9-11-68 koushien, Hyogo, Japan*

ARTICLE INFO

Article history:
Received 29 October 2007
Received in revised form 11 January 2008
Accepted 12 March 2008
Available online 18 March 2008

Keywords:
Curcuma
Curcumin
P-gp

ABSTRACT

Curcumin is a phenolic compound isolated from rhizomes of *C. longa*, *C. aromatica* and other *Curcumas* except *C. zedoaria*. Recently, both curcumin and *Curcumas* have become prevalent as supplement. P-gp has been reported as an important determinant for drug absorption in small intestine. In this study, Caco-2 cell monolayers were treated with methanol extracts of *Curcumas* (0.1 mg/ml) or curcumin (30 μM) for 72 h to investigate the relationship between the potential affects of *Curcumas* and curcumin on P-gp. [^{3}H]-digoxin and rhodamine 123 were used to evaluate P-gp activity. All *Curcumas* significantly increased the activity of P-gp by up-regulating the expressions of P-gp protein and *MDR1* mRNA levels. Interestingly, contrary to *Curcumas*, curcumin treatment inhibited the activity of P-gp with a decrease in P-gp protein and *MDR1* mRNA expression levels. *Curcumas* might alter the pharmacokinetics of co-administrated drugs by up-regulating the function and expression levels of intestinal P-gp. However, curcumin has no relationship with the inductive effect of *Curcumas* since curcumin showed an opposite effects. Caution should be exercised when *Curcumas* or curcumin are to be consumed with drugs that are P-gp substrates because *Curcumas* and curcumin might regulate the function of P-gp in completely opposite ways.

1. Introduction

In East Asia, *Curcuma* species (*C. longa*, *C. aromatica, C. zedoaria*) have been used as traditional medicines because of their various pharmacological activities including enhancing wound healing, promoting digestion, anti-cancer, anti-oxidant, hepatoprotective etc. (Araujo and Leon, 2001; Matsuda et al., 1998). Recent pharmacological studies have demonstrated that the rhizomes of *C. longa* expressed antiallergic (Ram et al., 2003) and antidepressant activities (Yu et al., 2002). Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the major yellow pigment from rhizomes of *C. longa*, *C. aromatica* and other *Curcumas* except *C. zedoaria*. Curcumin possesses wide range of pharmacological activities (Maheshwari et al., 2006). Especially, its effectivity as cancer chemopreventive agent has been affirmed by in vitro experiments and clinical trials (Thangapazham et al., 2006; Garcea et al., 2005). However, although both *Curcuma* drugs and curcumin have become prevalent as supplements in the world, little is known about the correct application of them because it is always thought that *Curcuma* drugs and curcumin share the same effects since curcumin is the main component from *Curcuma* drugs.

As herbal medicinal products have become prevalent throughout the world, the fatalness of herb–drug interactions stands out (Mills et al., 2005; Fugh-Berman and Ernst, 2001). Clinical trials have showed that many herbal supplements or over-the-counter remedies may modulate the activity of drug metabolism enzymes and/or drug transporters and further influence the bioavailability of co-administrated drugs (Gurley et al., 2004; Saruwatari et al., 2003; Anderson et al., 2003). In another of our report, we have indicated that *C. longa* and *C. zedoaria* might inhibit the activity and protein expression of cytochrome P450 3A4, which is the major phase I metabolizing enzyme and responsible for the metabolism of about 60% of drugs in current clinical use (Hou et al., 2007). On the other hand, Naganuma et al. (2006) reported that *C. longa* and curcumin inhibited the function of both sulfotransferase (SULT) and UDP-glucuronosyl transferase (UGT) activity in Caco-2 cells.

P-glycoprotein (P-gp) is a drug transporter belonging to the ATP-binding cassette transporter family. In humans, it is the product of

* Corresponding author. Tel.: +81 6 6879 8163; fax: +81 6 6879 8253.
E-mail address: Azuma@phs.osaka-u.ac.jp (J. Azuma).
[1] Present address: Department of Pharmaceutics, School of Pharmaceutical Science, Mukogawa Women's University, Hyogo, Japan.

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.03.010

multi-drug resistance gene *MDR1*. P-gp is expressed in the apical membrane of many pharmacologically important epithelial barriers, including the intestinal epithelium, hepatocytes and renal tubular cells. In intestinal epithelial cells, P-gp is expressed on the apical surface and excretes drugs and xenobiotic compounds to the apical side. Thus P-gp plays an important role in regulating the intestinal absorption of xenobiotic compounds (Chan et al., 2004). The inhibition/induction of intestinal P-gp has been reported as a significant reason for herb–drug interaction (Jodoin et al., 2002; Perloff et al., 2001; Han et al., 2006).

Caco-2 cell model is a well-established in vitro model to study drug absorption and related mechanism in small intestine (Hilgers et al., 1990; Neuhaus et al., 2006). Since Caco-2 cell expresses P-gp as much as normal intestinal epithelial cells do, it has become a well-used model to evaluate the affect of drugs on intestinal P-gp. In this study, Caco-2 cells were utilized to detect the *Curcuma*–drug interaction mediated by intestinal P-gp. The major goals of this study are as following: (1) To show some information for the correct application of *Curcuma* drugs. (2) To investigate the relationship between the application of curcumin and *Curcuma* drugs.

2. Materials and methods

2.1. Materials and chemicals

Caco-2 cells (HTB37) were obtained at passage 18 from American Type Culture Collection (Manassas, VA). *C. longa* (Okinawa, Japan), *C. zedoaria* (Okinawa, Japan) and *C. aromatica* (Okinawa, Japan) used in this study were correctly identified by the molecular biological method previously reported (Cao et al., 2001). All drugs are stored in the Museum of Material Medica, Institute of Natural Medicine, Toyama University, Japan. Dulbelcco's modified Eagle's medium (DMEM), non-essential amino acid (NEAA), antibiotic–antimycotic mixed stock solution, glucose, ethlenediaminertetracetic acid (EDTA), trypsin, rhodamine 123, liquid scintillation cocktail were obtained from Nacalai Tesque (Kyoto, Japan). Fetal bovine serum (FBS) was from ICN Biomedicals, Inc. (Aurora, Ohio). [^{3}H]-digoxin (specific radio activity of 21.8 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA, USA). Verapamil was from Sigma Chemical Co. (St. Louis, MO, USA). Transwell polycarbonate cell culture inserts (24 mm diameter, 0.4 μm pore size) were from Costar Corp. (Bedford, MA, USA). Millicell ERS device was obtained from Millipore (Bedford, MA, USA). Stock solution of *Curcuma* extracts and curcumin were prepared at 50 mg/ml and 100 mM in dimethyl sulfoxide (DMSO) and stored in −20 °C.

2.2. Cell culture condition

Caco-2 cells at passage 35–44 were used for all experiments. Cell cultures were maintained in a humidified 37 °C incubator with a 5% carbon dioxide in air atmosphere. Caco-2 cells were grown in plastic tissue culture dishes in a medium consisting of DMEM containing 25 mM glucose, 4 mM L-glutamine, 0.1 mM nonessential amino acids, 100 units/ml penicillin, 100 units/ml streptomycin, 250 nM amphotericin and supplemented with 10% heat-inactivated FBS. When the cells reached 80% confluence, they were removed using 0.2% trypsin/EDTA, diluted with 1:4 and reseeded onto fresh tissue culture dishes. Medium was changed at 2–3 days.

2.3. Preparation of Curcuma extracts

The methanol extracts of *Curcuma* rhizomes were prepared and analyzed as previously described (Sasaki et al., 2003). In briefly, powdered drugs were dipped in methanol (1 l × 2) for 12 h at room temperature, the combined supernatants were evaporated to obtain the methanol extracts. The methanol extracts were freeze-dried into resultant powders. Samples were analyzed using a HPLC apparatus consisting of pumps (Shimadzu LC-10AT, Japan), a degasser (Shimadzu DGU-12A, Japan) an autosampler (Shimadzu SIL-10A, Japan), and a UV–vis detector (Shimadzu SPD-10A, Japan). The reversed-phase separation was performed in Waters Symmetry C_{18} column (4.6 mm × 150 mm, 5 μm particles). The mobile phase was composed of 0.25% acetic acid (A) and acetonitrile (B). The gradient was as follows: 0 min: 60% A, 40% B; 15 min, 40% A, 60% B; 35 min, 0% A, 100% B; 40 min, 60% A, 40% B. The elution was performed at a rate of 1 ml/min. Curcumin was detected at 410 nm and identified according to the retention time. A methanol stock solution of curcumin was prepared at 100 μM. Concentrations were obtained by extrapolation of peak area from a standard curve.

2.4. Rhodamine 123 efflux and accumulation

Caco-2 cells were seeded at 5×10^5 cells/cm^2 onto Transwell insert. *Curcuma* drugs (0.1 mg/ml) or curcumin (30 μM) were supplied to the apical compartment on day 18. Control groups were treated with 0.2% DMSO. After 72 h, *Curcuma* drugs/curcumin were removed and transport experiments were conducted on monolayers with transepithelial electrical resistance (TEER) of 800–1000 Ω cm^2. Monolayer TEER was measured before and after transport experiments.

To start transport experiment, Transwell inserts were washed with warmed PBS twice, and equilibrated with transport medium (HBSS buffer with 10 mM HEPES and adjusted to pH 7.4 with 1 N NaOH) for 30 min. Transport buffer (2.6 ml) with 5 μM rhodamine 123 was then added to the basolateral compartment and transport buffer (1.5 ml) was added to the apical compartment. Transwell plate was incubated at 37 °C. At 15, 30, 60, 90 min, 50 μl aliquots were withdrawn from the apical compartment for analysis. Positive control groups were pretreated with verapamil (100 μM) for 30 min and during the experiments. Then the cells were thoroughly washed three times with ice-cold PBS. Cells were then solubilized with 1% triton X for analysis of rhodamine 123 fluorescence and P-gp total protein.

2.5. Bi-directional [^{3}H]-digoxin transport study

Experimental protocol for bi-directional [^{3}H]-digoxin transport was similar to that for the rhodamine 123 efflux except that [^{3}H]-digoxin was added to the apical (for apical to basolateral transport: A–B) or basolateral (for basolateral to apical transport: B–A) compartments. At 15, 30, 60, 90 min, 50 μl aliquots were removed from the receiver compartments and mixed with 3 ml of scintillation cocktail and analyzed in a liquid scintillation counter. Apparent permeability coefficient (P_{app}) was calculated as $P_{app} = (dQ/dt)/(AC_0)$ (cm/s) where dQ/dt (nmol/s) was the flux rate, A (cm^2) was the effective surface area of the cell monolayer, and C_0 (nmol/ml) was the initial drug concentration in the donor chamber. Net efflux was expressed as the quotient of P_{app} (B–A) to P_{app} (A–B).

2.6. Rhodamine 123 fluorometric analysis

Rhodamine 123 was quantified with fluorometric analysis, 485 nm (exitation) and 530 nm (emission), using a PerkinElmer LS50B luminescence spectrophotometer. Rhodamine 123 in samples was determined based on calibration curves constructed from a series of standards.