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Improvement of intestinal absorption of curcumin by cyclodextrins and the mechanisms underlying absorption enhancement

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ABSTRACT

Curcumin is known to possess a wide range of pharmacological activities for the treatment of chronic or inflammatory diseases, Alzheimer's disease, and various cancers. However, the therapeutic efficacy of curcumin is restricted by its poor bioavailability after oral administration. In this study, the effects of various cyclodextrins on the intestinal absorption of curcumin were evaluated in rat intestine by an *in situ* closed-loop method. Among the tested cyclodextrins, 50 mM α-cyclodextrin significantly enhanced the absorption of curcumin without inducing any intestinal toxicity. The analysis of cellular transport across Caco-2 cell monolayers showed that 50 mM α-cyclodextrin reduced the transepithelial electrical resistance value of cell monolayers and improved the permeability of 5(6)-carboxyfluorescein, a poorly absorbable drug, which is mainly transported via a paracellular pathway. Furthermore, the western blotting analysis showed that α-cyclodextrin decreased the expression of claudin-4, a tight junction-associated protein, in brush border membrane vesicles. Additionally, α-cyclodextrin increased the membrane fluidity of lipid bilayers in brush border membrane vesicles and may also have promoted the permeation of drug molecules via a transcellular pathway. These results suggested that α-cyclodextrin might enhance the intestinal absorption of curcumin via both paracellular and transcellular pathways.

1. Introduction

Curcumin (CUR) is a polyphenolic compound that is naturally obtained from the dried rhizome of *Curcuma longa*, a medicinal plant used for many centuries in India and China. CUR has been reported to possess a wide range of biological activities for the treatment of several chronic inflammatory diseases and the modulation of signaling pathways in cancer cells (Anand et al., 2007; Esatbeyoglu et al., 2012; Kunnumakkara et al., 2017). However, the pharmacological effects of CUR are restricted by low oral bioavailability that results from low aqueous solubility and poor permeation across the intestinal membrane (Wahlang et al., 2011; Li et al., 2017). In order to develop a potential CUR delivery system, various nano-formulations have been designed; the majority have focused on the solubilization of this hydrophobic compound in aqueous solution (Naksuriya et al., 2014). Recently, we developed amorphous nanoparticles of CUR to improve the poor permeation (Kimura et al., 2016). The permeability of CUR was enhanced by the direct transport of the amorphous solid particles across the absorptive membrane, which was not observed in the presence of crystalline drug particles or supersaturated drug solution. Since the crystalline state is more stable in dosage forms than the amorphous form, it is of interest to study the absorption mechanisms of CUR in a natural crystalline state, which might contribute to a promising strategy for oral administration.

In recent studies, we have reported that many absorption enhancers could improve the intestinal absorption of hydrophilic drugs and macromolecular drugs, including alendronate, insulin, and calcitonin by an *in situ* closed-loop method (Alama et al., 2016; Fetih et al., 2005; Gao et al., 2008; Lin et al., 2011; Nakaya et al., 2016). Of these absorption enhancers, cyclodextrins (CDs) are a unique type of macrocyclic nano-carriers and some have been applied as pharmaceutical excipients by US, European, and Japanese regulatory agencies. They are widely used in pharmaceutical formulations owing to their versatile functions for the solubilization and stabilization of drug molecules by their central non-polar cavity and for the potential enhancement of drug permeation via paracellular and transcellular routes (Doi et al., 2011; Loftsson et al., 2005, 2007; O'Neill et al., 2011; Réti-Nagy et al., 2015). The favorable effects of CDs on the solubility of CUR were demonstrated by a solubility enhancement ratio of more than 10,000 (Tønnesen et al., 2002). As reported in the same study, the stability of

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CUR was also ameliorated by the presence of different 0.1% β-CD derivatives in alkaline solution. However, few studies have examined the influence of CDs on the intestinal absorption of CUR with respect to its low permeability.

In the present study, the effects of various CDs, including α-, β-, γ-CD, hydroxypropyl (HP)-β-CD, and dimethyl (DM)-β-CD, on the intestinal absorption of CUR were studied using an *in situ* closed-loop experiment. Additionally, to elucidate the absorption-enhancing mechanisms of CDs via a paracellular pathway, we examined the cellular transport of 5(6)-carboxyfluorescein (CF) in Caco-2 cell monolayers and the expression of claudin-4 in brush border membrane vesicles (BBMVs) in the presence of 50 mM α-CD. Furthermore, the effect of 50 mM α-CD on the membrane fluidity of BBMVs was also evaluated to determine whether α-CD could enhance the intestinal absorption of CUR via a transcellular pathway.

2. Materials and methods

2.1. Materials

α-CD, β-CD, and HP-β-CD were provided by Nihon Shokuhin Kako Co., Ltd (Tokyo, Japan). 2,6-Di-O-methyl-β-CD (DM-β-CD), γ-CD, Dulbecco's modified Eagle's medium (DMEM), non-essential amino acids (MEM-NEAA), antibiotic-antimycotic mixture stock (10,000 U/mL penicillin, 10,000 mg/mL streptomycin, and 25 mg/mL amphotericin B in 0.85% sodium chloride), 0.25% trypsin/1 mM ethylenediaminetetraacetic acid (EDTA) solution, and dansyl chloride (DNS-Cl) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Curcumin, albumin (from bovine serum, Cohn Fraction V, pH 7.0), lactate dehydrogenase (LDH) (from chicken heart), Coomassie brilliant blue G-250 (CBB G-250), and 1-(4-(trimethylamino) phenyl)-6-phenylhexa-1,3,5-hexatriene-p-toluenesulfonate (tma-DPH) were obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan). 5(6)-Carboxyfluorescein was supplied by Eastman Kodak Company (Rochester, NY, USA). The cytotoxicity detection kit was manufactured by Roche Diagnostics GmbH (Penzberg, Germany). Fetal bovine serum was purchased from Life Technologies Corporation (Carlsbad, CA, USA) and the Caco-2 cell line was purchased from Dainippon Sumitomo Pharma Co., Ltd (Osaka, Japan). 1,6-Diphenyl-1,3,5-hexatriene (DPH) and Hank's balanced salt (HBSS) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Claudin-4 Mouse Monoclonal Antibody-Unconjugated and HRP-Rabbit Anti-Mouse IgG (H + L) Conjugate were purchased from Invitrogen™ (Carlsbad, CA, USA). α-Tubulin (DM1A) Mouse mAb was produced by Cell Signaling Technology, Inc. (Danvers, MA, USA). Polycarbonate membrane Transwell inserts (12 wells, 12 mm in diameter, 0.4-μm pore size, sterile) were manufactured by Corning Inc. (Corning, NY, USA). All other reagents used in the experiments were of analytical grade.

2.2. Solubility of CUR in the presence of CDs

An excess of CUR was added into 1 mL of each CD solution/suspension in a closed vial. The drug suspension was protected from light and sonicated at 40 kHz at 30 °C for 2 h (US-5R; AS ONE Corporation, Osaka, Japan) (Maria et al., 2017). The suspension in each vial was centrifuged at 9660 × g for 2 min and then filtered through a 0.45-μm filter. The initial filtrate was discarded and the remaining solution was collected and diluted with 5% acetic acid for the assay. The CUR concentration was determined by RP-HPLC (Zhang et al., 2009). The HPLC system was configured with a binary pump (LC-20AB) and a fluorescence detector (RF-10A; Shimadzu Corporation, Kyoto, Japan). CUR was eluted by a C18 reverse-phase column (150 mm × 4.6 mm, 5C18-AR-II; Nacalai Tesque Co. Ltd, Kyoto, Japan) warmed to 35 °C. The column eluent was analyzed with an excitation wavelength of 420 nm and an emission wavelength of 530 nm. The mobile phase was a mixture of 5% acetic acid and methanol (32:68, v/v) with a flow rate of 1 mL/min. The solubility enhancement ratio (ErS) was calculated from the following equation:

$$ErS = \text{Solubility with CD}/\text{Solubility without CD}$$

2.3. Effects of CDs on the intestinal absorption of CUR

The intestinal absorption was studied in male Wistar rats, weighing 220–260 g (SLC Inc., Hamamatsu, Japan), by an *in situ* closed-loop method (Yamamoto et al., 1994, 1996). All experiments were conducted in compliance with the guidelines of the Animal Ethics Committee at Kyoto Pharmaceutical University. To administer CUR, 16.67 mg/mL of drug suspension was prepared in various CD vehicles dissolved in pH 6.5 phosphate-buffered saline (PBS). The control consisted of CUR prepared in PBS. All formulations were treated with ultrasound at 40 kHz at 30 °C for 2 h. The animals were fasted for 16 h prior to the experiment but allowed access to water. During the experiment, the animals were anesthetized using an intraperitoneal injection of sodium pentobarbital (32 mg/kg body weight) and kept warm under a heating lamp. The small intestine was exposed through a midline abdominal incision. After ligation of the bile duct, polyethylene cannulas were inserted into the cut of the small intestine at both the duodenal and ileal ends and the intestine was washed with PBS. Three milliliters of the drug suspension, with or without CD, was administered to the small intestine and the cannulas were then closed by forceps (Diehl et al., 2001). Blood samples (~ 0.4 mL) were withdrawn from the jugular vein at the specified time points for up to 240 min after administration. Plasma was separated immediately by centrifugation at 9660 × *g* for 5 min and stored at -30 °C until assay. CUR concentration in plasma was determined by the same HPLC method as described in 2.2.

The maximal plasma concentration of CUR (C_{max}) and the time to maximal plasma concentration (T_{max}) were read directly from the curve of plasma drug concentration *versus* time. The area under the curve (AUC) was calculated manually by the trapezoidal method in the range of 0–240 min. The absorption enhancement ratio (ErA) was calculated from the following equation:

$$ErA = (AUC_{0\rightarrow 240\ \text{min with CD}})/(AUC_{0\rightarrow 240\ \text{min without CD}})$$

2.4. Toxicity study of CDs on the intestinal membrane

To explore the intestinal toxicity of CDs, the activity of LDH and the protein leakage from the intestinal tissue were measured. At the end of the intestinal absorption procedure as described in Section 2.2, the small intestine was washed with 30 mL PBS (pH 7.4). The washing solution was recovered and centrifuged at 200 × *g* for 7 min at 4 °C. An aliquot of the supernatant was collected, from which the level of LDH was assayed by using the working solution of the cytotoxicity detection kit and the amount of protein was determined using the Bradford method (Bradford, 1976). 3% (v/v) Triton X-100 (TX-100) was administered into rat small intestines as a positive control.

2.5. Cellular transport of CF in the presence of α-CD

The permeation of CF in α-CD formulations was studied in the direction from the apical side to the basolateral side across Caco-2 cell monolayers. As reported previously, Caco-2 cells were cultured in DMEM containing 10% (v/v) FBS, 1% (v/v) antibiotic-antimycotic mixed stock solution, and 100 mM MEM non-essential amino acid solution in a filter cap cell culture flask under a humidified atmosphere of 5% CO_2 at 37 °C (Alama et al., 2016; Nakaya et al., 2016; Zhao et al., 2016). The cells, used at passage numbers 57–68, were seeded at an initial density of 1×10^5 cells/insert onto 12-well plates fitted with polycarbonate inserts. The cultivated cells were grown for 21 d with a