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Research paper

Transport of curcumin derivatives in Caco-2 cell monolayers

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ABSTRACT

Curcumin (Cur) is a strong natural antioxidant, who can prevent multiple diseases such as anti-cancer, anti-inflammatory, have a resistance to alzheimer's disease and various malignant diseases. But it has poor oral bioavailability due to its poor aqueous solubility, as well as instability. While its novel derivatives (CB and FE), showed better anti-tumor activity, better anti-oxidant activity and better stability than the original drug (Cur). The aim of this study was to study the intestinal transport of Cur, CB and FE using an *in vitro* Caco-2 cell monolayer model. The results showed that Cur had a lower permeability coefficient ($1.13 \times 10^{-6} \pm 0.11 \times 10^{-6}$ cm/s) for apical-to-basolated (AP-BL) transport at 25 μM, while the transport rate for AP to BL flux of CB ($3.18 \times 10^{-6} \pm 0.31 \times 10^{-6}$ cm/s) and FE ($5.28 \times 10^{-6} \pm 0.83 \times 10^{-6}$ cm/s) were significantly greater than that of Cur. The efflux ratio (ER) value at the concentration of 25 μM was 1.31 for Cur, 1.26 for CB and 1.33 for FE, suggesting there was no active efflux involved in the translocation across the Caco-2 cell monolayers for the three compounds. Furthermore, the transport flux of CB and FE was in a concentration dependent manner, suggesting the intestinal transport mechanism in them was passive transport. In summary, the results demonstrated that both the intestinal permeability of CB and FE across Caco-2 cell monolayers was significantly improved compare to Cur. Thus they might show a higher oral bioavailability *in vivo*, and show the potential application in clinic or nutraceutical.

1. Introduction

Curcumin (Cur, Fig. 1(A)), a yellow polyphenolic compound, derived from the rhizome of *longa*, is a strong natural antioxidant, which contribute to the prevention of multiple diseases such as anti-oxidant, anti-cancer, anti-inflammatory, resistance to atherosclerosis, resistance to alzheimer's disease and various malignant diseases [1–7]. And it is widely used in foods, dishes, cakes, candies, beverages, as well as cosmetics, medicine's coloring, except in medicine due to its instability. Furthermore, the poor aqueous solubility also limited the "Cur molecule" transform into a "medicine" [8].

In the past decades, several strategies have been used to overcome these problems, which divided into two categories. On one side, some scholars were focus on the pharmaceutical preparation technology such as β-cyclodextrin inclusion technique, emulsion, solid dispersions, nanoemulsionsand nanoparticles [9–13]. Which appear to provide longer circulation, better permeability, and resistance to metabolic processes. On the other side, there were many researchers who intent on the structural modifications of curcumin, hoped to improve the biological activity of curcumin. Numerous curcumin derivatives studies about enhancing the biological activity can be found in the literatures [14–17]. But only a few studies reported the pharmacokinetics and bioavailability evaluation of a curcumin analogues [8]. In this study, we evaluated the bioavailability of curcumin and its derivatives through their permeability and transport characteristics.

Moreover, the intestinal permeability plays a critical role in oral bioavailability of a compound. Caco-2 cell line, which is derived from human colon adenocarcinoma, resembles morphologically the enterocytes of the small intestine. Formed monolayers have intercellular tight junctions, microvilli and exhibit brush-border characteristics at the apical side after confluence. For this reason,

Abbreviations: AP, apical; BL, basolateral; CB, (1E,4E)-1,5-bis (3-methoxy-4-hydroxy phenyl)-1,4-diene-3-pentanone; Cur, curcumin; DMEM, dulbecco's modified eagle's medium; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; ER, efflux ratio; FBS, fetal bovine serum; FE, (1E,4E)-1-(3,4-methoxyphenyl)-5-(4-hydroxy-3,5-methoxyphenyl)-1,4-diene-3-pentanone; HBSS, hank's buffered salt solution; HPLC, high performance liquid chromatography; LY, lucifer yellow; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; NEAA, non-essential amino acids; PBS, phosphate-buffered saline.

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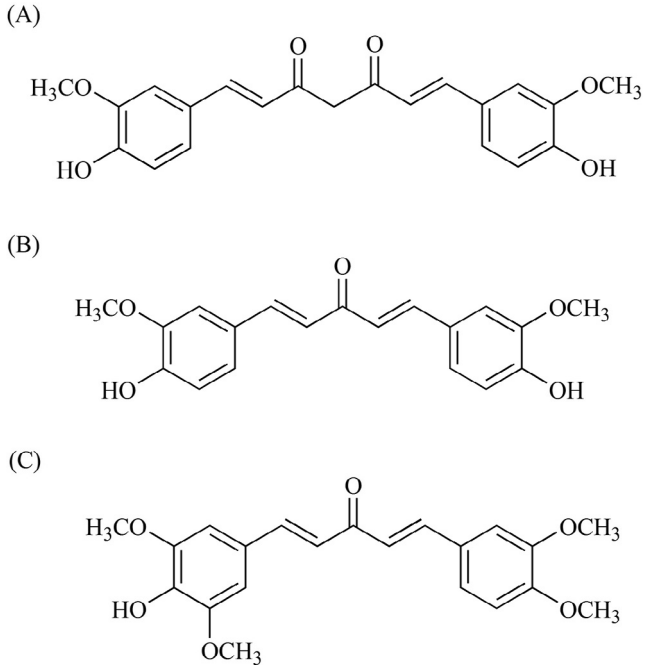

Fig. 1. Chemical structures of Cur (A), CB (B) and FE (C).

it is a good intestinal absorption model for studying permeability and transport characteristics of drugs [18–20].

In our previous study [21], CB (Fig. 1(B)) and FE (Fig. 1(C)) are two new curcumin derivatives, which were identified with better anti-tumor activity, better aqueous solubility and better stability. They were obtained from a series of structure optimization and bioactivity screening based on the purpose to improve the curcumin derivatives' stability, antioxidant activity and antitumor activity. They are single carbonyl analogues of curcumin, and have a 5-carbon spacer and a single carbonyl group. Among them, CB is a symmetrical monocarbonyl curcumin analogue, which has the same substituent groups with Cur. While FE is an unsymmetrical monocarbonyl curcumin analogue, which has added a methoxyl group in the ortho position of a 4-OH and replaced another 4-OH with a methoxyl group. Our previous studies have demonstrated that the anti-proliferative activities of CB to MCF-7 cells was 2–3 times higher than Cur, while FE was about 8 times higher.

The aim of this paper was to investigate the intestinal transport process of the two kinds of curcumin derivatives CB and FE compared with Cur by using human intestinal Caco-2 cell monolayers as an *in vitro* model. We also explored the absorption mechanisms and transport properties, predicted the absorption of CB and FE *in vivo* compared with Cur. So that they might show a higher oral bioavailability *in vivo*, and show the potential application in clinic or nutraceutical, thus make it a prospective medicine candidate and provide an approach for the therapy of multiple diseases.

2. Materials and methods

2.1. Chemicals and materials

The human colon adenocarcinoma cell line, Caco-2, was purchased from the China Center for Type Culture Collection, CCTCC (Wuhan, China). Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco BRL Life Technology (Carlsbad, CA). DMSO, Lucifer yellow (LY) and Non-essential amino acids (NEAA) were obtained from Sigma–Aldrich (St. Louis, MO). Fetal bovine serum (FBS) was purchased from Hyclone (Thermo Fisher Scientific). Hank's buffered salt solution (HBSS), phosphate-buffered saline (PBS), 100 U/mL of penicillin and 100 μg/mL of streptomycin were purchased from Genom. 0.25% trypsin with ethylenediaminetetraacetic acid (trypsin–EDTA) and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Solarbio (Beijing Solarbio Science & Technology Co., Ltd., China). All reagents for HPLC (high performance liquid chromatography) were of analytical grade. Bis-(1E,4E)-1-(3-methoxy-4-hydroxyphenyl)-3-one (CB), Curcumin (Cur) and (1E,4E)-1-(4-hydroxy-3,5-dimethoxyphenyl)-5-(3,4-dimethoxy-phenyl)-1,4-pentadien-3-one (FE) (purity > 98%) were synthesized by our lab [21].

Blood counting chamber was purchased from QIU JING® (Shanghai). 6-well cell culture cluster, 96-well cell culture cluster, T-25 cm^2 cell culture flask and Transwell® permeable support (3401, 0.4 μm pore size polycarbonate membrane, 12 diameter inserts, 12 well plate) were purchased from Costar® (Corning Incorporated, USA). Millicell ERS-2 Epithelial volt-Ohm meter with Ag/AgCl electrodes purchased from Millipore (USA) was used.

2.2. Drug pretreatment

Cur, CB and FE were first dissolved in DMSO at a concentration of 5 mM. For the cell viability assay, samples were diluted with DMEM culture medium (containing 2% (v/v) FBS, 1% (v/v) NEAA, 100 U/mL of penicillin, and 100 μg/mL of streptomycin) to prepare the different concentrations. For the permeation experiments, samples were diluted with HBSS to prepare the final concentrations. The final concentrations of DMSO in different samples were controlled below 0.3% (v/v) to ensure the safety to the cells.

2.3. Caco-2 cell culture

The human colon adenocarcinoma cell line Caco-2 were grown in T-25 cm^2 cell culture flasks (Corning®) containing DMEM supplemented with 15% (v/v) FBS, 1% (v/v) NEAA, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cell cultures were maintained at 37 °C in a CO_2 incubator (Thermo Fisher Scientific). The incubator has an atmosphere of 95% air/5% CO_2 and 95% humidity. Culture medium was changed after washed with PBS every two days, the non-adherent cells were removed by washing with PBS and medium changes. The cells were passaged every 4 days when the cell density reached 80–90% confluence at a 1:3 split ratio by treating with a solution of 0.25% trypsin and 0.02% EDTA for 3 min after washed with PBS (pH = 7.4).

For transport experiments, Caco-2 cells from passage number 30–45 were seeded at a density of 5×10^5 cells per well on inserts with polycarbonate membranes using a blood counting chamber (QIU JING®, Shanghai). A 0.5 mL of culture medium with cells was added to the apical (AP) side and 1.5 mL of blank culture medium was added to the basolateral side (Fig. 2). The medium in both the AP and BL sides was changed every 2 days for the first week and every day after during 21 days of incubation.

2.4. HPLC analysis of samples

Samples from the intestinal permeability study were directly applied to HPLC analysis, performed on a Shimadzu HPLC system equipped with a LC solution software, a LC-20AT binary gradient pump, a CTO-10AS column oven, and a SPD-20A UV/VIS detector (Shimadzu, Kyoto, Japan). The HPLC separation was performed on a C18 (250 mm × 4.6 mm, 5 μm, Elite, Dalian) reverse-phase column maintained at 40 °C. The mobile phase consisted of 0.5% acetic acid and acetonitrile (40:60 v/v) with a 1.0 mL/min flow rate. The absorbance detector wavelength was set at 420 nm, and the injection volume was 20 μL.