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Lecithin in mixed micelles attenuates the cytotoxicity of bile salts in Caco-2 cells

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ABSTRACT

This study was designed to investigate the cytotoxicity of bile salt–lecithin mixed micelles on the Caco-2 cell model. Cell viability and proliferation after mixed micelles treatments were evaluated with the MTT assay, and the integrity of Caco-2 cell monolayer was determined by quantitating the transepithelial electrical resistance and the flux of tracer, FITC-dextran 4400. The apoptosis induced by mixed micelles treatments was investigated with the annexin V/PI protocol. The particle size of mixed micelles was all smaller than 100 nm. The mixed micelles with lower than 0.2 mM sodium deoxycholate (SDC) had no significant effects on cell viability and proliferation. When the level of SDC was higher than 0.4 mM and the lecithin/SDC ratio was lower than 2:1, the mixed micelles caused significant changes in cell viability and proliferation. Furthermore, the mixed micelles affected tight junctions in a composition-dependent manner. Specifically, the tight junctions were transiently opened rather than damaged by the mixed micelles with SDC of between 0.2 and 0.6 mM. The mixed micelles with more lecithin also induced less apoptosis. These results demonstrate that relatively higher concentrations of mixed micelles are toxic to Caco-2 cells, while phospholipids can attenuate the toxicity of the bile salts.

1. Introduction

Nanotechnology has been widely applied in medications and drug delivery systems. The demand for nanotechnology in medical products is expected to grow by more than 17% annually, with the largest share in pharmaceutical applications that may reach $18 billion in 2014 (Jones and Grainger, 2009). However, the effects of nanomaterials or nanodrug delivery systems on tissues or cells require further evaluations for their safe and effective applications. Indeed, the properties of particles, including pharmacology and toxicity, will change significantly from the normal states when their sizes are reduced to nanoscales. Different reports have reached distinct conclusions towards the toxicity of nanoparticles (Gelperina et al., 2002; Renwick et al., 2001). This controversy clearly needs new studies to be settled.

The mixed micelles of bile salts (BSs) and phosphatidylcholine (PC) have been widely used in the oral delivery of insoluble drugs to increase their dissolution and bioavailability (Mrestani et al., 2010; van Hasselt et al., 2009; Wiedmann et al., 2002; Yu et al., 2010). The concentration of bile salts can reach 14 mM in human bile, and they can form micelles spontaneously when their concentrations exceed 2–5 mM, the critical micellar concentrations (CMC) (Coleman et al., 1979; Dial et al., 2008). PC is another main ingredient of the bile (Balint et al., 1965), which binds to bile salts to form mixed micelles that can improve the dissolution of insoluble drugs (Alkanonyuksel and Son, 1992; Li et al., 1996; Magee et al., 2003).

Although bile salts can dissolve the membrane lipids and disrupt the gastric mucosal barrier (Duane, 1980), the mucosal lining of the gastrointestinal (GI) tract is not damaged by the bile under physiological conditions (Martin et al., 1992). PC has been suggested to prevent the toxicity of bile salts on gastrointestinal epithelia and membrane (Dial et al., 2008; Narain et al., 1998).

In this study, we evaluated the effects of lecithin on the bile salt, sodium deoxycholate, and the effects of mixed micelles formed by lecithin and bile salts on cell viability, proliferation, and the integrity of monolayer and apoptosis in Caco-2 cells. The Caco-2 cell model was chosen to evaluate the effects of mixed micelles on gastrointestinal epithelia and membrane for the following two reasons: Caco-2 cells were derived from a human colon carcinoma thus serve as a cell culture model of intestinal epithelia cells; and the morphology and other properties of the Caco-2 monolayer mimic those of the intestinal epithelia (Shah et al., 2006; Turco et al., 2010).

Abbreviations: SDC, sodium deoxycholate; BS, bile salts; PC, phosphatidylcholine; TEER, transepithelial electrical resistance; P_{app}, apparent permeability coefficient; FD-4, FITC-dextran 4400.

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2. Materials and methods

2.1. Chemicals and regents

Lecithin (Lipoid S100, average Mw 770) and sodium deoxycholate (SDC) were purchased from Lipoid (Germany) and Sionpharm chemical Co. Ltd (Shanghai, China), respectively. Methyl tetrazolium (MTT) and FITC-dextran (Mw 4400) were obtained from Sigma (St. Louis, MO, USA). Annexin V-FITC apoptosis detection kit was purchased from KeyGEN Company (Nanjing, China). The HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany).

2.2. Preparation and characterization of mixed micelles

Different concentrations of lecithin (0.4, 0.2, 0.1, 0.05 mM) and 0.1 mM sodium deoxycholate (SDC) were dissolved in 10 mL organic solvent containing equal amounts of methanol and dichloromethane (v/v). Then, the organic solvent was removed by reduced-pressure evaporation for 12 h. At last, a series of mixed micelles with different ratios of lecithin/SDC were obtained by adding 10 mL deionized water. The micelles were treated with ultrasound (285 W, 60 s) (ultrasonic instrument, Scientz IID, China) to reduce the particle size and homogenize the micelles. The size and zeta potential were evaluated by Zeta-Sizer Nano ZS (Marlven, UK). Besides, control group (0/1 of lecithin to SDC) was prepared by dissolving SDC in deionized water and then diluted to different concentrations.

Each mixed micelle was diluted to various concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mM, calculated by SDC) with culture medium without fetal bovine serum for the cell experiments.

2.3. Cell culture

Human colonic carcinoma Caco-2 cells were purchased from the American Type Culture Collection (Rockville, MD). The cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2 in Dulbecco's modified minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Gibco), 4.5 mg/L glucose, 1% (v/v) nonessential amino acids, and antibiotics (penicillin 200 U/mL and streptomycin 100 mg/mL). The cells were grown in 25 cm^2 culture flasks. The medium was changed every other day, and the cells were trypsinized every 5 days for passage. The Caco-2 cells with passage numbers of 45–52 were used in the experiments.

2.4. Assessment of cell viability

The cell viability of Caco-2 cells was measured by the MTT assay to evaluate the cytotoxicity of mixed micelles. 1×10^4 cells in 200 μL culture medium per well were seeded into 96-well plate. Three days later, the medium was replaced with the same volume of mixed micelles in culture medium. Blank culture medium was used as negative control. After 2 h of micelle treatment, 20 μL of 5 mg/mL MTT solution was added to each well, and the medium was removed after 4 h. Then, the formazan crystals were dissolved in 150 μL of DMSO. The absorbance was measured at 490 nm.

2.5. Assessment of cell proliferation by MTT assay

The cells were seeded in 96-well plates at a density of 1×10^4 cells per well and cultured for 12 h before the mixed micelles samples were added. Cells were thereafter cultured for 3 days and underwent MTT assay (Yin et al., 2009). The cells without micelle treatment served as a control. The MTT assay was performed in sextuplicates for each sample, and the proliferation ratio was defined as the proportion of treated cells to the control cells.

2.6. Examination of the integrity of Caco-2 monolayer

The Caco-2 cells were cultured on porous polycarbonate filter membranes (Millicell®-PCF, 12 mm diameter, cell culture insert, Millipore) with a pore size of 0.4 μm and a diameter of 12 mm in 24-well culture plate (Corning Costar, Cambridge, MA) for the measurement of cell monolayer integrity. The seeding density on the filter was 1×10^4 cells/cm^2. The cells were cultured for 21 days under normal conditions as in Section 2.3 before the measurement. The medium was changed every second day for the first week and every day for the following two weeks.

The transepithelial electrical resistance (TEER) of Caco-2 cell monolayer was measured once every three days during the culturing with a Millicell®-ERS (Millipore Corp., Bedford, MA) connected to a pair of chopstick electrodes. On the day of measurement, the culture medium was replaced with equal volume of HBSS (0.4 mL apically and 0.6 mL basolaterally), and the cells were allowed to equilibrate for 1 h. Then, the TEER was measured to reveal the integrity of the monolayer formed on the filters. TEER measurements were also performed during the experiment to check the effects of mixed micelles on the integrity of Caco-2 cell monolayers at the intervals of 0, 30, 60 and 120 min. After 120 min, the donor solutions were removed carefully, and the monolayers were rinsed with HBSS and re-fed with culture medium for monolayer recovery. Twenty-four hours later, TEER was measured again.

The permeability of FITC-dextran MW 4400 (FD-4) was determined after mixed micelle treatments to further characterize the integrity of Caco-2 cell monolayers. Briefly, the HBSS solution of FD-4 (5 mg/mL) containing different mixed micelles was added to the apical side. After 15, 30, 45, 60, 90, and 120 min, 400 μL samples in the basolateral side were collected and replaced with 400 μL blank HBSS. The concentration of samples was determined by HPLC-FLD (Agilent, USA). Apparent permeability (P_{app}) for each substance was calculated according to the following formula:

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{AC_0}$$

where P_{app} is the apparent permeability (cm/s); dQ/dt is the permeability rate; A is the diffusion area of the monolayer (cm^2); and C_0 is the initial concentration of the compound in the feed.

2.7. Determination of FD-4

FD-4 in the medium was isolated and characterized by using Agilent 1100 HPLC system (Agilent, USA) consisting of a ternary pump, an automatic sampler, a fluorescence detector and a column heater. The separation of FD-4 was carried out using an analytical RP 18 column (150 × 4.6 mm, i.d. 5 μm) with a mobile phase of 10% acetonitrile and 90% KH_2PO_4 (2 mM, pH 7.2). The flow rate of the mobile phase was 1 mL/min, and the detector was operated at an excitation wavelength of 495 nm and an emission wavelength of 515 nm. The injection volume was 20 μL, and the detection limit of FD-4 was 5 ng/mL.

2.8. Apoptosis assay

The Caco-2 cells were cultured in 6-well plates at a dendity of 1×10^6 cells per well. After 5 days of incubation, the culture medium was removed, and the cells were washed 3 times with D-Hank's buffer solution. Then the mixed micelle treatments started (0.2 mM mixed micelles) with blank culture medium as the negative control. After being treated for 24 h, the cells were detached