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Study on the Establishment of the Transport Model of Hizikia Fusifarme Polysaccharide in Caco-2 Cells Model

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Abstract

Intestinal absorption is a key step in the process of drug absorption. The most commonly used model is human colon adenocarcinoma Caco-2 cell model. After being cultured for a period of time, Caco-2 cells have similar morphology, marker enzyme expression, uptake and transport and permeability characteristics to small intestinal epithelial cells, so they are often used to study the mechanism of drug absorption through small intestinal epithelial cells. Based on previous work, this topic established. The uptake and transport model of Caco-2 cells to investigate the uptake and transport characteristics of Hizikia fusifarme polysaccharides in intestinal epithelial cells provides a theoretical basis for the absorption and transport mechanism of Hizikia fusifarme polysaccharides.

Keywords

Hizikia fusifarme polysaccharides; Caco-2 cells; model.

1. INTRODUCTION

Caco-2 is derived from human colon cancer cells. When cultured in vitro, it can grow into a complete cell monolayer. It has a structure similar to small intestinal epithelial cells. It has a microvilli structure and can express the iconic protein carrier and enzymes of epithelial cells. Is a commonly used in vitro cell model for studying drug absorption. Based on the research purpose of this subject, this study constructed a Caco-2 cell uptake and transport model, and carried out index evaluation and toxicity testing on the model to provide a certain basis for later uptake and transport experiments [1-2].

2. MATERIALS AND EQUIPMENT

Hizikia fusifarme polysaccharide extract: purchased from Jiangsu Jianjiang Pharmaceutical Co., Ltd.;Caco-2 cell line: purchased from Shanghai Guandao Biological Co., Ltd.;DMEM (DULBECCO'S MODIFIED OF EAGLE'S MEDIUM) high-sugar medium, fetal bovine serum, 100X penicillin-streptomycin mixed solution, pancreatin, L-glutamine, Hank's balanced salt solution was purchased from Beijing Soleibao Technology Co., Ltd.;CCK-8 (cell counting kit-8) kit, dimethyl sulfoxxide (DMSO), verapamil, trifluoroacetic acid, MK-571, phenylarsenic oxide, sodium deoxycholate, alkaline Phosphatase (alkaline phosphatese, ALP) kit, acetonitrile, methanol (all chromatographically pure), and the rest of the reagents are domestic analytical pure.

ZCP-270WIR carbon dioxide incubator: Shanghai Zhetu Scientific Instrument Co., Ltd.;1260 High Performance Liquid Chromatograph: Beijing Keyi Hengda Technology Co., Ltd.;GL-21M centrifuge: Jinan Qike Instrument Equipment Co., Ltd.;HH-A constant temperature water bath: Changzhou Yinggeer Instrument Manufacturing Co., Ltd. HG202-1A electric heating blast drying oven: Shanghai Zhetai Machinery Manufacturing Co., Ltd.; Inverted optical microscope: Shanghai Optical Instrument Factory; Millicell ERS-2 cell resistance meter: Beijing Mingyang

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Kehua Biotechnology Co., Ltd.; Transmission electron microscope: Thermo Fisher Scientific Co., Ltd.

3. METHOD

3.1. Determination of the Stability of SFPS in the Gastrointestinal Tract

3.1.1 Determine the stability of SFPS in different pH close to the gastrointestinal environment

Take 0.9ml phosphate buffer at different pH values (1.2, 2.5, 5.0, 6.5, 7.4-8.0), add 0.1ml 10mg/ml SFPS solution to the phosphate buffer, and place it at the temperature Heat in a 37°C water bath, take samples at 0, 10, 30, 60, 120, and 180 minutes respectively, and then inactivate the enzyme at 100°C for 15 minutes. Use the HPLC standard curve to determine the mass concentration of SFPS. The final mass concentration of SFPS is compared with the initial The ratio of the mass concentration is the remaining percentage [3].

3.1.2 Determine the stability of SFPS in artificial gastric juice and artificial intestinal juice

Add SFPS to artificial gastric juice and artificial intestinal juice to ensure that its mass concentration is 1mg/ml, vortex for 2s, and incubate at 37°C for 0, 10, 30, 60, 120, and 180 minutes to sample, and place it at 100°C to kill the enzyme In 15 minutes, use the HPLC standard curve to determine the mass concentration of SFPS. The ratio of the final mass concentration of SFPS to the initial mass concentration is the remaining percentage [4].

3.1.3 Caco-2 cell culture

Human colon cancer Caco-2 cells were inoculated in a culture flask with a medium of 10% FBS-DMEM. Complete the culture medium and place it in a 37°C, 5% CO2 incubator, and cultivate under the conditions of relative saturated humidity. The cells are in a state of adherent growth. Change the medium every two days until the cells grow to 80%~90%. Use 0.25% trypsin solution for digestion and passage, and passage every 5-7 d [5-6].

4. RESULTS AND ANALYSIS

4.1. Analysis of the Stability of SFPS in the Gastrointestinal Tract

Table 1. The stability of SFPS in a solution of pH

Training time	pH 1.2	pH 2.5	pH 5.0	pH 6.5	pH 8.0	pH 1.2
10min	100%	100%	100%	100%	100%	100%
30min	99.4%	98.5%	98.6%	98.4%	98.6%	98.4%
60min	97.5%	97.9%	96.8%	97.6%	98.9%	98.4%
120min	97.6%	97.7%	97.5%	97.5%	97.6%	97.8%
180min	96.8%	96.6%	96.4%	96.8%	96.8%	96.3%

As shown in table 1, the result of the HPLC method is that the remaining percentage of Hizikia fusifarme polysaccharide is basically maintained in an environment close to the pH value of the human gastrointestinal tract Between 95% and 100%, the remaining percentage within 180 minutes showed a slow downward trend, but at the 180th minute, more than 95% of Hizikia fusifarme polysaccharides were still in the prototype state. It can be seen that Hizikia fusifarme Vegetable polysaccharides remain basically stable in a pH environment similar to that of the human gastrointestinal tract [7].

4.1.2 Stability analysis of SFPS in artificial gastric juice and artificial intestinal juice

As shown in table 2, the degradation index of Hizikia fusifarme polysaccharide in artificial gastric juice and artificial intestinal juice is about 5%, and the extraction solvent is 95%. When ethanol and material-to-liquid ratio are at the 180th minute [8], the remaining percentage of

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Hizikia fusifarme polysaccharide in artificial gastric juice is 94.37%, and the remaining percentage in artificial intestinal juice is 94.63%. From this result, it can be concluded that the artificial gastrointestinal juice is effective. The stability of Hizikia fusifarme polysaccharides did not cause a significant effect. In addition, the results of Hizikia fusifarme polysaccharides in the artificial gastrointestinal fluid are basically consistent with the results of Hizikia fusifarme in the pH environment close to the human gastrointestinal tract.

Table 2 Stability results of SFPS in artificial gastric juice

Training time	Artificial gastric juice	Artificial intestinal juice
10min	100%	100%
30min	98.42%	98.51%
60min	97.54%	96.90%
120min	95.65%	95.72%
180min	94.37%%	94.63%

4.2. Caco-2 Cell Culture Morphology

The Caco-2 cells in the culture flask grow to about 80%. At this time, the Caco-2 cells grow densely and most of them are in a state of adherent growth, and are The cells are closely connected, but there is a clear boundary between each other, which shows that the Caco-2 cells at this time have the conditions for establishing a cell monolayer model [9].

It is the state of Caco-2 cells after digestion and passage with a trypsin solution with a mass fraction of 0.25%. When the cells are not blown away, they show a high density of single clumps. Morphology, there are very obvious boundaries between each cell, and there is a large blank area, which means that the cells have been digested [10].

4.3. The Effect of Different Mass Concentrations of SFPS on the Survival Rate of Cells

The results of the CCK-8 test kit showed that the survival rate of Caco-2 cells will gradually decrease with the increase of the mass concentration of Hizikia fusifarme polysaccharide, and the mass concentration of Hizikia fusifarme polysaccharide is 0.10mg/mL and 0.25 respectively. At mg/mL, the survival rate of Caco-2 cells was above 90%. When the mass concentration of Hizikia fusifarme polysaccharides is 1.00 mg/mL, the cell survival rate has dropped to 76.43%. Compared with the blank control group, this data has a significant difference ($P<0.5$). In addition, when the mass concentration is above 0.25, the time to carry out the transport experiment is significantly less than the transport time of other mass concentrations. Therefore, 0.25mg/mL is the highest mass concentration that can be selected.

Table 3. The effect of different mass concentrations of SFPS on the survival rate of cells

SFPS mass concentration /(mg/mL)	0	0.10	0.25	0.50	1.00	
Survival rate /%	100 ±5.60	101.65 ±1.41	94.68 ±3.53	87.58 ±3.78	76.43	±4.83

4.4. Validation and Evaluation Results of the Caco-2 Cell Monolayer Model

4.4.1 Validation results of Caco-2 cell monolayer model

After Caco-2 cells were cultured on a 12-well Transwell culture plate for 21 days, an inverted microscope and a transmission electron microscope combined to observe the formation of a good brush border on the surface of the Caco-2 cells, and the cells were tightly connected, showing a paving stone-like shape And there is a clear boundary between each other, forming a dense monolayer of cells. All the results show that the Caco-2 cells cultured on the Transwell culture plate for 21 days have appeared polarized characteristics and are unevenly distributed. Already have some structural characteristics of small intestinal epithelial cells.

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5. CONCLUSION

Caco-2 cells are derived from human colon adenocarcinoma epithelial cells. After a period of culture, their morphology, marker enzyme expression, uptake and transport, and permeability characteristics are similar to those of small intestinal epithelial cells. Therefore, the Caco-2 cell model is selected for research in this experiment. The absorption mechanism of Hizikia fusifarme polysaccharides. The established Caco-2 cell monolayer is usually evaluated by using the following two indicators: TEER and ALP activity determination. When the resistance value is between 500 Ω·cm2-1000 Ω·cm2 and the ratio of ALP (AP/BL) is greater than 3, the cell monolayer molecular layer forms a complete and compact cell monolayer and has polar characteristics, which is consistent with transport Experimental requirements. In this experiment, this model can be used for drug delivery experiments. Cell morphology observation results show that the cell uptake model is successfully established; TEER and ALP (AP/BL) measurement results show that the cell transport model is successfully established, which can be used for subsequent uptake and transport experiments.

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