Enhancement of sodium taurocholate to the absorption of cefquinome

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Abstract: The objective of the research was to detect the enhancement effect of sodium taurocholate on the absorption of cefquinome both in Caco-2 cells and rats. The absorption efficiency of cefquinome was determined by high performance liquid chromatography and calculated with apparent permeability coefficients (P_{app}) after Caco-2 cell monolayers treated odium taurocholate (2 mmol/L) and cefquinome. The results showed that the absorption of cefquinome in Caco-2 cell monolayers was significantly increased with the sodium taurocholate (2mmol/L). Similar results were also detected in the rats orally administrated with 1 mL PBS of cefquinome (20mg/mL) containing different concentration of sodium taurocholate (5 mmol/L, 10mmol/L and 20mmol/L) respectively. Compared with control group, sodium taurocholate at 10 and 20 mmol/L increased the absorption of cefquinome in rats from 0.26±0.04μg/mL to 0.57±0.03μg/mL, 0.78 ±0.07μg/mL respectively. These results indicated that sodium taurocholate could increase the intestinal permeability in a concentration-dependent mode, which will be useful for clinical treatment.

Keywords: Caco-2 cells; cefquinome; sodium taurocholate; absorption

INTRODUCTION

Cefquinome is a new broad-spectrum cephalosporin that was widely used for animals (Murphy *et al*., 1994), which showed a higher activity against bacteria, also highly stable to β-lactamases. Cefquinome was usually conducted through intravenous and intramuscular administrations in clinical (Limbert *et al*., 1991). Oral drug delivery is considered as the preferred route of administration because of great advantage of convenience to perform and non-invasive to animals.

Recently it was reported that sodium taurocholate could be used as an absorption enhancer to enhance the permeability of mucosal tissues to poorly soluble drugs (Deli, 2009; Meaney & O'Driscoll, 2000). It was reported that bile acids could influence the integrity of tight junction, even disrupt epithelial barrier (Araki *et al*., 2005).

The Caco-2 cell monolayers have been widely employed in studying drug absorption (Ward *et al*., 2000), which possessed brush border, tight junctions and transporters (Meunier *et al*., 1995). Therefore, the aim of this research was to evaluate the potentiality of cefquinome through oral administration and the characteristic of absorption efficiency enhanced by sodium taurocholate both in Caco-2 cell monolayers and rats.

MATERIALS AND METHODS

Reagents and chemicals

Cefquinome ($C_{23}H_{24}N_6O_5S_2$) standard powder was provided by China Institute of Veterinary Drug Control. Acetonitrile (ACN) and methanol were chromatographic grade and purchased from Sigma. The fluorescence markers (FITC, 389.4Da), sodium taurocholate ($C_{24}H_{39}NaO_5$, 430.57) and MTT were purchased from Sigma.

Cell culture

Cells cultured in DMEM supplemented with 10% fetal bovine serum, were incubated at 37°C with 5% CO_2 and 95% humidity. The confluent monolayer of Caco-2 cells were removed from with trypsin, washed and re-suspended, and then seeded on the Millicell filter inserts (Millipore, USA, growth area 0.3cm^2, pore size 0.4μm). Cells reached confluence in 7-8 days and were used for experimentation between days 14 and 21.

The cytotoxicity of cefquinome to caco-2 cell monolayers

The cytotoxicity of cefquinome to the viability of Caco-2 monolayer was determined by MTT assay. The Caco-2 cells were cultured for 21 days on the Millicell filters until formation of confluent monolayer. 100μL cefquinome at different concentration solution containing DMEM (5×10^{-3}, 1×10^{-3}, 5×10^{-4}, 1×10^{-4}, 5×10^{-5} and 8×10^{-6} mol/L, pH=7.2) was added to the apical compartment of the Millicell filters respectively. The cells were placed at 37°C with 5% CO_2 and 95% humidity to ensure adequate mixing for 48h. 20μL (5mg/ml) MTT was added to the apical compartment of the Millicell filters. Then the cultures were placed at 37°C with 5% CO_2 and 95% humidity to ensure adequate mixing for 4h. After medium removed, 100μL DMSO were added, completely dissolve the dark blue crystals.

Then the plates were measured with BioTek FL600-fluorescence/absorbance plate reader. Cell viability was expressed as the percentage of control. The reference blanks without cells, contained additional wells with

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media and chemical only in same plate. The integrity of the Caco-2 monolayer was determined by measuring trans-intestinal transportation of FITC. The FITC was unable to permeate the cellular membrane at physiologic pH due to its hydrophilicity. It had to be transported exclusively by paracellular route across intestinal epithelia. The Caco-2 cells were cultured for 21 days on the Millicell filters until formation of confluent monolayer. 0.5mL (20μg/mL) FITC was added to the apical compartment of the Millicell filters. Then the monolayer was placed at 37°C with 5% CO_2 and 95% humidity, to ensure adequate mixing. The permeation was determined over a 120 min period. The permeability was expressed as the percent ratio of the concentration in the basolateral side versus that in the apical side. Samples (100μL) of the apical and basolateral chambers were diluted in 1mL water and analyzed for fluorescence with Biotek fluorescence spectrophotometer (excitation wavelength 495 nm, emission wavelength 525nm).

The effect of sodium taurocholate on ultra-structure of caco-2 monolayer

The effect of sodium taurocholate on ultra-structure of Caco-2 monolayer was determined by transmission electron micrograph. The Caco-2 cells were cultured for 21 days on the Millicell filters until formation of confluent monolayer. The monolayer was exposed to culture medium only (control group), while 0.5mL sodium taurocholate solution diluted in DMEM (2 mmol/L, pH=7.2) was added to the apical compartment of the Millicell filters for 2h at 37°C. After medium was removed, the monolayer was washed three times with HBSS at 37°C, fixed in 0.05% glutaraldehyde for 1h at 4°C, post-fixed with 1% osmium tetroxide for 45min at room temperature. The monolayer was stained and sections handled as previous reported (Hilgers *et al*., 1990), examined with a JEM-100CX II electron microscope.

The effect of sodium taurocholate on transport of cefquinome in caco-2 monolayer

The Caco-2 cells were cultured for 21 days on the Millicell filters until formation of confluent monolayer and treated as described in table 1. Then 100μL culture medium from apical or basolateral receiver chamber were sampled from each group at 30 min, 60 min, 90 min and 120 min and analyzed by high performance liquid chromatography.

The mobile phase consisted of 0.1% formic acid in water-ACN at a ratio of 90:10 (v/v) and the flow rate was setted at 0.8mL/min with column temperature was 30°C. Standard curve and inter-day and intra-day reproducibility were detected at 270 nm wavelength as previous reported (Li *et al*., 2008). The apparent permeability coefficient (Papp) of parent cefquinome was calculated using the following equation (Heimbach *et al*., 2003): $Papp=V/AC_0 \cdot dC/dt$ (cm/s). Where A is the surface area of the cell monolayer, C_0 is the initial concentration of parent drug in the donor chamber, and dC/dt is the linear appearance rate of parent drug mass in the receiver chamber.

The effect of sodium taurocholate on transport of cefquinome in rats

The *in vivo* effect of the sodium taurocholate on absorption enhancement was performed on rats. All animal experiments were performed according to the guidelines of the local authorities. In this study, thirty-two clinically healthy rats were divided into four groups. After fasting for 24h, the rats were orally administrated with 1mL cefquinome (20mg/mL, pH=7.2) containing different concentration of sodium taurocholate individually (5mmol/L, 10mmol/L and 20mmol/L) respectively. After 0.5h, blood samples of rats were collected and serum samples were obtained by centrifugation at 10,000×g and 4°C for 10 min. Serum samples were handled and analyzed as previous method (Li, *et al*., 2008).

RESULTS

The cytotoxicity of cefquinome to caco-2 monolayers

We firstly tested the cytotoxicity of cefquinome on the Caco-2 monolayers. The Caco-2 cells viability was negatively correlated with concentration of cefquinome (fig. 1). The Cell survival rate was increased following the decreasing concentration of cefquinome. Cell survival rate exceeded 98% when the concentration of cefquinome was 8×10^{-6} mol/L.

The effect of sodium taurocholate to ultra-structure of caco-2 monolayer

The tight junction is the key component of the cell junction, which controls the permeability of paracellular pathway. We found that tight junction was integrated without treatment. However, the tight junction of Caco-2 cell monolayers treated with taurocholate (2mmol/L sodium) for 2h was disrupted (fig. 2). It suggested that the sodium taurocholate enhanced the absorption of medicine through modulating the functions of tight junction.

The effect of sodium taurocholate on transportation of cefquinome

The chromatograms of cefquinome in rat serum and culture medium were sharp and no interference. The retention time of cefquinome was about 10.69 min and the calibration curve was linear between 0.01 and 10.0μg/mL cefquinome with correlation coefficient more than 0.9998. On the basis of signal to noise ratio of 3:1 and 6:1. The limit of detection and limit of quantification for cefquinome in rat serum and culture medium were 0.01 and 0.05μg/mL respectively. Fortified at concentrations of 0.5, 5 and 10μg/mL, the recoveries of cefquinome were 92.35±1.27%, 93.89±2.01%, 91.23±1.04% (n=5) respectively.

Sodium taurocholate could significantly increase the transportation of cefquinome both in the apical or basolateral side of Caco-2 cell monolayers compared with control groups (P<0.01) (fig. 3). However, no significant difference was detected between group II and IV. Furthermore, the Papp tendency of cefquinome in Caco-2 monolayer was decreased in 2 h (table 2). The sodium taurocholate also increased the cefquinome absorption in rats compared with control group (fig. 4). The absorption of cefquinome were increased significantly (P<0.01) by 10 and 20mmol/L sodium taurocholate from 0.26±0.04μg/mL to 0.57±0.03μg/mL and 0.78±0.07μg/mL respectively.

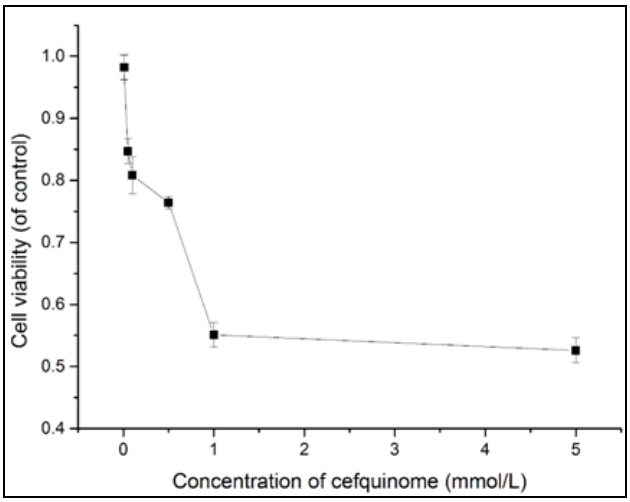

Fig. 1: The cytotoxicity of different concentration of Cefquinome to Caco-2 monolayer. Cell viability was negatively correlated with concentration of Cefquinome.

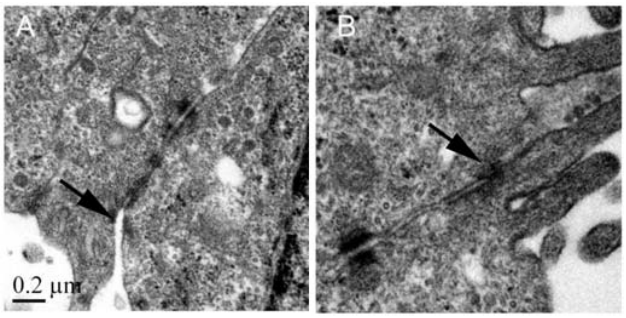

Fig. 2: Transmission electron micrograph images of Caco-2 monolayers. The bar indicates 0.2μm. (A) Treated with 2mmol/L sodium taurocholate for 2h. The tight junction (arrowheads) of apical side was disrupted. (B) The tight junction (arrowheads) of apical side was integrated without sodium taurocholate treatment.

DISCUSSION

The intestinal epithelium, particularly the tight junctions between the epithelium, is a major barrier to the absorption of medicine (Ward, *et al*., 2000). The absorption enhancers were studied to improve the absorption of medicine in intestinal epithelium (Araki, *et al*., 2005). Recently it was reported that bile acids modulated functions of the tight junction, increased intracellular reactive oxygen species (ROS), and then affect the epithelial barrier, improved the medicine absorption (Araki, *et al*., 2005; Catalioto *et al*., 2008). Bile salts could also increased the otilonium bromide paracellular absorption in Caco-2 cells (Catalioto, *et al*., 2008). In our study, the tight junctions of Caco-2 monolayer treated with 2mmol/L sodium taurocholate for 2 h was disrupted, whereas the tight junctions between in control groups kept integrated. The results indicated that the sodium taurocholate may increase the paracellular absorption of drugs through disrupting the tight junction momentarily.

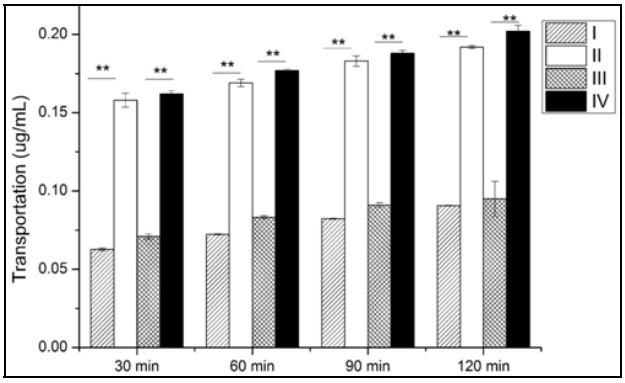

Fig. 3: The transportation of Cefquinome in Caco-2 monolayer. 2mmol/L Sodium taurocholate significantly increased the transportation of Cefquinome from apical side to basolateral side or basolateral side to apical side respectively. **, P<0.01, as determined by ANOVA.

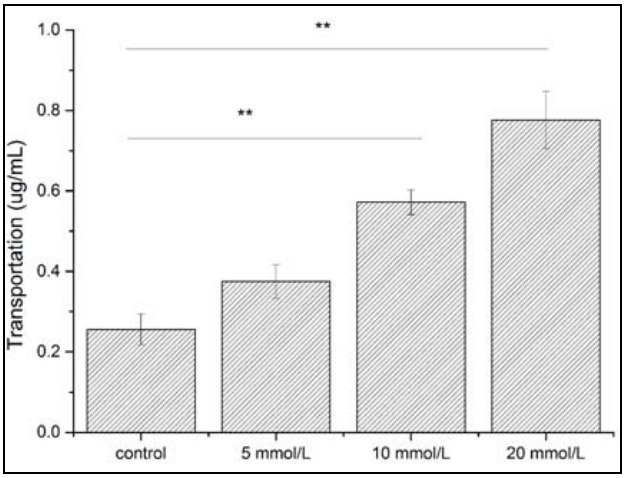

Fig. 4: The sodium taurocholate enhanced the Cefquinome absorption in mice. All treatments except 5 mmol/L were significantly different from control. The absorption of Cefquinome in mice was increased by 10 and 20mmol/L sodium taurocholate from 0.26±0.04ug/mL to 0.57±0.03ug/mL and 0.78±0.07ug/mL respectively. **, P <0.01, as determined by ANOVA.

Table 1: The method of Caco-2 cells treatment

	Cefquinome	Sodium taurocholate	Cell side
Group I	4×10^{-6} mmol		Apical
Group II	4×10^{-6} mmol	2×10^{-3} mmol	Apical
Group III	8×10^{-6} mmol		basolateral
Group IV	8×10^{-6} mmol	2×10^{-3} mmol	basolateral

Table 2: The Papp values of cefquinome

	30 min	60 min	90 min	120 min
Group I	2.85±0.03a	2.11±0.11c	1.31±0.08d	1.03±0.09d
Group II	7.28±0.14a	4.37±0.23c	3.28±0.21c	2.21±0.18d
Group III	3.27±0.08a	2.22±0.16c	1.45±0.18e	1.09±0.05e
Group IV	8.94±0.24a	4.49±0.18c	3.53±0.19e	2.32±0.12g

The same lowercase letters are not significantly; different adjacent lowercase letters differ at $P<0.05$; different interphase lowercase letters differ at $P<0.01$.

The cefquinome is a fourth generation cephalosporin with a C-3 bicyclic pyridinium group, use as an antibacterial drug in cattle and pigs (Limbert, *et al*., 1991; Thomas, *et al*., 2006). It was usually conducted by intravenous and intramuscular administrations in clinical practice. The P_{app} tendency of cefquinome in Caco-2 monolayer was decreased in 2h. Furthermore, the P_{app} value form basolateral side to apical side exceeded that from apical side to basolateral side. It indicated that cefquinome uptake in Caco-2 cells may involve multiple transport pathways (Catalioto, *et al*., 2008; Hayashi & Tomita, 2007). Furthermore, the transportation of sodium glycocholate in Caco-2 cells was in a concentration-dependent manner, and had a momentarily effect (Lindhardt & Bechgaard, 2003). *In vivo*, the absorption of cefquinome in rats by oral deliver was increased with 10 and 20mmol/L sodium taurocholate from 0.26±0.04μg/mL to 0.57±0.03μg/mL and 0.78±0.07 μg/mL respectively, which exceeded the concentration against *streptococci* (MIC_{90}=0.032μg/mL) and *enterobacteriaceae* (MIC_{90}=0.03μg/mL MIC_{90}=0.13 μg/mL) (Thomas *et al*., 2006). These results suggested the oral delivery could be a promising candidate method for cefquinome administration in clinical, improving the efficiency and range of cefquinome use in clinical because of great advantage of convenience to produce and non-invasive to animals.

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