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Research paper

Improvement of lipophilicity and membrane transport of cefuroxime using in vitro models

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Abstract

Most β-lactam antibiotics cannot be absorbed orally and, therefore, must be administered intravenously (i.v.) or intramuscularly (i.m.). Because of the obvious drawbacks of drug delivery by injection, the development of alternatives with enhanced oral bioavailability is receiving much attention in pharmaceutical research. Cefuroxime exhibiting significant advantages in the parental treatment of common infections, was used as model drug in the present study. The effect of the cationic absorption enhancers (four quaternary ammonium salts) on the lipophilicity of cefuroxime was investigated by means of the *n*-octanol/water system. The results on partitioning coefficients in the *n*-octanol/buffer system were confirmed using an in vitro transport model with artificial (dodecanol collodium membrane) and biological membranes (Charles-River guinea pig).

Keywords: Cefuroxime; Partition coefficients; In vitro transport

1. Introduction

Most cephalosporins were administered intravenously or intramuscularly [1–3]. Only a small number of cephalosporins can be administered orally [4–6]. In the recent years, the potentials of co-administration of absorption-enhancing agents have been investigated extensively. In the majority of such studies, antibiotics, peptides and proteins have been used as model substances. Because of their hydrophilic properties, ionic charge and high molecular weight, the absorption-limiting barriers of these drugs are likely to be located in the mucouse layer, the apical cell membrane and the tight junction. Surfactants are widely used as additives in the preparation of pharmaceuticals. Polyoxyethylene ethers were reported to enhance gastric or rectal absorption of penicillins and cephalosporins [7]. In general, the non-ionic surfactants exert benign effects on the membrane structure, in comparison with cationic surfactants and anionic surfactants [8,9]. In rats, bioavailability of rectally infused cephalosporins proved to be considerably improved by medium-chain fatty acids [10]. Beskid et al. demonstrated an enhancing effect of glyceryl-1-monooctanoate on cephalosporin absorption after oral intraduodenal and rectal delivery in various animal species [11]. In the rat large intestine, mixed micelles containing sodium taurocholate and glyceryl monooleate or oleic acid promoted the absorption of cephalosporin [12]. In the present study, cefuroxime was selected as a hydrophilic model drug. Cefuroxime is not accepted by the H^+/peptide transporter PEPT1. Bretschneider et al. reported a very low affinity of cefuroxime at PEPT1 ($K_i = 26 \pm 4$ mmol/l) [13]. The effect of cationic absorption enhancers on the lipophilicity of the model drug was investigated using the system *n*-octanol/buffer. The experimental results on the partition coefficients were confirmed using in vitro standard transport model systems with artificial and biological membranes.

Abbreviations: Cefu, cefuroxime; BAC, hexadecyldimethylbenzylammonium chloride; NCP, *N*-hexadecylpyridinium bromide; LTB, dodecyltrimethylammonium bromide; CTB, hexadecyltrimethylammonium bromide; CZE, capillary zone electrophoresis; HPLC, high-performance liquid chromatography; cmc, critical micelle concentration.

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2. Experimental section

2.1. Materials

Cefuroxime (Cefu) was obtained from Hoechst (Germany). Hexadecyldimethylbenzyl-ammonium chloride (BAC) and hexadecyltrimethylammonium bromide (CTB) were obtained from Sigma-Aldrich Chemie (Germany). *N*-Hexadecylpyridinium bromide (NCP) was obtained from Merck (Germany). Dodecyltrimethylammonium bromide (LTB) was purchased from Fluka (Switzerland). Collodium 4%, ether, ethanol, dodecanol and *n*-octanol were obtained from Caesar and Lorentz (Germany). The small intestine (Charles-River guinea pig) was obtained from the Julius-Bernstein-Institute of Physiology of the Martin-Luther-University Halle-Wittenberg (Germany).

2.2. Sample preparation

Standard solutions of cefuroxime were prepared at 200 μg/ml in phosphate buffer at pH 7.4 (ionic strength, $I = 0.048$) with or without absorption enhancers.

2.3. Analytical assay

2.3.1. Capillary zone electrophoresis (CZE)

Capillary electrophoresis experiments were performed on a Hewlett Packard Model G1600A (Waldbronn, Germany) ^{3D}CE system. The detection wavelength was at 264 nm. Fused-silica capillaries from Hewlett Packard (Waldbronn, Germany) with a total length of 48.5 cm, a length to the detector of 40 cm and an internal diameter of 50 μm were used. Buffer, 20 mM phosphate (pH 7.4), 30 kV, a temperature of 25 °C and an injection time of 9 s at 50 mbar were used for the determination of Cefu [14,15].

2.3.2. High-performance liquid chromatography (HPLC)

A liquid chromatograph equipped with a diode array detector (Lichrograph, MERCK-Hitachi) was used. For the stationary phase, a reversed phase column (RP-18, nucleosile) was used. The mobile phase consisted of acetonitrile: water:phosphoric acid (30:70:0.5). Cefuroxime was determined by measuring the UV absorption at 260 nm [16].

2.4. Determination of partition coefficients

The partitioning coefficients of cefuroxime with or without absorption enhancers in 1:1, 1:5, and 1:10 molar ratios were determined between water and *n*-octanol (Table 1). These two phases were saturated with each other. The compounds were dissolved in the water phase (200 μg/ml). The *n*-octanol/buffer solutions (phosphate, pH 7.4) (3 ml/3 ml) were filled into suitable vials and shaken for 12 h at 37 °C. After separation of the samples into the two phases, the drug content was analysed by CZE and HPLC.

The partitioning coefficient was calculated using the following equation:

$$P_{ow} = \frac{a_o}{a_w} \quad (1)$$

where a_o and a_w were the concentrations of the drugs in the *n*-octanol and in the aqueous phases, respectively.

2.5. Determination of permeation coefficients

The transport model system was described by Neubert et al. [17]. The donor and the acceptor compartments were separated by a dodecanol collodium membrane. The effective permeation area of the dodecanol collodium membrane was 15.8 cm^2. For permeation, cells were simultaneously used at 37 °C. 20 ml of this solution (200 μg/ml of drugs) were placed in the donor compartment and 20 ml of the buffer (phosphate, pH 7.4) were filled into the acceptor compartment. Samples (2.0 ml) were periodically removed from the acceptor compartments over 4 h, after 4 h from the donor compartments, and the drug content was analysed by HPLC and CZE [14–16].

2.6. Determination of the content of cefuroxime in the membrane

The membrane was removed from the model after 4 h and shaken in 20 ml water for 30 min. After 30 min shaking, the membrane was removed and repeatedly washed with water. The membrane was then dried and dissolved in 2 ml ethanol:water in a ratio of 90:10. After 30 min the solution was filtered and measured using HPLC.

Table 1
Partitioning coefficients of cefuroxime in *n*-octanol/buffer solution

Absorption enhancers	P_{ow}				
	1:2*	1:5*	1:10*	1:40*	cmc [mM]
Cefuroxime (Cefu)	0.4 ± 0.04	–	–	–	
Cefu-CTB	0.46 ± 0.20	1.80 ± 0.40	3.60 ± 0.30	–	0.92
Cefu-NCP	0.50 ± 0.20	0.60 ± 0.10	2.40 ± 0.20	–	0.58
Cefu-BAC	0.90 ± 0.10	0.30 ± 0.10	2.80 ± 0.20	–	6.9
Cefu-LTB	1.00 ± 0.10	1.60 ± 0.20	2.40 ± 0.30	4.8 ± 0.40	17.9

*, Molar ratio: Cefuroxime:absorption enhancer pH = 7.4; $T = 37$ °C, $C_0 = 200$ μg/ml, $n = 8$. CTB, Hexadecyltrimethylammonium bromide; NCP, *N*-Hexadecylpyridinium bromide; BAC, Hexadecyldimethylbenzyl-ammonium chloride; LTB, Dodecyltrimethyl-ammonium bromide.

2.7. Calculation of the transepithelial flux

Eq. (2) was used for the calculation of the transepithelial flux. The slope of the linear regression gives the permeation rate. The transepithelial flux ($J_{m\rightarrow a}$) can be calculated according to the following equation:

$$J_{m\rightarrow a} = \frac{dQ}{dt \times A} \quad (2)$$

$J_{m\rightarrow a}$ is the transepithelial flux, dQ/dt is the permeation rate, A represents the membrane area.

2.8. Calculation of the permeation coefficient

The slope was determined through linear regression. That is, the permeation instalment was related to the area (dQ/dtA). The permeation coefficient was calculated using the following equation:

$$P_G = \frac{dQ}{dt \times A \times C_0} \quad (3)$$

A represents the membrane area, $A = 15.8$ cm^2 (dodecanol collodium membrane), $A = 0.1963$ cm^2 (small intestine), C_0 is the starting concentration.

2.9. The mucus membrane model

The model was described by Wagner et al [18]. A Charles-River guinea pig with a weight of 300–400 g was killed through cerebral dislocation. 30 cm of small intestine was taken out. Buffer, 5 mM KCl, 1 mM KH_2P0_4, 26 mM $NaHCO_3$ and 122 mM NaCl were used for these experiments. 200 μg/ml Cefu was investigated alone or in combination with surfactants in ratios of 1:2 and 1:10. The experiments were performed at 37 °C for 3 h.

3. Results

To measure the hydrophobic/hydrophilic properties of Cefu, partition coefficients (P_{ow}) in the *n*-octanol/water system were investigated (Table 1). Cefu is a very hydrophilic drug and exhibits very small partition coefficients. The partition coefficients of Cefu were determined through the combination with cationic absorption enhancers below the cmc (ion-pair formation) and above the cmc (aggregation form). Cefu alone shows a small P_{ow} of 0.4. The combination with cationic absorption enhancers below the cmc increases the lipophilicity of Cefu till 9-fold. The combination with cationic absorption enhancers above the cmc leads to an increase of the P_{ow} and of the lipophilicity of Cefu till 15-fold (Table 1).

Cefu alone exhibited no absorption via the lipid membranes (artificial lipid membranes). The combination with LTB leads to a permeation rate of Cefu of 54 μg/cm^2

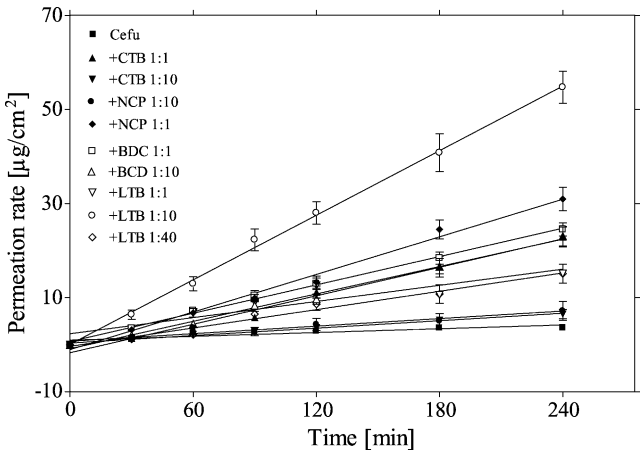

Fig. 1. Permeation rate of cefuroxime (μg/cm^2) using artificial lipid membrane ($C_0 = 200$ μg/ml, $n = 8$).

(Fig. 1) and a permeation coefficient ranging from 0.92×10^{-7} to 1.93×10^{-7} m/s (Fig. 2). Above the cmc, the permeation coefficient decreases to 0.64×10^{-7} m/s (Fig. 2). In the membrane, Cefu was found at all molar ratios between 117 and 300 μg. Using a combination with CTB in molar ratios of 1:1 and 1:10, Cefu was transported with a permeation rate of 22.9 and 6.5 μg/cm^2, respectively. The combinations with NCP and BAC lead also to a transport of Cefu across the dodecanol collodium membrane. The content of Cefu in this membrane above the cmc was significantly smaller than below the cmc (Fig. 3).

In the in vitro transport model at native intestinal epithelium (Charles-River guinea pig), it turnes out that the largest permeation rate of Cefu was reached using LTB below the cmc with about 45 μg/cm^2 after 3 h (Fig. 4). The permeation coefficients of Cefu using a combination with absorption enhancers were in the range from 0.125×10^{-7} to 1.36×10^{-7} (Fig. 5). The content of Cefu in the mucus increases significantly with all cationic absorption enhancers (Table 2). Cefu with LTB in a ratio of 1:40 with about 4.47% reached the largest dimension followed by BAC in a ratio of 1:10 with 4.6%. Similar to mucus, also in connective tissue (Table 2) the content of Cefu through the combination

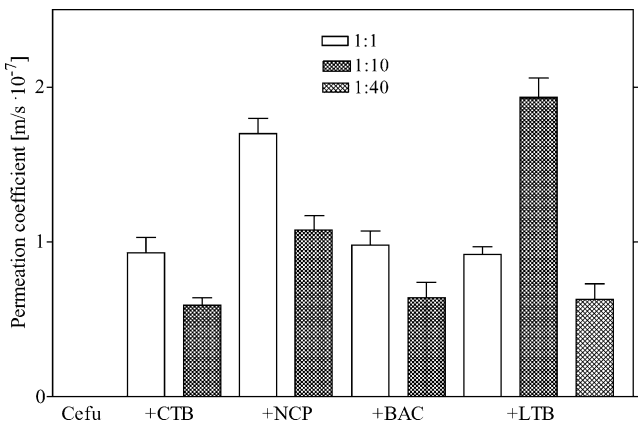

Fig. 2. Permeation coefficient of cefuroxime (m/s × 10^{-7}) using artificial lipid membrane ($C_0 = 200$ μg/ml, $n = 8$).

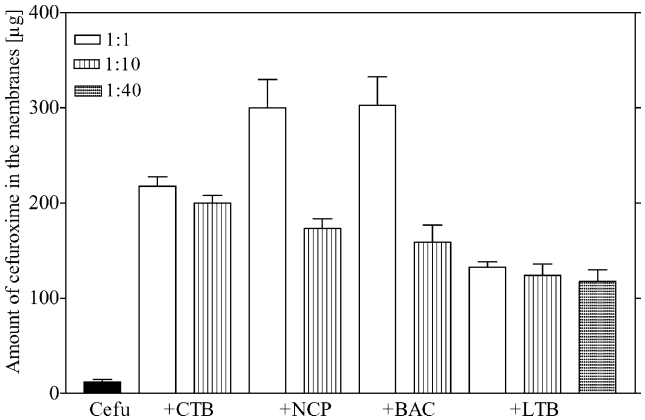

Fig. 3. Content of cefuroxime in the membrane (µg) using artificial lipid membrane.

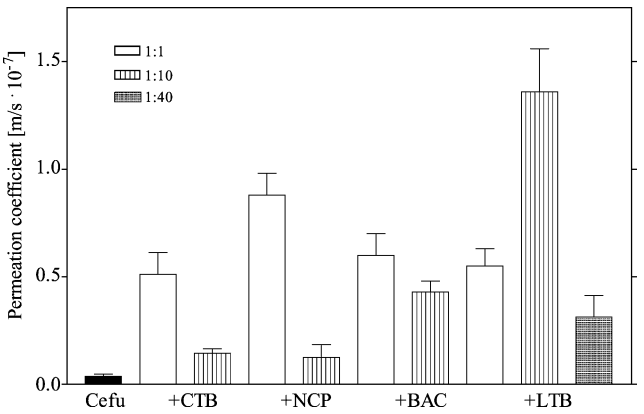

Fig. 5. Permeation coefficient of cefuroxime (m/s × 10^{-7}) using biological membrane (C_0 = 200 µg/ml, n = 8).

with LTB (1:10) shows the largest content with 2% followed by CTB in a ratio of 1:10 with 1.95%.

Furthermore, the influence of the absorption enhancers on the active D-glucose transport using guinea pig intestinal epithelium was studied. The concentration of D-glucose was measured in the acceptor using a radioactive substance marker [19]. The results show that the concentration profile of the glucose in the acceptor corresponds to that concentration given in the literature [20,21].

4. Discussion

The influence of enhancers on the absorption of cefuroxime was studied below and above the critical micelle concentration (cmc). It was observed that the partition coefficients in the n-octanol/water system were increased both below and above the cmc. Here, electrostatic interactions (ion-pair formation) and hydrophobic interactions (aggregation) play a significant role. The investigation using the model with artificial lipid membranes shows an exprimary enriching of Cefu in the membrane through the combination with cationic absorption enhancers. The investigation using the model with biological lipid membranes leads to the enrichment of Cefu in the mucus membrane on the one hand, and the permeation into the acceptor on the other hand. The results with LTB confirmed that the optimal effect was obtained through the combination with absorption enhancers of an alkyl chain length of C12 [10]. D-glucose is transported actively via SGLT1. Here, we have investigated the influence of absorption enhancers on the active D-glucose transport using guinea pig intestinal epithelium. LTB influenced the active D-glucose transport only at a concentration of 20 mM. The other absorption enhancers did not significantly influence the active D-glucose transport at the supported concentrations (5 mM) (1:11) (Fig. 6) [22]. Tsuji et al. showed that the micellar solution of the cetyltrimethylammonium bromide protects β-lactam antibiotics from acidic decomposition [23]. The study of Park et al. supported our results [24]. Park et al. improved the nasal and intestinal resorption of cefotaxime through ion-pair formation with cetylpyridinium chloride, cetyltrimethylammonium bromide and benzalkonium chloride. Malcchiodi et al. showed that CTB does not affect adversely the tissue morphology of

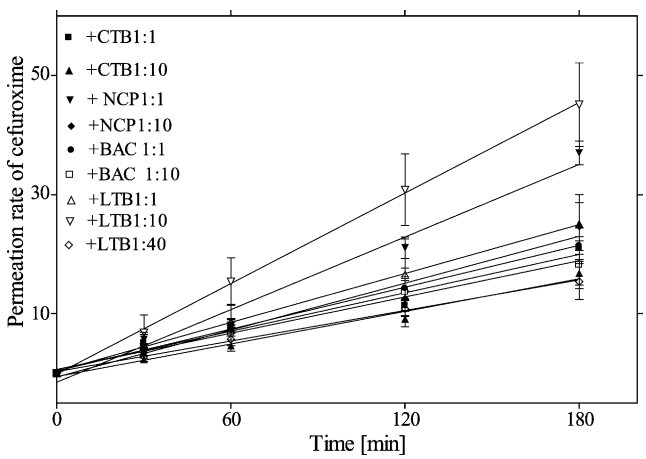

Fig. 4. Permeation rate of cefuroxime (µg/cm^2) using biological membrane (C_0 = 200 µg/ml, n = 8).

Table 2
Influence of cationic absorption enhancers on the permeation amount (%) of cefuroxime using guinea pig small intestine membrane (n = 8)

	Mucus	Connective tissue
Cefuroxime (only)	1.0 ± 0.1	0.40 ± 0.02
Cefuroxime-CTB 1:1	2.8 ± 0.4	1.73 ± 0.3
Cefuroxime-CTB 1:10	3.3 ± 0.5	1.95 ± 0.2
Cefuroxime-NCP 1:1	3.5 ± 0.8	1.0 ± 0.1
Cefuroxime-NCP 1:10	4.2 ± 0.9	1.3 ± 0.1
Cefuroxime-BAC 1:1	3.0 ± 0.3	0.97 ± 0.1
Cefuroxime-BAC 1:10	4.6 ± 0.4	0.86 ± 0.16
Cefuroxime-LTB 1:1	2.82 ± 0.5	1.84 ± 0.15
Cefuroxime-LTB 1:10	3.26 ± 0.7	1.99 ± 0.1
Cefuroxime-LTB 1:40	4.47 ± 0.6	1.88 ± 0.2

*, Molar ratio: Cefuroxime, absorption enhancer. pH = 7.4; T = 37 °C; n = 8. CTB, Hexadecyltrimethylammonium bromide; NCP, *N*-Hexadecylpyridinium bromide; BAC, Hexadecyldimethylbenzyl-ammonium chloride; LTB, Dodecyltrimethyl-ammonium bromide.

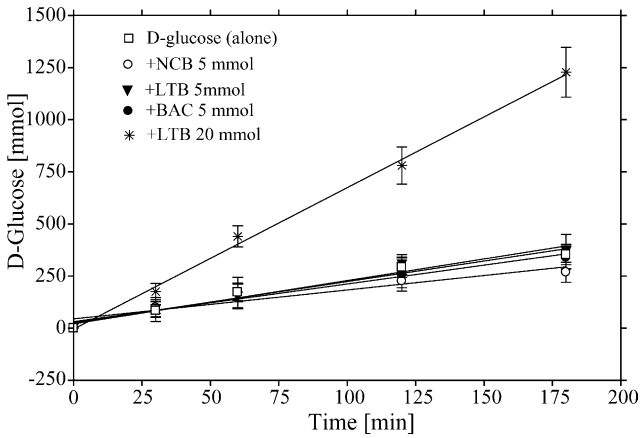

Fig. 6. Influence of surfactants on the transport of D-glucose using guinea pig intestinal epithelium ($n = 4$).

human colon mucus [25]. In addition, CTB and NCP were used as local mouth antiseptic having contact also with the intestine mucus [26].

5. Conclusion

The influence of the cationic absorption enhancers on the transport of cefuroxime was tested on in vitro transport models using artificial lipid membranes and biological lipid membranes. Different interactions between the cationic absorption enhancers and the membranes were observed. The ionic interactions between cefuroxime and cationic absorption enhancers below the cmc and hydrophobic interactions above the cmc strongly influence the permeation into and through the membranes. Tolerated concentrations of the absorption enhancers for the model of the active D-glucose transport with biological lipid membranes using guinea pig intestinal epithelium were 5 mM (1:11). As an alternative for the confirmation of the results, the influence of cationic absorption enhancers on the pharmacokinetics of cefuroxime in rabbits (in vivo) should be examined.

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