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Influence of enhancers on the absorption and on the pharmacokinetics of cefodizime using in-vitro and in-vivo models

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Abstract

In the development of novel antibiotics, more and more compounds have been found that cannot be absorbed orally and, therefore, must be administered intravenously or intramuscularly. Because of the obvious drawbacks of drug delivery by injection, the development of alternatives with enhanced oral bioavailability has received much attention in pharmaceutical research. Cefodizime, a novel third-generation cephalosporin with significant advantages in the parenteral treatment of common infections, was used as a model drug. Cefodizime behaves as a highly hydrophilic compound, as shown from its extremely low partition coefficient. The effect of cationic absorption enhancers (hexadecyldimethylbenzylammonium chloride, *N*-hexadecylpyridinium bromide, dodecyltrimethylammonium bromide and hexadecyltrimethylammonium bromide) on the lipophilicity of cefodizime was investigated by means of the n-octanol/water system. Results showed that the counter-ions had a positive influence on the solubility of cefodizime. These results on partitioning coefficients in the n-octanol/buffer system were confirmed using an in-vitro transport model with artificial and biological membranes (Caco-2-cells). Furthermore, the physiological compatibility of the absorption enhancers was investigated using the active D-glucose transport. The pharmacokinetic profile of cefodizime was evaluated in rabbits after intraduodenal administration with and without an absorption enhancer.

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Introduction

In recent years, a number of β-lactamase-stable, highly-active broad-spectrum cephalosporins have been developed and introduced into therapy (Durckheimer et al 1988; Kavi et al 1988; King & Cohen 1995). Most cephalosporins are administered intravenously or intramuscularly (Bryskier et al 1990; Brockmeier & Dagrosa 1992; Isert et al 1992); only a small number can be administered orally (Palin et al 1986; Eugenie 1996; Yamamoto et al 2000). The therapeutic use of very polar cephalosporins is generally restricted to parenteral delivery because of their poor passage through epithelial membranes. To improve the passage across epithelia, extensive research programs have been undertaken to develop more appropriate methods of delivery, including intestinal delivery, by using absorption-enhancing agents (Palin et al 1986; Nishihata et al 1987; Van Hoogdalem et al 1989). Cefodizime was administered to rats and dogs, either intravenously or intramuscularly, at various doses for one month. No toxicity resulted except for venous irritation and sporadic vomiting in some dogs receiving 2000 mg kg^{-1} daily (Abe et al 1988). In general, cefodizime can be compared favourably with other injectable antibiotics in terms of activity against common clinical pathogens (Wise et al 1990). It has an excellent pharmacokinetic profile making it suitable for use in a variety of infections and in the majority of patients (Bakk & Phillips 1990). In this study, we searched for possible influences on the lipophilicity and the absorption of cefodizime. In the literature, many possibilities for the improvement of the lipophilicity and the bioavailability of hydrophilic drugs are described. Improvement of the lipophilicity and the bioavailability of hydrophilic drugs has been obtained through manufacturing of prodrugs (Auterhoff et al 1991) and drugs have been conjugated to physiological substrates like peptides and bile salts (Smith et al 1992, 1996; Tacnet et al

1993; Lee et al 1996). In recent years, many studies were carried out to investigate the influence of absorption enhancers on the intestinal absorption of drugs (Kakemi et al 1969; Van Hoogdalem et al 1989; Sancho et al 1995). In this study, cefodizime was selected as a hydrophilic model drug (the solubility is $2.5 \pm 0.5\,g\,mL^{-1}$). Cefodizime is not accepted by the H^+/peptide transporter PEPT1. At the intestinal epithelium, this transport system is responsible for the uptake of many antibiotics, such as cephalosporins and penicillins, into the cell. Bretschneider et al (1999) reported a very low affinity of cefodizime at PEPT1 ($K_i = 22 \pm 3\,mmol\,L^{-1}$). In agreement, the cefodizime uptake into the cell and the transepithelial flux across Caco-2 monolayers were very low (flux = $0.3\,nmol\,cm^{-2}\,h$) at an extracellular concentration of 1 mM). In our work, cationic absorption enhancers were used for the development of new vehicle systems for the oral application of cefodizime. In-vivo and in-vitro studies were carried out. The effect of the cationic absorption enhancers on the lipophilicity of the model drug was investigated using the system n-octanol/buffer. The experimental results on the partition coefficient were confirmed using in-vitro standard transport model systems with artificial and biological membranes. We also investigated the influence of the most effective absorption enhancers on the enteral bioavailability of cefodizime after intraduodenal administration in rabbits.

Materials and Methods

Materials

Cefodizime was obtained from Hoechst (Germany). Hexadecyldimethylbenzylammonium 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 & Loretz, Hilden (Germany). The small intestine (Charles-River guinea-pig) was obtained from the Julius-Bernstein-Institute for Physiology, University Halle-Wittenberg (Germany). The human colon carcinoma cell line Caco-2 was obtained from the German collection of microorganisms and cell cultures (Braunschweig, Germany). Culture media and supplements were purchased from Life Technologies, Inc. (Germany).

Sample preparation

Standard solutions of cefodizime were prepared at $200\,\mu g\,mL^{-1}$ in phosphate buffer at pH 7.4 with or without absorption enhancers.

Analytical assay

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, temperature 25 °C and injection time of 9 s at 50 mbar were used for the determination of cefodizime (Mrestani et al 1996, 1997).

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). Cefodizime was determined by measuring the UV absorption at 264 nm (Van Krimpen et al 1987).

In-vitro models

Determination of partition coefficients

The partition coefficients of cefodizime with or without absorption enhancers in 1:2, 1:10, and 1:20 molar ratios were determined between water and n-octanol (Table 1). These two phases were saturated with each other. The compounds

Table 1 Influence of cationic absorption enhancers on the partition coefficient (P_{ow}) of cefodizime using n-octanol/water system.

Absorption enhancer	P_{ow}			CMC (mM)
	Molar ratio of cefodizime:absorption enhancer			
	1:2	1:10	1:20	
Cefodizime	0.06 ± 0.01	—	—	—
Cefodizime + CTB	0.12 ± 0.03	1.11 ± 0.21	4.14 ± 0.63	0.92
Cefodizime + NCP	0.84 ± 0.20	1.53 ± 0.12	6.86 ± 0.51	0.58
Cefodizime + BAC	0.31 ± 0.04	0.60 ± 0.12	5.36 ± 0.84	6.9
Cefodizime + LTB	0.18 ± 0.05	1.22 ± 0.44	1.98 ± 0.47	17.9

Data are means ± s.d., n = 8. pH = 7.4; T = 37 °C. CTB, hexadecyltrimethylammonium bromide; NCP, *N*-hexadecylpyridinium bromide; BAC, hexadecyldimethylbenzylammoniumchloride; LTB, dodecyltrimethylammonium bromide.

were dissolved in the water phase (200 μg mL^{-1}). The n-octanol/buffer solutions (phosphate, pH 7.4) were filled into suitable vials and shaken for 12 h at 37 °C. After separation of the samples into both phases, the drug content was analysed by CZE and HPLC.

The partition coefficient was calculated using equation 1:

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

where a_o and a_w are the concentrations of the drug in the n-octanol and in the aqueous phases, respectively.

Determination of permeation coefficients

The transport model system was described by Neubert & Fürst 1989. The donor and the acceptor compartments were separated by a dodecanol collodium membrane (effective permeation area 15.8 cm^2). For permeation, cells were simultaneously used at 37 °C. Twenty millilitres of a solution containing 200 μg mL^{-1} of drugs were placed in the donor compartment and 20 mL of the buffer (phosphate, pH 7.4) were placed in the acceptor compartment. Samples (2.0 mL) were periodically removed from the acceptor compartments over 4 h. After sample removal, the sample volume (2 mL) was topped up. The drug content of every sample was analysed by HPLC and CZE (Van Krimpen et al 1987; Mrestani et al 1996, 1997).

Determination of the content of cefodizime in the membrane

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

Calculation of transepithelial flux

Equation 2 was used for the calculation of the transepithelial flux. The slope of the linear regression gives the permeation rate.

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

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

Calculation of the permeation coefficient

For the calculation of the permeation coefficients (artificial lipid membranes, Charles-River guinea-pig and Caco-2) the permeation amount of the drug per cm^2 surface area was replotted versus the time. The slope (dQ/dt) is, here, the permeation rate. A linear calibration curve was obtained with a correlation coefficient of 0.993–0.998. The slope (dQ/dt) was determined through linear regression. That is, the permeation instalment was related to the area (dQ/dtA). The permeation coefficient was calculated using equation 3.

$$P_G = dQ/dt \cdot A \cdot C_0 \quad (3)$$

A is the membrane area (A = 15.8 cm^2 for dodecanol collodium membrane, A = 0.1963 cm^2 for small intestine, A = 4.71 cm^2 for Transwell cell) and C_0 is the starting concentration.

Mucous membrane model

The model was described by Wagner (1996). A Charles-River guinea-pig, 300–400 g, was killed by cerebral dislocation and a 30-cm section of small intestine was taken out. Buffer (5 mM KCl, 1 mM KH_2PO_4, 26 mM $NaHCO_3$ and 122 mM NaCl) was used for these experiments; cefodizime 200 μg mL^{-1} was investigated alone or in combination with surfactants in ratios of 1:2 and 1:20. The experiments were performed at 37 °C for 3 h. For the mucous membrane model, 0.5 mL of this solution was placed in the donor compartment and 5 mL of the buffer were placed into the acceptor compartment. Samples (200 μL) were periodically removed from the acceptor compartment over 3 h (30, 60–180 min). The sample volume (200 μL) was topped up after sample was removed. The solutions in the acceptor were set under an atmosphere of 95% O_2 and 5% CO_2. The drug content was analysed by CZE and HPLC (Van Krimpen et al 1987; Mrestani et al 1996, 1997). The D-glucose experiments were performed similarly. The D-glucose content was determined using the radioactive substance marker [^{14}C]mannitol (Doerffel et al 1994). In the acceptor compartment 10 mM mannitol was used. In the donor compartment 10 mM D-glucose was used alone or in combination with surfactants at concentrations of 5 mM and 20 mM.

Caco2-cell system

The human colon carcinoma cell line Caco-2 (passages 64–78) was routinely cultured in 75-cm^2 culture flasks in minimum essential medium supplemented with 10% fetal bovine serum, penicillin (100 U mL^{-1}), streptomycin (100 μg mL^{-1}) and 1% non-essential amino-acid solution (Bretschneider et al 1999). Subconfluent cultures were treated for 5 min with Dulbecco's phosphate-buffered saline followed by a 2-min incubation with 0.25% trypsin solution. Cells were seeded in 35-mm disposable petri dishes (Becton Dickinson, UK) at a density of 0.75×10^6 cells per dish. Under these conditions cells reached confluence the next day. Microscopic examination, after incubation with surfactants (0.1–5 mM) for 10 min, was performed on the 7th day after confluence. The uptake buffer contained (in mM): 25 Hepes/Tris (pH 7.5), 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.8 $MgSO_4$ and 5 glucose. Caco-2 cells were also cultured on permeable polycarbonate Transwell cell culture inserts (diameter 24.5 mm, pore size 3 μm; Costar GmbH, Bodenheim, Germany) (Bretschneider et al 1999). Subcultures were started at a cell density of 43 000 cells/cm^2 and cultured for 17–23 days. The lower (receiver) compartment contained 2.6 mL medium and the upper (donor) compartment 1.5 mL medium. All experiments were performed at 37 °C in a shaking water bath. After washing the inserts with buffer for 10 min, uptake was started by adding uptake buffer (in mM: 25 Hepes/Tris, pH 7.5, 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.8 $MgSO_4$ and 5 glucose) containing cefodizime (1 mM) with or without surfactants (0.25 mM) to the donor side. At time intervals of 10, 30, 60 and 120 min, 200 μL of the samples were taken from the receiver compartment and replaced with fresh buffer

(pH 7.5). Stability of cefodizime in the experimental system was checked by capillary electrophoresis. The effect of surfactants on the integrity of the Caco-2 cell monolayers was studied by measuring the transepithelial electrical resistance (TEER). Moreover, the effect of the surfactants on simple diffusion was determined by measuring the transepithelial flux of [^{14}C]mannitol (5 μM) from the apical (donor) to the basolateral compartment in buffer (in mM: 25 Hepes/Tris, pH 7.5, 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.8 $MgSO_4$ and 5 glucose). Samples for radioactivity measurements by liquid scintillation counting were taken from the receiver compartment (10–120 min).

In-vivo models

Rabbits (feminine) (Chinchilla Bastard and New Zealand White, 3–5 kg; Charles River Kisslegg, Germany) were fasted for 18 h. For the experimental preparation, the rabbits were narcotised with 50 mg kg^{-1} pentobarbital sodium (SPOFA, United Pharmaceutical Works Prague, Czech Republic). The rabbits were fixed onto an operation table which was kept at constant temperature (38 °C). Polyethylene tubes of different diameters were inserted into a carotid artery, common bile duct, ureters and duodenum. To prevent clotting and to stabilise the blood circulation, an infusion of 36 mL h^{-1} of heparinized physiological saline solution was administered using an infusion pump (Program2; Becton Dickinson, France).

Drug administration and sampling

Cefodizime with or without absorption enhancers was administered intraduodenally to rabbits via an inserted polyethylene tube in the duodenum. The dose of cefodizime was 20 mg kg^{-1} in phosphate buffer at pH 7.4 with or without surfactants. Cationic absorption enhancers were used as model enhancers to demonstrate basal absorption increasing ability in ratios of 2:1 (LTB–cefodizime, BAC–cefodizime) and 20:1 (NCP–cefodizime). The volume of the solution was 10 mL kg^{-1} ethanol–Soerensen phosphate buffer (1:5 v/v) at a pH of 7.4. For comparison, cefodizime (20 mg kg^{-1}) was also injected as bolus intravenously via the femoral vein. Following the administration of cefodizime, 3 mL blood were withdrawn from the carotid artery with a syringe containing 3 mL sodium citrate solution (3.13%) at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5 and 6 h. The blood was centrifuged at 3000 rev min^{-1} for 10 min to obtain plasma, which was kept at −20 °C until analysis. Bile and urine were sampled via the cannulas in the ductus choledochus or ureters at different intervals between 0 and 6 h after dosing and the samples were kept at −20 °C until further processing.

Calculation of the pharmacokinetic parameters

Where mean results are given, the values are shown together with values of standard deviation (s.d.). The pharmacokinetic parameters C_{max}, t_{max}, and AUC (area under the plasma concentration–time curve) were calculated using the PC program TOPFIT 2.0 (Heinzel et al 1993).

Calculation of the amount (%) of cefodizime in bile and urine

The amount of cefodizime in rabbit bile and urine was calculated for every sample using equation 4.

$$m = c.V \quad (4)$$

where m and c were the amount and the concentration of cefodizime, respectively, and V is the volume of the sample. The total amount of cefodizime after 6 h was calculated by the addition of the amounts of cefodizime at different times.

Analytical assays

The determination of cefodizime in rabbit plasma, bile and urine was performed by CZE (Mrestani et al 1996, 1997) and HPLC (Van Krimpen et al 1987).

Statistical methods

For statistics, the one-way analysis of variance post-hoc test, the Tukey's test (GraphPad Prism 03, biostatistics, curve fitting and scientific graphing) was used for the determination of significant differences in this work. A significance level of $P < 0.5$ denoted significance in all cases. The analysis of variance test (Tukey's test) exhibited significant differences for the partitioning coefficients of cefodizime with absorption enhancers in 1:2, 1:10 and 1:20 molar ratios (Table 1). The effect of the various absorption enhancers on the permeation through the artificial lipid membranes differed significantly (Table 2). The permeation rates of the various systems were also significantly different at different times. No significant differences were observed in the effect of various ratios on the content and on the permeation of cefodizime below and above the critical micelle concentration (CMC) at the guinea-pig small membrane model (mucous, connective tissue and acceptor) and at artificial lipid membranes (Table 3, Figures 1 and 2). Significant differences were

Table 2 The permeation amount (%) of cationic absorption enhancers through artificial lipid membranes.

	Donor (%)	Membrane (%)	Acceptor (%)
NCP–cefodizime 2:1	75.0 ± 1.3	6.7 ± 1.4	17.9 ± 1.5
NCP–cefodizime 20:1	62.3 ± 1.7	25.4 ± 1.8	11.6 ± 1.2
BAC–cefodizime 2:1	60.6 ± 1.2	7.7 ± 1.1	29.7 ± 1.4
BAC–cefodizime 20:1	45.3 ± 1.5	22.8 ± 1.6	31.9 ± 2.2
LTB–cefodizime 2:1	61.5 ± 1.2	0.0	38.3 ± 3.1
LTB–cefodizime 20:1	66.2 ± 1.5	2.5 ± 0.6	29.8 ± 2.1

Data are means ± s.d., n = 8. CTB, hexadecyltrimethylammonium bromide; NCP, *N*-hexadecylpyridinium bromide; BAC, hexadecyldimethylbenzylammonium chloride; LTB, dodecyltrimethylammonium bromide.

Table 3 Influence of cationic absorption enhancers on the permeation amount (%) of cefodizime using guinea-pig small intestine membrane.

	Mucus	Connective tissue	Acceptor
Cefodizime (only)	0.0	1.1 ± 0.3	0.5 ± 0.1
Cefodizime–NCP 1:2	11.0 ± 1.1	14.7 ± 1.7	15.6 ± 2.1
Cefodizime–NCP 1:20	4.9 ± 0.6	13.9 ± 1.6	20.0 ± 2.4
Cefodizime–BAC 1:2	9.6 ± 1.1	12.1 ± 1.2	13.2 ± 1.7
Cefodizime–BAC 1:20	3.3 ± 0.8	11.4 ± 1.3	16.3 ± 2.1
Cefodizime–LTB 1:2	1.6 ± 0.8	2.5 ± 0.9	9.9 ± 1.2
Cefodizime–LTB 1:20	14.4 ± 1.2	16.7 ± 1.6	13.0 ± 1.6

Data are means ± s.d., n = 8. CTB, hexadecyltrimethylammonium bromide; NCP, *N*-hexadecylpyridinium bromide; BAC, hexadecyldimethylbenzylammonium chloride; LTB, dodecyltrimethylammonium bromide.

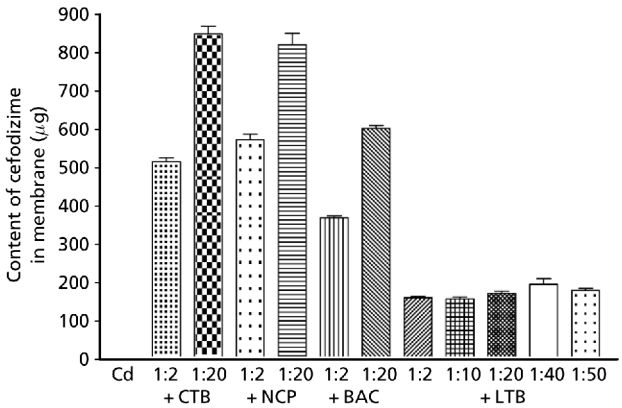

Figure 1 Content of cefodizime (Cd) in the artificial lipid membrane. A dodecanol collodium membrane with an effective permeation area of 15.8 cm^2 separated a donor compartment containing 20 mL of a solution containing 200 $\mu g\,mL^{-1}$ of cefodizime (alone or in combination with surfactants) from an acceptor compartment containing 20 mL of buffer (phosphate, pH 7.4). The experiments were performed at 37 °C for 4 h.

observed in the effect of various ratios on the permeation coefficient using artificial membrane. The influence of absorption enhancers on the active D-glucose transport exhibited a significant difference (Figure 3). The change in the plasma concentration of cefodizime seen in Figure 4, using various absorption enhancers, led also to significant differences. Furthermore, the analysis of variance post-hoc test (Tukey's test) was used for the comparison of all models with one another (partition coefficients, transport model at artificial lipid membranes, Charles-River guinea-pig and rabbit models). For all models used in this study, the transport of cefodizime using absorption enhancers exhibited significant differences ($P < 0.05$).

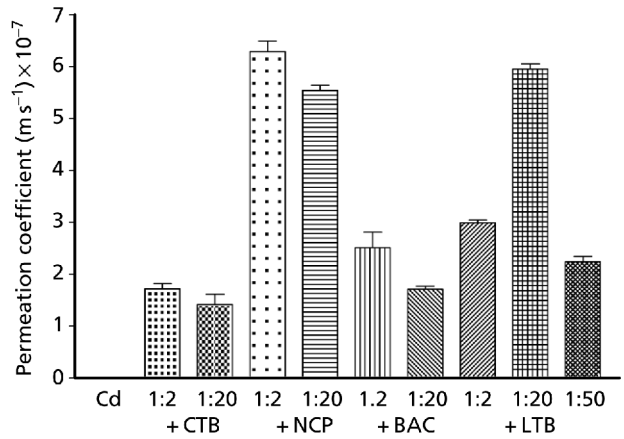

Figure 2 Permeation coefficient of cefodizime (Cd) using guinea-pig intestinal epithelium ($C_0 = 200\,\mu g\,mL$). Data are means ± s.d., n = 8. Cefodizime (0.5 mL of a solution containing 200 $\mu g\,mL^{-1}$) alone or in combination with surfactants was placed in the donor compartment and 5 mL of buffer (5 mM KCl, 1 mM KH_2PO_4, 26 mM $NaHCO_3$ and 122 mM NaCl) was placed in the acceptor compartment. The experiments were performed at 37 °C for 3 h. The solutions in the acceptor were set under an atmosphere of 95% O_2 and 5% CO_2.

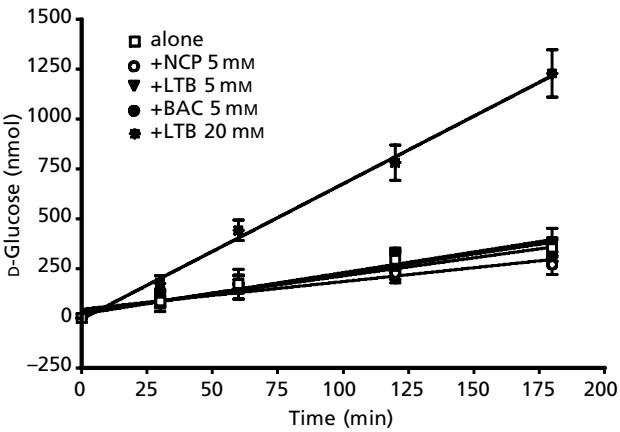

Figure 3 Influence of surfactants on the transport of D-glucose using guinea-pig intestinal epithelium ($C_0 = 200\,\mu g\,mL$). Data are means ± s.d., n = 4. Experiments were performed similarly to those studying the permeation coefficient of cefodizime; in the acceptor compartment 10 mM mannitol was used and in the donor compartment 10 mM D-glucose was used alone or in combination with surfactants at concentrations of 5 mM and 20 mM.

Results

In-vitro models

Partition coefficients (P_{ow})

The partition coefficients of cefodizime (Table 1) were investigated in the n-octanol/water system to measure the hydrophobic/hydrophilic properties. Cefodizime exhibited very small partition coefficients. It is a very

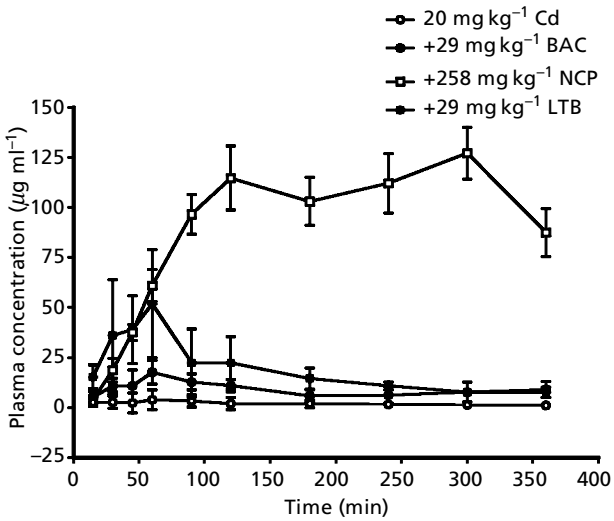

Figure 4 The mean arterial plasma concentration–time profiles of cefodizime (Cd) 20 mg kg^{-1} after intraduodenal administration to rabbits (Chinchilla). BAC, hexadecyldimethylbenzylammonium chloride; NCP, *N*-hexadecylpyridinium bromide; LTB, dodecyltrimethylammonium bromide.

hydrophilic drug and contains a number of polar groups. It is anionic at pH 7.4 (Mrestani et al 1998).The combination with cationic absorption enhancers leads to a considerable improvement in the partition coefficients. The partition coefficients of cefodizime were determined through the combination with cationic absorption enhancers below the CMC (ion pair formation) and above the CMC (aggregation form). Cefodizime alone shows a small P_{ow} of 0.06. The combination with cationic absorption enhancers below the CMC in a ratio of 1:2 increases the solubility of cefodizime up to 14 fold. The combination with cationic surfactants above the CMC leads to an increase of the P_{ow} of cefodizime, with NCP about 115 fold, with BAC about 89 fold and with LTB about 33 fold.

In-vitro transport model at artificial lipid membranes

For calculation of the permeation coefficients, the permeation amount of the drug per cm^2 surface area was replotted versus the time. The slope (dQ/dt) represents here the permeation rate. In Figures 2 and 3 the results with artificial lipid membranes are shown. Cefodizime alone was not transported via the lipid membranes. The combination with LTB led to a permeation rate for cefodizime of 98 μg cm^{-2} and a permeation coefficient ranging from 1.06×10^{-7} m s^{-1} (1:2) to 5.01×10^{-7} m s^{-1} (1:40). In the combinations with LTB at 1:10 and 1:20, the permeation coefficients were 1.9×10^{-7} m s^{-1} and 3.5×10^{-7} m s^{-1}. Above the CMC at a concentration of 50 mM, the permeation rate decreased to 26 μg cm^{-2} and the permeation coefficient to 1.18×10^{-7} m s^{-1}. In the membrane, cefodizime was found at all molar ratios between 4 and 21%. Using a combination with CTB in a molar ratio of 1:2, cefodizime was transported with a permeation rate of 6 μg cm^{-2} and a permeation coefficient of 0.3×10^{-7} m s^{-1}. No transport of cefodizime via the lipid membranes was observed above the CMC. The combination with NCP and BAC led to no transport of cefodizime across the dodecanol collodium membranes. The content of cefodizime in the membrane below the CMC was also significantly smaller than above the CMC (Figure 1). The transport of the absorption enhancers used here was also investigated using artificial lipid membranes. Table 2 summarises the data on the content of the cationic absorption enhancers in the donor, in the membrane and in the acceptor.

In-vitro transport model using native intestinal epithelium (Charles-River guinea-pig)

All cationic absorption enhancers influenced the transport of cefodizime in a positive manner using the biological membranes. The largest permeation rate of cefodizime was achieved with NCP below the CMC (70 μg cm^{-2} after 3 h); the smallest permeation rate of cefodizime was observed with CTB below the CMC (18.5 μg/cm^2 after 3 h). The permeation coefficients of cefodizime ranged from 1.3×10^{-7} to 6.5×10^{-7} m s^{-1} (Figure 2). The content of cefodizime in the mucus decreased significantly with all cationic absorption enhancers (Table 3). Cefodizime with LTB in a ratio of 1:20 resulted in the largest amount (about 14.4%), followed by NCB in a ratio of 1:2 (11%). Similar to mucus, also in connective tissue the content of cefodizime in combination with LTB 1:20 resulted in the largest content (16.7%), followed by NCP in a ratio of 1:2 (14.7%).

Influence of the absorption enhancers on the active D-glucose transport

D-Glucose is transported actively via the Na^+/glucose cotransporter SGLT1. Here, we have investigated the influence of the absorption enhancers on active D-glucose transport using guinea-pig intestinal epithelium. The concentration of D-glucose was measured in the acceptor using a radioactive substance marker. Figure 3 shows that LTB influenced the active D-glucose transport only at a concentration of 20 mM. The other absorption enhancers did not influence the active D-glucose transport at the supported concentrations (5 mM).

In-vitro transport model with Caco-2-cells

The influence of the cationic absorption enhancers on the transport of cefodizime was investigated in Caco-2-cells. In the cell culture investigations it was observed that the absorption enhancers influence the structure and the composition of the Caco-2 cell very strongly. At high concentrations of absorption enhancers the Caco-2 cells were damaged. The macroscopic and microscopic investigations showed that the test surfactants should be used only in a concentration of 0.25–0.5 mM. Concentrations larger than 0.5 mM led to damage of the Caco-2 cell layer. The permeation coefficients of cefodizime were determined with or without absorption enhancers in the Caco-2 cell model. The diffusion of cefodizime through the Caco-2 cell layer was very slow ($P_G = 1.5 \times 10^{-9}$ m s^{-1}). The combination of cefodizime with 0.5 mM NCP ($P_G = 2.0 \times 10^{-7}$ m s^{-1})

or 0.5 mM CTB ($P_G = 2.2 \times 10^{-7}$ m s^{-1}) led to a 120-fold increase in the permeation coefficient in comparison with cefodizime without enhancer.

In-vivo models

Cumulative bile excretion

In bile, cefodizime was detected after intraduodenal administration with absorption enhancers or alone. The concentration of cefodizime in bile after use alone was 0.1–0.94 μg mL^{-1}. BAC and NCP increased the biliary elimination of cefodizime. The concentration of cefodizime in bile after co-administration with BAC and NCP was 0.5–4.97 μg mL^{-1}. The concentration of cefodizime in bile after administration with LTB was 1.33–8.66 μg mL^{-1}. The cumulative biliary excretion of cefodizime after 6 h was 0.18% with LTB, 0.16% with NCP and 0.08% with BAC, expressed as a percentage of the administered dose in comparison with cefodizime used alone (0.02%).

Cumulative urinary excretion

The combination of cefodizime with absorption enhancers led to about a 12-fold increase in the amount of cefodizime in urine with LTB (25% total), about seven-fold with NCP (15%) and about three-fold with BAC (7% total) in comparison with cefodizime used alone (2.1% total) after 6 h.

Plasma concentration–time profiles

The pharmacokinetics and absolute bioavailability of cefodizime were determined after intravenous and intraduodenal administration to rabbits (Chinchilla) (Figure 4). The AUC of cefodizime alone was 835 ± 121 μg min mL^{-1} after intraduodenal administration (n = 6) and 36013 ± 12872 μg min mL^{-1} (n = 6) after intravenous administration. T_{max} and C_{max} were about 58 ± 8 min and 4 ± 3 μg mL^{-1} after intraduodenal administration of cefodizime alone. After intraduodenal administration of cefodizime in combination with absorption enhancers, the plasma concentration was significantly higher than after administration without absorption enhancers. When cefodizime was administered simultaneously with BAC, C_{max} was 19 ± 6 μg mL^{-1}, T_{max} was 61 ± 6 min and the AUC was four-fold higher (3069 ± 1022 μg min mL^{-1}, n = 4) than when cefodizime was used alone. After co-administration of cefodizime with LTB the averaged maximum concentration was 52 ± 27 μg mL^{-1}. This combination of cefodizime with LTB led to an increase in C_{max} of about 13 fold compared with cefodizime used alone. Between 30 and 60 min after dosing the T_{max} was observed. The AUC of the cefodizime–LTB combination was ten times (8348 ± 3821 μg min mL^{-1}, n = 4) that of cefodizime used alone. Co-administration of cefodizime with NCP led to the enhancement of C_{max} by 32 fold (127 ± 13 μg mL^{-1}), of T_{max} by around 50 fold (300 ± 12 min) and of the AUC by around 40 fold (33476 ± 8749 μg min mL^{-1}, n = 4) in comparison with cefodizime without absorption enhancers. The absolute bioavailability of cefodizime used alone after intraduodenal administration was 3%, for the co-administration with BAC it was 9%, with LTB 23% and with NCP 88%. Table 4 summarises the pharmacokinetic parameters of cefodizime used alone or co-administered with absorption enhancers.

Table 4 Pharmacokinetic parameters of cefodizime after separate or simultaneous administration with absorption enhancers to rabbits.

	T_{max} (min)	C_{max} (μg mL^{-1})	AUC (μg min mL^{-1})
Cefodizime (i.v.) (n = 6)	—	—	36013 ± 12872
Cefodizime (i.d.) (n = 6)	58 ± 8	4 ± 3	835 ± 121
Cefodizime–BAC (i.d.) (n = 4)	61 ± 6	19 ± 6	3069 ± 1022
Cefodizime–NCP (i.d.) (n = 4)	300 ± 12	127 ± 13	33476 ± 8749
Cefodizime–LTB (i.d.) (n = 4)	60 ± 5	52 ± 27	8348 ± 3821

i.v., intravenous; i.d., intraduodenal; NCP, *N*-hexadecylpyridinium bromide; BAC, hexadecyldimethylbenzylammonium chloride; LTB, dodecyltrimethylammonium bromide.

Discussion

In this study, cefodizime was selected because it was not accepted as a substrate by the intestinal H^+/peptide transporter PEPT1, and no other active transport mechanism has been shown. However, cefodizime can overcome the biological membrane barriers by passive diffusion. The combination of cefodizime with absorption enhancers was used below and above the critical micelle concentration (CMC). The partition coefficients in the n-octanol/water system were increased both below and above the CMC. We assumed that electrostatic interactions (ion-pair formation) and hydrophobic interactions (aggregation) play a significant role. Using a model with artificial lipid membranes, primary enrichment of cefodizime in the membrane through the combination with cationic absorption enhancers was observed. The same combination of this model with biological lipid membranes led to both enrichment of cefodizime in the mucous membrane and permeation into the acceptor. The results with LTB confirmed that the optimal effect was obtained through the combination with absorption enhancers of an alkyl chain length of C12 (Van Hoogdalem et al 1989). Tsuji et al (1982) showed that the micellar solution of cetyltrimethylammonium bromide protects β-lactam antibiotics from acidic decomposition. The study of Park et al (1995) supported our results. Park et al (1995) improved the nasal and intestinal resorption of cefotaxime through ion-pair formation with cetylpyridinium chloride, cetyltrimethylammonium bromide and benzalkonium chloride. The improvement of the transport of cefodizime by the cationic absorption enhancers used here was also investigated using a model with Caco-2-cells. However, the cells were

very sensitive to these absorption enhancers, so we could not apply the same concentrations of cefodizime and of the cationic absorption enhancers that were used in the other models. It is known that amphiphilic organic surfactants can bind to an unspecific site of the luminal cell membrane via hydrophilic or lipophilic interactions. This binding strength decreases with an increasing in the alkyl chain length (Saitoh et al 1990). The influence of the surfactant on active D-glucose transport has been examined in biological lipid membranes (Brot et al 1986). The results showed that the concentration profile of the glucose in the acceptor corresponded to that concentration given in the literature (Kakemi et al 1969; Grass & Sweetana 1998). Our results show that cells remain viable for at least 180 min in this model. The model system can be used for the experiment described above. The influence of the absorption enhancers on D-glucose transport through the small intestine layer demonstrated that LTB, at a concentration of 20 mM, can damage the cell membrane. The other absorption enhancers do not influence significantly the D-glucose transport at the concentration used (Figure 3). The membrane model with Caco-2-cells was applied to study the influence of the absorption enhancers on the cells. Here, we measured the flux of the paracellular diffusion marker mannitol. The epithelium was damaged at certain concentrations of the absorption enhancer (0.25–0.5 mM). Our results indicate that Caco-2-cells are very sensitive to cationic absorption enhancers, although Malchiodi-Albedi et al (1991) showed that CTB does not adversely affect the tissue morphology of human colon mucus. In addition, CTB and NCP have been used as local mouth antiseptics having contact also with the intestinal mucus (Collins & Deasy 1990). In this study, the rabbit model was found to be very useful for parallel investigation of biliary and renal excretion in comparison with plasma concentration–time profiles of drugs after separate or simultaneous administration. The intestinal absorption of cefodizime after intraduodenal administration in combination with BAC, LTB or NCP was significantly higher than when cefodizime was administered alone. The absolute bioavailability of cefodizime was enhanced to 88% through co-administration with NCP in comparison with cefodizime used alone (3%). The co-administration of cefodizime and NCP turned out to be useful for increasing the enteral bioavailability of cefodizime. The evaluation of transport systems is very important for the development of different pharmaceutical formulations in different vehicle systems.

Conclusion

The influence of cationic absorption enhancers on the transport of cefodizime was tested on in-vitro and in-vivo transport models using artificial lipid membranes, biological membranes, Caco-2-cells and the rabbit model. The rabbit model was used because intraduodenal administration and also simultaneous sampling of plasma, bile and urine are possible. Caco-2-cells are very sensitive to cationic absorption enhancers in comparison with other membranes. Tolerated concentrations of the cationic absorption enhancers for the Caco-2-cell model were found to be 0.2–0.5 mM. Ionic interactions between cefodizime and cationic absorption enhancers below the CMC (ion-pair formation) and hydrophobic interactions above the CMC (aggregation) have a very strong influence on the permeation in and through the membranes. In-vivo experiments have to be performed to determine the effect of absorption enhancers on the pharmacokinetics of cefodizime. An absolute bioavailability of 88% for cefodizime was achieved by the combination with NCP. These results could serve as evidence for an increased bioavailability of cefodizime using absorption enhancers in an in-vivo animal model.

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