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Influence of absorption enhancers on the pharmacokinetic properties of non-oral β-lactam-cefpirom using the rabbit (Chinchilla) in vivo model

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Abstract

The oral application is the application of the first choice for drug administration. A lot of drugs exhibit relatively low bioavailability. This may be caused by binding of the drug in the gastro-intestinal tract, by poor penetration of the intestinal mucose or by highly hydrophilic properties. Therefore, problem drugs were only used for i.v. administration (intravenously) or for i.m. administration (intramuscularly). In the present study, cefpirom was investigated as a model substance. Cefpirom (Cp) is a semi-synthetic amino-2-thiazolyl-methoxyimino cephalosporin. It exhibits highly hydrophilic properties ($P_{ow} = 0.02 \pm 0.01$) and a very low bioavailability (AUC = 524 ± 403 μg min/ml). It was only applied i.v. or i.m. In this work, the influence of absorption enhancers (aggregation and ion-pair formation) on the bioavailability and on the hydrophilic properties of Cp was investigated. The bioavailability of cefpirom was improved through the combination with absorption enhancers (hexadecyldimethylbenzylammonium chloride, BAC; hexylsalicylic acid, HSA). The absolute bioavailability of the Cp combination with absorption enhancers was 21 times larger for BAC and 15 times larger for HSA than in the case when Cp was used alone.

Keywords: Cefpirom; Partition coefficients; Pharmacokinetics

1. Introduction

A large number of important cephalosporins show no or only poor absorption from the intestinum. Therefore, they have to be applied intravenously or intramuscularly (Bryskier et al., 1990; Brockmeier and Dagrosa, 1992; Isert et al., 1992). Only a small number of cephalosporins can be administered orally (Eugenie, 1996; Yamamoto et al., 2000; Palin et al., 1986). To enable oral administration, one has to search for modifications which improve the absorption of such drugs. In the literature, many possibilities for the improvement of the lipophilicity and the bioavailability of hydrophilic drugs are described. For instance, improvement of the bioavailability of hydrophilic drugs was obtained through manufacturing of prodrugs Auterhoff et al. (1991). Nishihata et al. studied the effect of absorption enhancers on rectal absorption of β-lactam antibiotics Nishihata et al. (1987). In recent years, a lot of studies was carried out to investigate the influence of absorption enhancers on the intestinal absorption of drugs (Van Hoogdalem et al., 1989; Sancho et al., 1995; Kakemi et al., 1969). Tsuji et al. showed that the micellar solution of cetyltrimethylammonium bromide protects β-lactam antibiotics from acidic decomposition Tsuji et al. (1982). Park et al. improved the nasal and intestinal resorption of cefotaxim through ion-pair formation with cetylpyridinium chloride, cetyltrimethylammonium bromide and benzalkonium chloride Park et al. (1995). We reported recently on our investigations of the influence of bile salts (anionic absorption enhancers) on the transport of the extremely hydrophilic cephalosporin cefpirom in in vitro and in vivo transport models using artificial lipid membranes and in the rabbit model Mestani et al. (2004). The effect of cationic absorption enhancers and HSA on the bioavailability and on the hydrophilic properties of the model drug cefpirom was investigated using the *n*-octanol/buffer sys-

Abbreviations: Cp, cefpirom; i.d., intraduodenal; BAC, hexadecyldimethylbenzylammonium chloride; HSA, hexylsalicylic acid; CZE, capillary zone electrophoresis; HPLC, high performance liquid chromatography; cmc, critical micelle concentration

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tem. The experimental results on the partition coefficients were confirmed using in vitro and in vivo model systems with artificial membranes and rabbits.

2. Materials and methods

2.1. Materials

Cefpirom (Cp) was obtained from Hoechst (Frankfurt, Germany). Hexadecyldimethylbenzylammonium chloride (BAC) and hexylsalicylic acid (HSA) were obtained from Sigma-Aldrich Chemie (München, Germany). Collodium 4%, diethyl ether, ethanol, dodecanol and *n*-octanol were obtained from Caesar & Lorentz (Hilden, Germany).

2.2. Sample preparation

Standard solutions of cefpirom were prepared at 200 μg/ml in phosphate buffer at pH 7.4 for the determination of partition coefficients (P_{ow}) and for the application in the permeation model.

2.3. Analytical assays

2.3.1. CZE

The 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.

A 20 mM phosphate buffer (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 Cp (Mrestani et al., 1996, 1997).

2.3.2. HPLC

A liquid chromatography system 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 a mixture of acetonitrile:water:phosphoric acid (15:85:0.5). Cefpirom was determined by measuring the UV absorption at 260 nm Van Krimpen et al. (1987).

2.4. In vitro models

2.4.1. Determination of partition coefficients (P_{ow})

The partitioning coefficients of cefpirom with absorption enhancers in 1:1, 1:10, and 1:20 molar ratios were determined between buffer (phosphate, pH 7.4) and *n*-octanol Mestani et al. (2004).

2.4.2. Permeation model

The transport model system was described by Neubert and Fürst (1989). The donor and the acceptor compartments were separated by a dodecanol collodium membrane. The effective permeation area of dodecanol collodium membrane was 15.8 cm^2. For permeation, cells were simultaneously used at 37 °C. Twenty milliliters of the corresponding solution (200 μg/ml of the drug) 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 sample removal, the sample volume (2 ml) was filled up. The drug content was analysed for every sample by HPLC and CZE.

2.4.3. Determination of the content of cefodizim 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.

2.5. In vivo models

2.5.1. Rabbit model

Female rabbits (Chinchilla Bastard and New Zealand White, 3–5 kg body weight (b.w.), Charles River, Kisslegg, Germany) were fasted for 18 h. For the experimental preparation, the rabbits were narcotised by 50 mg/kg b.w. pentabarbitone sodium (SPOFA, United Pharmaceutical Works Prague, Czech Republic). The animals 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. In order to prevent clotting and to stabilise the blood circulation, an infusion of 36 ml/h of heparinized physiological saline solution was administered using an infusion pump (Program2, Becton Dickinson, France). The investigations were approved by the Thüringer Landesverwaltungsamt, Germany, 74043-2684.04-52/99. Cp with absorption enhancers was administered i.d. via an inserted polyethylene tube in the duodenum. The doses of Cp were 100 mg/kg in phosphate buffer at pH 7.4. The volume of the solution was 10 ml/kg ethanol/Soerensen phosphate buffer (1:5, v/v) at a pH of 7.4. For comparison, Cp (100 mg/kg b.w.) was also injected as bolus intravenously (i.v.) via the femoral vein. Following the Cp administration 3 ml blood was 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 rpm for 10 min to obtain plasma, which was kept at −20 °C until analysis. Bile and urine were sampled via the cannulas in ductus choledochus or ureters at different intervals between 0 and 6 h after dosing and the aliquots were kept at −20 °C until further processing. The determination of Cp in plasma, bile and urine was performed by CZE (Mrestani et al., 1996, 1997) and HPLC Van Krimpen et al. (1987). The pharmacokinetic parameters C_{max}, t_{max}, and area under the plasma concentration–time curve (AUC) were calculated using the PC program TOPFIT 2.0 (Heinzel et al., 1993).

Table 1
Partition coefficients of cefpirom in the system *n*-octanol/water at pH 7.4

	P_{ow}			cmc (mM)
	1:1[a]	1:10[a]	1:20[a]	
Cefpirom (Cp)	0.02 ± 0.01			
Cp + BAC	0.532 ± 0.42	0.660 ± 0.35	0.356 ± 0.45	4.2 ± 1.1
Cp + HSA	0.215 ± 0.11	0.333 ± 0.14	0.456 ± 0.12	–

Data are mean ± S.D., $n = 8$, pH 7.4, $T = 37\,^{\circ}C$. BAC, hexadecyldimethylbenzylammonium chloride; HSA, hexylsalicylic acid.
[a] Molar ratio: cefpirom:absorption enhancer.

3. Results and discussion

The combination with bile salts as anionic absorption enhancers below and above the cmc leads to an improvement of the permeation, of the lipophilicity properties and of the bioavailability of cefpirom. We have found recently that the AUC of the Cp combination with anionic absorption enhancers was 17 times larger than that when Cp was used without absorption enhancers (Mestani et al., 2004). In the present study, the effect of cationic absorption enhancers (below and above the cmc) and of HSA as ion-pair formation on the improvement of the bioavailability of cefpirom was investigated. For the characterisation of the hydrophobic/hydrophilic properties of Cp with absorption enhancers, partition coefficients (P_{ow}) in the *n*-octanol/buffer system were used. Cp is a very hydrophilic drug and exhibits very small partition coefficients ($P_{ow} = 0.02 \pm 0.01$). The partition coefficients of Cp were determined through the combination with cationic absorption enhancers below the cmc and above the cmc (aggregation form). The combination with BAC below the cmc increases the lipophilicity of Cp till 27-fold. The combination with BAC above the cmc leads to an increase of the P_{ow} and of the lipophilicity of Cp till 18-fold. The combination with varying concentrations of HSA (ion-pair formation) led to an improvement of the lipophilicity of Cp till 23-fold (Table 1). Electrostatic interactions (ion-pair formation) and hydrophobic interactions (aggregation) between HSA, BAC and Cp led to an increase of the lipophilicity of Cp.

Furthermore, the influence of BAC and HSA on the permeation of cefpirom through artificial lipid membranes (dodecanol collodium membranes) (Table 2) was studied. Cp without absorption enhancers showed no transport via the lipid membranes. The combination with BAC and HSA led to an improvement of the permeation of Cp via the lipid membranes. Only Cp was found in the membrane. The content of Cp in the membrane was between 0.3 and 6%.

Table 2
Amount of cefpirom (%) in the artificial lipid membranes after 4 h

	In the membrane (%)	In the acceptor (%)
Cp (4 mg, total amount of Cp in the donor)	0	0
Cp + BAC, 1:1	0.3 ± 0.31 (12 μg of Cp)	0
Cp + BAC, 1:10	0.7 ± 0.45 (28 μg of Cp)	0
Cp + HSA, 1:1	4.0 ± 0.15 (160 μg of Cp)	0
Cp + HSA, 1:10	6.0 ± 0.25 (240 μg of Cp)	0

Data are mean ± S.D., $n = 8$; pH 7.4, $T = 37\,^{\circ}C$. BAC, hexadecyldimethylbenzylammonium chloride; HSA, hexylsalicylic acid.

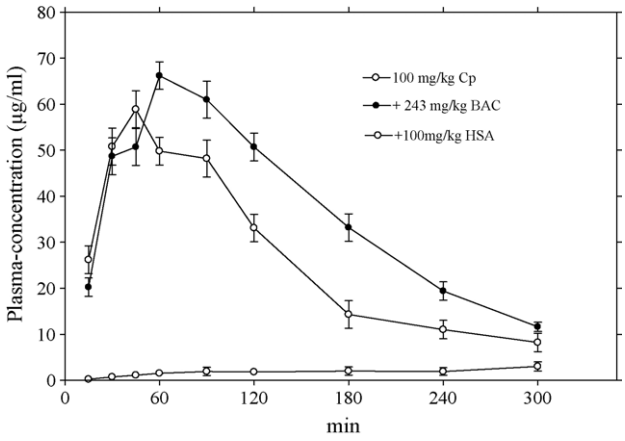

Fig. 1. The mean arterial plasma concentration–time profiles of cefpirom after i.d. administration using rabbits (Chinchilla). BAC, hexadecyldimethylbenzylammonium chloride; HSA, hexylsalicylic acid.

In the present study, the rabbit model was found to be very useful for parallel investigation of biliary and renal excretion in comparison to plasma concentration–time profiles of drugs after separated or simultaneous administration. The cumulative biliary excretion of Cp after 6 h was 0.34% with BAC and 0.4% with HSA of the administrated dose in comparison to Cp used without absorption enhancers (0.05%). The combination of Cp with BAC leads to an increase of the amount of Cp in urine of about 16%, with HSA of about 10% in comparison to Cp when it was used without absorption enhancers (2.1% total) after 6 h. The pharmacokinetics and the absolute bioavailability of Cp were detected after i.v. and i.d. administration using Chinchilla rabbits (Fig. 1). The AUC of Cp used without absorption enhancers after i.d. administration was 524 ± 403 μg min/ml ($n = 6$) and after i.v. administration 18907 ± 9368 μg min/ml ($n = 6$). After i.d. administration of Cp in combination with BAC and HSA, the plasma concentration was significantly higher than in the case without absorption enhancers (Fig. 1). When Cp was administered simultaneously with BAC, C_{max} was 66 ± 3 μg/ml, T_{max} was 60 ± 2 min and the AUC was 20-fold higher (10903 ± 1220 μg min/ml, $n = 4$) than in the case when Cp was used without absorption enhancers (Table 2). The combination of Cp with HSA led to an increase of C_{max} of about 20-fold compared to Cp used without absorption enhancers (Table 2). The absolute bioavailability of Cp used without absorption enhancers after i.d. administration was 2.8%, for the co-administration with BAC 59% and with HSA 42%. The investigations in the present study demonstrated that non-oral β-lactam-cefpirom can be used for peroral administration through the combination with BAC and HSA. A lot of other studies supported our results. Investigations of Tsuji and Park showed that cationic absorption enhancers can be used in order to protect β-lactam antibiotics from acidic decomposition and for the improvement of nasal and intestinal resorption of cefotaxime through ion-pair formation (Tsuji et al., 1982; Park et al., 1995). In addition, cationic

Table 3
Pharmacokinetic parameters of cefpirom after separate or simultaneous administration of absorption enhancers

	T_{max} (min)	C_{max} (μg/ml)	AUC_{0-300} (μg min/ml)
Cp, i.v. ($n = 6$)			18907 ± 9368
Cp, i.d. ($n = 6$)	90 ± 2.0	3 ± 2.0	524 ± 403
Cp:BAC i.d. ($n = 3$)	60 ± 2.0	66 ± 3.0	10903 ± 1220
Cp:HSA i.d. ($n = 4$)	45 ± 2.0	59 ± 4.0	7864 ± 3151

i.v., Intravenous; i.d., intraduodenal; BAC, hexadecyldimethylbenzylammonium chloride; HSA, hexylsalicylic acid.

absorption enhancers were also used as local mouth antiseptics Collins and Deasy (1990) (Table 3).

4. Conclusion

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. The administration (intravenously or intramuscularly) of non-oral β-lactam-cephalosporins is accompanied by many problems and high costs. The aim of the present work was to demonstrate that non-oral β-lactam-cefpirom should be used for peroral applications. Here, cationic absorption enhancers and HSA have been used for the development of new vehicle systems for the oral application of cefpirom. The absolute bioavailability of Cp in combination with BAC and HSA after i.d. administration shows that the aim of the work could be achieved.

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