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Research paper

Microemulsion and mixed micelle for oral administration as new drug formulations for highly hydrophilic drugs

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ABSTRACT

Microemulsions (MEs) and mixed micelles (MMs) have been used as new drug formulations for high hydrophilic drugs such as cefpirom and cefodizim for oral administration. Cefpirom and cefodizim are neither actively nor passively transported across cell membranes. Up to date, they can be only administrated intravenously (i.v.) or intramuscularly (i.m.). The rabbit (Chinchilla) *in vivo* model was used in the present work to investigate ways of overcoming the poor oral absorption of these cephalosporins. The cephalosporins at 100 mg/kg were formulated in MEs and MMs and administered intraduodenally (i.d.). Very low bioavailability (2.5–3.0%) was observed, if cefpirom or cefodizim i.d. were applied without colloidal vehicle. However, the addition of the cephalosporins to ME or MM is shown to be highly effective in increasing the bioavailability values (up to 64% absolute bioavailability) of the model drugs. In conclusion, MEs and MMs improve essentially the oral bioavailability of the high hydrophilic drugs.

1. Introduction

Cephalosporins such as cefpirom (Cp) and cefodizim (Cd) (Fig. 1) are highly hydrophilic drugs. Most of them were only administered intravenously (i.v.) or intramuscularly (i.m.). Only a few numbers of cephalosporins are suitable for oral administration. Therefore, we investigated if it is possible to improve the lipophilicity as well as the pharmacokinetic properties of highly hydrophilic drugs. In the present study, Cp and Cd were selected as highly hydrophilic model drugs. Cp and Cd are not accepted as a substrate by the intestinal H^+/peptide symporter PEPT1, and no other active transport mechanism could be identified for these drugs [1]. A lot of studies were carried out in order to investigate the influence of absorption enhancers on the intestinal absorption of drugs [2–4]. Absorption enhancers are widely used as additives in the preparation of pharmaceuticals.

Seulki et al. studied the pharmacokinetics of a new, orally available ceftriaxone formulation in physical complexation with a cationic analogue of bile acid in rats [5]. Mrestani et al. investigated the effect of the anionic and cationic absorption enhancers on the absorption and on the pharmacokinetics of Cp and Cd using *in vitro* and *in vivo* models [6,7]. The use of mixed micelles (MMs) for the solubilization of highly lipophilic drugs is known four decades [8]. Mixed micelles were developed only for highly lipophilic drugs [9]. Examples are Valium® MM [10] and Konakion® MM [11]. These MM for i.v. application exhibit good stability and compatibility [9,12,13]. Sandimmun Optoral (cyclosporine formulation) and Immunosporin® (microemulsion) have been used for peroral application [14,15]. Microemulsions (MEs) are transparent fine dispersions stabilized by surfactant molecules. Such dispersions are easy to formulate, and various structures (oil/water or water/oil) can be obtained. In contrast to emulsions, MMs and MEs are thermodynamically stable. This stability makes them interesting as drug carrier systems. The application of MEs has also drawn attention in the field of solubilization techniques. For instance, the ME formulations of cyclosporine, a highly lipophilic and water-insoluble drug, have been shown to improve oral bioavailability and decrease absorption variation [16,17]. In recent years, lipid MEs incorporating medium-chain glycerides have attracted much interest as oral dosage forms to improve dissolution of highly lipophilic drugs and/or intestinal absorption [18]. While the number of publications on the possible application of aerosol OT MEs for topical drug delivery is already large, aerosol OT applicability for oral ME drug delivery still needs to be studied [19–23].

In the present work, MMs and MEs for the development of new drug formulations for oral administration of highly hydrophilic drugs such as Cp and Cd were used. For highly hydrophilic drugs, such drug formulations were not developed until now [19,20].

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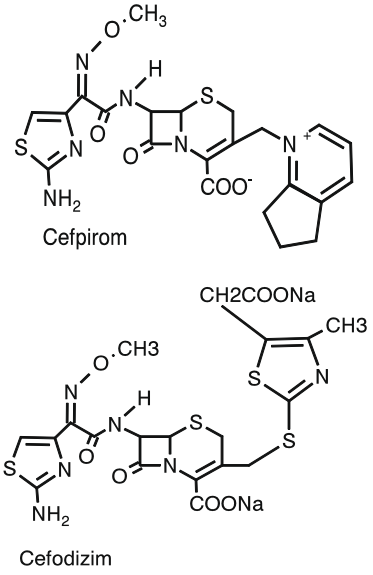

Fig. 1. Chemical structure of cefpirom and cefodizim.

2. Materials and methods

2.1. Materials

Cefpirom (Cp) and cefodizim (Cd) were obtained from Hoechst (Germany). Sodium salt of glycodeoxycholic acid was obtained from Sigma–Aldrich Chemie (Germany). Oleic acid and polysorbate 20 (Tween® 20) were purchased from Fluka (Buchs, Switzerland) (see Figs. 2 and 3).

2.2. Analytical assays

2.2.1. Capillary zone electrophoresis (CZE)

A Hewlett Packard Model G1600A (Waldbronn, Germany)® CE system was used for the determination of the cephalosporins. The cephalosporins were detected 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 [24,25].

2.2.2. High pressure liquid chromatography (HPLC)

The HPLC method (Lichrograph, MERCK-Hitachi) was used for the determination of the cephalosporins. A reversed-phase column (RP-18, nucleosile) was used for the stationary phase. The mobile phase consisted of acetonitrile–water–phosphoric acid (15:85:0.5). The cephalosporins were determined by measuring the UV absorption at 264 nm. The injection volume was 20 μl, the temperature of the column was 25 °C and the flow rate was 1 ml/min [26].

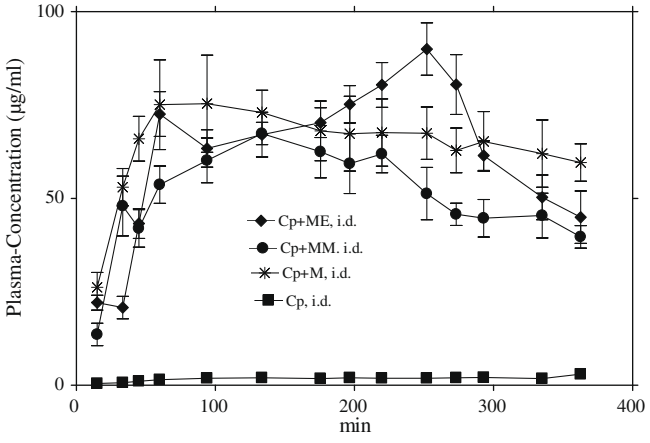

Fig. 2. Plasma concentration–time profiles of cefpirom after i.d. administration (means ± s.d.).

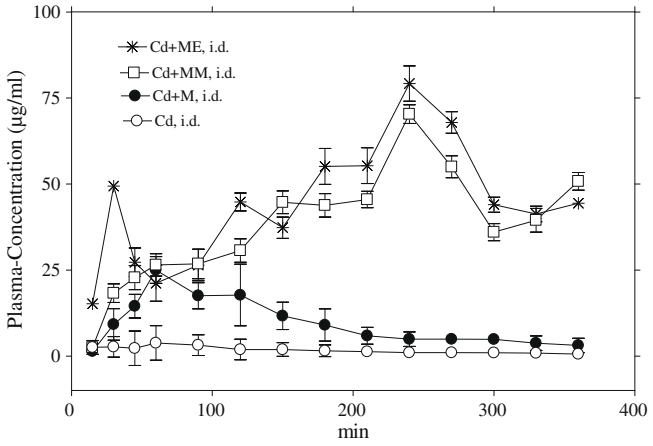

Fig. 3. Plasma concentration–time profiles of cefodizim after i.d. administration (means ± s.d.).

2.2.3. Affinity capillary electrophoresis (ACE)

The technique of affinity capillary electrophoresis was used to determine the partitioning behaviour of Cp and Cd to microemulsion and to mixed micelles. The partitioning behaviour was characterized through calculating the capacity factor of cephalosporins [27].

2.2.3.1. Calculation of capacity factor. Capacity factor represents the ratio between bound and free analyte in the micellar solution.

The capacity factor k' of analyses was determined from the calculated effective ion mobility using the following equation [27]:

$$k' = \frac{\mu_0 - \mu_m}{\mu_m - \mu_{mc}}$$

where μ_m is the calculated effective ionic mobility of the solute in micellar solution, μ_0 is the effective ionic mobility of solute in the buffer and μ_{mc} is the effective ionic mobility of the micelle [27].

2.3. Sample preparation (solution)

Cp or Cd (488 mg) were dissolved in the buffer solution, filling up to a volume of 100 ml.

2.4. Preparation of drug formulations

2.4.1. Preparation of the micelle (M)

A 2% (w/v) solution of sodium glycodeoxycholic acid in phosphate buffer pH 7.4 was prepared.

Cephalosporins (488 mg) were dissolved in the M solution, filling up to a volume of 100 ml. The solution was stirred for 25 min using ultrasonic bath. The solution was then kept at 2 °C in the fridge until further processing.

2.4.2. Preparation of the mixed micelle (MM)

A 2% (w/v) of sodium glycodeoxycholic acid and 10% (w/v) polysorbate 20 solution in phosphate buffer pH 7.4 was prepared.

Solution A: the amount of sodium glycodeoxycholic acid (2.0 g) was dissolved in 20 ml phosphate (pH 7.4).

Solution B: 10 g of polysorbate 20 were also dissolved in 20 ml of the buffer solution (pH 7.4).

(1) Solution A was added to solution B under stirring until a clear solution was formed. The solution was left to stand for 18 h at room temperature filling up to a volume of 100 ml.

(2) Cephalosporin (488 mg) were dissolved in the MM solution, filling up to a volume of 100 ml. The solution was stirred for 25 min using ultrasonic bath. The solution was then kept at 2 °C in the fridge until further processing.

2.4.3. Preparation of the microemulsion (ME)

A 0.9432% (w/v) of sodium glycodeoxycholic acid, 10% (w/v) polysorbate 20 and 1% (w/v) oleic acid solution in phosphate buffer pH 7.4 was prepared.

Solution A: the amount of sodium glycodeoxycholic acid (0.9432 g) was dissolved in 20 ml of the buffer solution (pH 7.4).

Solution B: 10 g polysorbate 20 were also dissolved in 20 ml of the buffer solution (pH 7.4).

(1) Solutions A and B were added to 1 g of oleic acid under stirring (15 min, ultrasonic bath). Phosphate buffer was added to the mixture (to a volume of 95 ml). This mixture was stirred for 20 min using ultrasonic bath. The solution was left to stand for 18 h at room temperature until clear solution was formed filling up to a volume of 100 ml.

(2) Cephalosporin (488 mg) was dissolved in the ME solution, filling up to a volume of 100 ml. This solution was stirred for 25 min using ultrasonic bath.

2.5. In vivo models (rabbit model)

Female rabbits (Chinchilla Bastard and New Zealand White, 3–5 kg body weight (b.w.), Charles River, Kisslegg, Germany) were used for the *in vivo* model [6,7]. Cephalosporin with absorption enhancers (M, MM, ME) was administered i.d. via an inserted polyethylene tube in the duodenum. The doses of cephalosporin were 100 mg/kg in phosphate buffer at pH 7.4. The volume of the solution was 10 ml/kg ethanol/Sorensen phosphate buffer (1:5 v/v) at a pH of 7.4. For comparison, cephalosporin (100 mg/kg b.w.) was also injected as bolus intravenously (i.v.) via the femoral vein. Following the cephalosporin 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. The determination of cephalosporin in plasma was performed by HPLC [26]. 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 [28].

3. Results and discussion

For the determination of the affinity of Cp and Cd to MM and ME, the ACE technique was used [27]. This technique provides useful information on the partitioning behaviour of Cp and Cd to microemulsion and mixed micelle. The capacity factor values of Cp and Cd in M, MM and in ME are given in Table 1. Here, the capacity factor reveals the relationship between bound and non-bound drug in the colloidal vehicles. A high affinity of Cp and Cd in MM and ME was observed.

Microemulsions and mixed micelles were suited both as hydrophilic drugs and lipophilic drugs. That makes them very attractive as drug formulations. Its importance as drug formulations and particularly for hydrophilic drugs has not been investigated so far. For this purpose, we have developed a microemulsion system containing glycodeoxycholic acid sodium salt, polysorbate 20 and oleic acid as well as a mixed micelle containing glycodeoxycholic acid sodium salt and polysorbate 20. For the investigation of the influence of the colloidal vehicles on the pharmacokinetic properties of these hydrophilic drugs, the rabbit model was used. The rabbit model used in this study 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 pharmacokinetics and absolute bioavailability of Cp and Cd were determinated after i.v. and i.d. (intraduodenal) administration. It was observed that the concentration of Cp and Cd in plasma was significantly higher than in the case without vehicle systems.

The combination of Cp and Cd with mixed micelles and microemulsion led to an increase of C_{max} of about 29-fold compared to drugs used alone. Between 60 and 255 min after dosing, t_{max} was observed. Due to the higher plasma concentration of Cp and Cd, a definite increase of the AUC was observed. The AUC of the Cp and Cd combination with M, MM, and ME was between 19 and 23 times larger than that when Cp oder Cd was used alone (see Tables 2 and 3).

The absolute bioavailability of Cp or Cd without absorption enhancer after i.d. administration was 2.5% and 3%. The combination of Cp and Cd with mixed micelles and microemulsion led to an increase of the absolute bioavailability for Cp of 64% (Fig. 4) and for Cd of 54% (Figs. 4 and 5).

The high absolute bioavailability (>50%) of these extremely hydrophilic drugs studied in the present work opens a new possibility for the development and for the establishment of new and modern drug formulations.

These results are in good agreement with their k' values and confirm other *in vivo* research works which have shown that lipophilicity and bioavailability of cephalosporins have been strongly affected by combination with absorption enhancers. It was observed a linear relationship between k' and the absolute

Table 1
Capacity factor values (k') of Cp and Cd in various systems.

	M	MM	ME
Cefpirom	0.16	0.39	0.38
Cefodizim	1.99	18	19

M: 2% sodium glycodeoxycholic acid, MM: 2% sodium glycodeoxycholic acid, 10% polysorbate 20, ME: 20 mM sodium glycodeoxycholic acid, 10% polysorbate 20, 1% oleic acid.

Table 2
Pharmacokinetic parameters of cefpirom after separate or simultaneous administration of absorption enhancers.

	T_{max} (min)	C_{max} (μg ml^{-1})	AUC_{0-300} (μg min ml^{-1})
Cp, i.v. $n = 6$			18,906 ± 9367.8
Cp, i.d. $n = 6$	90	3	524 ± 402.8
Cp: ME, i.d. $n = 4$	255	90	12,059 ± 3487
Cp: MM, i.d. $n = 4$	140	67	11,533 ± 2050
Cp: M, i.d. $n = 4$	90	75	9437 ± 2985

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

	T_{max} (min)	C_{max} (μg ml^{-1})	AUC_{0-300} (μg min ml^{-1} l)
Cd, i.v. $n = 6$	–	–	36,013 ± 12,872
Cd, i.d. $n = 6$	58	4	995 ± 121
Cd: ME, i.d. $n = 4$	270	79	19,447 ± 2345
Cd: MM, i.d. $n = 4$	240	70	17,646 ± 1222
Cd: M, i.d. $n = 4$	60	25	3961 ± 3131

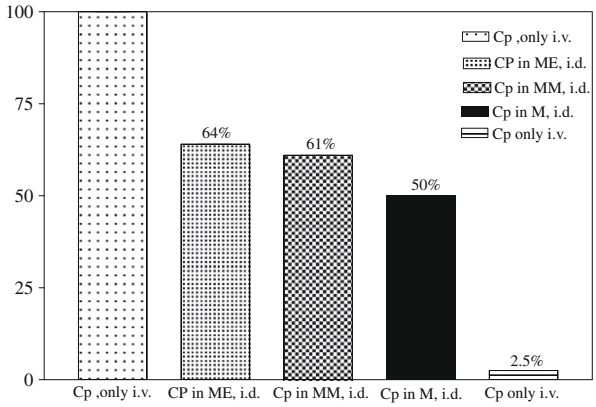

Fig. 4. Absolute bioavailability of cefpirom.

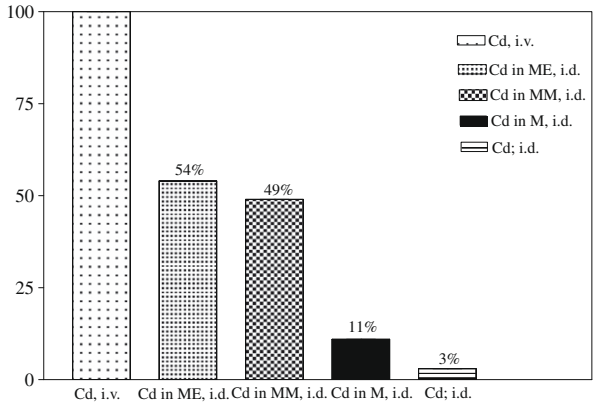

Fig. 5. Absolute bioavailability of cefodizim.

bioavailability. The correlation coefficient was for Cp 0.989 and for Cd 0.988. The bigger k' is the bigger is the AUC.

4. Conclusion

In the present study, cefpirom and cefodizim were investigated as model substances. The administration (intravenously or intramuscularly) of non-oral β-lactam–cephalosporins is accompanied by many problems and high costs. Therefore, the development of alternatives with enhanced oral bioavailability is receiving much attention in pharmaceutical research. The aim of the present study was to demonstrate that non-oral β-lactam–cephalosporins should be used for peroral applications using new drug formulations such as MM or ME. The influence of MMs and MEs on the bioavailability of the extremely hydrophilic cephalosporins was investigated using the *in vivo* rabbit model. Up to 50% absolute bioavailability of Cp and Cd was obtained by their combination with MMs and MEs. The results make the present work very interesting and open a new way for the development and generation of new drug formulations for extremely hydrophilic cephalosporins exhibiting very low bioavailability after peroral application.

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