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SIMULATED ABSORPTION OF ORAL CEPHALOSPORINS : DIFFUSION THROUGH ARTIFICIAL LIPID BARRIERS

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ABSTRACT

This paper describes the simulated gastro-intestinal absorption of cefradine, cefalexine and cephaloridine through artifical lipid barriers. Cefradine and cefalexine are usually administrated orally and cephaloridine was only used for parenteral injection. These experiments were carried out using a Sartorius absorption simulator and a partition coefficient system. At pH = 3 cefalexine and cefradine have a better diffusion in gastric phase than at pH = 6 in intestinal one. Coefficient partition values which were determined with Sartorius lipid phases confirmed these previous results. Cefalexine and cefradine are amphoteric drugs and are found in 98 % under Zwitterion species, the most liposoluble one at pH = 6 and 75 % at pH=3. Kp values were determined at pH = 5 and 6 using the surfactant of the gastric phase added to the intestinal phase. These experiments demonstrated the importance of tensioactif agent because the measured Kp

are about ten times higher than those determined without any surfactant. Moreover with this absorption simulator, initially used for passive transport, it was possible to verify a facilited transport for cefradine and cefalexine

INTRODUCTION

The purpose of biopharmaceutical investigations for oral cephalosporins was to research factors influencing bioavaibility of active drugs to optimize their pharmacokinetic and microbiological activity. The used previsional methods to simulate gastro-intestinal absorption of drugs is often relatively simple and may provide valuable information.

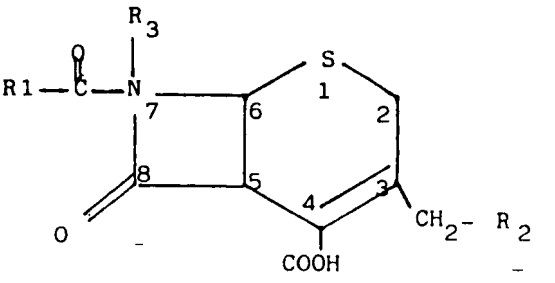
The aim of this study has been to simulate the gastro-intestinal absorption of cefradine and cefalexine using a Sartorius absorption simulator apparatus and a partition coefficient system. Cephaloridine,a parenteral cephalosporin has been chosen as a sample having an orally weak bioavaibility. The in vitro systems permitted to determine the area of the gastro-intestinal tract from which absorption was optimal, to explain the mechanism and to evaluate the kinetics of absorption.

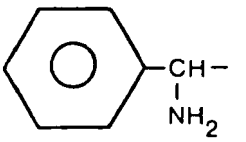
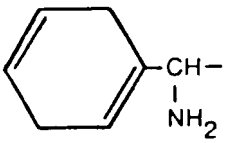
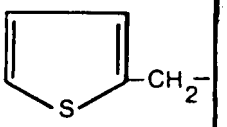
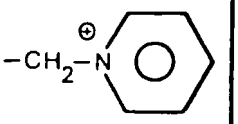
MATERIALS AND METHODS

Tested drugs

Three cephalosporins were experimented : cefalexine (CEX) (Eli Lilly), cefradine (CED) (Squibb) and cephaloridine (CER) (Glaxo). CER is usually administered by parenteral injection and the other ones are used for oral, intramuscular and intravenous administration. CEX and CED are amphoteric drugs and CER is a weak acid (Table 1).

Table 1
Chemical structures of cephalosporins



	R 1	R 2	R 3
CEFALEXINE pKa_1= 2,56 pKa_2= 6,88		$-CH_3$	-H
CEFRADINE pKa_1 = 2,53 pKa_2 = 7,30		$-CH_3$	-H
CEPHALO- RIDINE pKa_1 = 1,67			-H

The quantitative measurement of these antibiotics was made by an UV spectrophotometric assay at 260 nm for CEX and CED and 234 nm for CER. These measurements were compared with a control curve (1). The chemical stability of antibiotics in the experimental conditions was controlled by UV spectrophotometry.

Table 2
Composition of lipid phases

pH	1 and 2	3	5 and 6
Lipid gastric phase of Sartorius : dodecanol 4,2 g + surfactant 0,10 g	+	+	+
Lipid intestinal phase of Sartorius : dodecanol 0,92 g + caprylic acid 4 g		+	+
Other lipid phases:			
dodecanol		+	
dodecanol + caprylic acid + surfactant			+

Evaluation of the in vitro diffusion

The Sartorius absorption simulator was characterized by two compartments containing 100 ml of a buffered phase. The first simulated either the gastric (pH = 1 and 3) or the intestinal (pH = 5 and 6) phase and the second one the plasmatic phase (pH = 7.4). The composition of these aqueous buffers was described by Stricker (2, 3). These two compartments were divided by a lipid barrier composed with a cellulose nitrate membrane wetted by a mixture of dodecanol + surfactant for the artificial gastric barrier and dodecanol + caprylic acid for the intestinal one (Table 2).

The experimental procedure consisted essentially in following the concentration cephalosporin changes for 90 minutes in the two compartments. The plasma compart-

ment was bound to a spectrophotometric enregistrer which permitted to measure directly the increase of drug concentration in the recipient. The experimentation was carried out at 37°C using an initial cephalosporin concentration of 2 g/l for the intestinal simulation and 400 mg/l for the gastric one. These constatations were chosen taking in account the usual amount obtained after an oral administration of these drugs. The diffusion rate constant according to Stricker's works (3) or rate constant (Kd), through the artificial gastro-intestinal barrier was calculated from the slope of the straight line obtained plotting the variation drug concentrations in the simulated plasma phase versus time.
Kd is obtained according to the following equation :

$$Kd = \frac{C_{II_2} - C_{II_1}}{t_2 - t_1} = \frac{1}{C_{Io}} \times \frac{V_{IIo}}{A} \qquad (1)$$

C_{II}= concentration of the drug in the second phase (mg/ml)
C_{Io}= concentration of the drug in the first phase at the time = 0 (mg/ml) ;
V_{IIo}= initial volume of the second phase (ml)
A = effective barrier area (cm^2)

According to their diffusion drugs can be classified in three groups (2, 3) :
- drugs with a low diffusion $Kd < 1.10^{-3}$ $cm.min^{-1}$
- drugs with a medium diffusion :
  $1.10^{-3} < Kd < 5.10^{-3}$ cm. min^{-1}
- drugs with a high diffusion : $Kd > 5.10^{-3} cm.min^{-1}$

Evaluation of cephalosporins lipid phase/aqueous buffers partition coefficients

The partition coefficients (Kp) of cephalosporins were determined using a method derivated from the one described by Reese et al. (4). The antibiotic concentrations and the composition of the aqueous phases were identical to those used in the diffusion assays. The composition of lipid phases was described in table 2
5 ml of the buffered solution containing antibiotic were shaken with 3 ml of lipid phase in a stoppered pyrex tube at 37°C ± 1°C using horizontal shaking device. After 90 minutes, the two phases were separated by centrifugation 3 min at 3000 rpm. The initial and final concentrations of cephalosporins in the aqueous phase was determined spectrophotometrically. The apparent partition coefficient was determined according to the following equation :

$$Kp = \frac{C_o - C}{C_o} \qquad (2)$$

C_o = initial concentration of the cephalosporins in the aqueous phase

C = final concentrations of the drugs in the aqueous phase.

RESULTS

The Kd values obtained by the simulated intestinal barrier, pH = 6, are reported in table 3
For CED, the Kd was $0,7.10^{-3}$cm.min^{-1}; for CEX $0,40.10^{-3}$ cm.min^{-1} and for CER $0,17.10^{-3}$cm.min^{-1}. These results showed a best diffusion for CED than CEX, but a weakest diffusion for CER than the other ones.
A pH 3, with the simulated gastric barrier (Table 3)
CED had always the best diffusion Kd = $2,16.10^{-3}$cm.min^{-1} and CEX possessed a value a little lower Kd = $1,85.10^{-3}$

Table 3
Diffusion rate constants (Kd) x 10^{-3} $cm.min^{-1}$
means of 5 values

pH	1	2	3	6
cefradine	0,92	1,09	2,16	0,70
cefalexine	0,68	0,80	1,85	0,40
cephaloridine			0,68	0,17

$cm.min^{-1}$. These two antibiotics diffused better at pH = 3 than pH = 6 and they belong to the drugs having a medium diffusion, contrary to the CER which had a weak diffusion.

At pH = 1 and 2, experiments with CED and CEX are only undertaken because CER was unsteady. CED possessed always the best diffusion, only it was difficult to classify these two antibiotics according to the Strikers classification. For CED the Kd's values were equivalent at pH 1 and 2 and corresponded to the limit between a medium and a weak diffusion.

The partition coefficients obtained with the lipid phases of the Sartorius technique are shown in the table 4. Using the Sartorius gastric phase of Kp was higher for CED than CEX and the values increased from pH 1 to pH 3. With the intestinal phase (pH 5 and 6) Kp were very minimal CED possessed always higher values than CEX. Kp always appeared higher with gastric phase than intestinal one.

Other experimentations were undertaken using lipid phase different from those proposed by Sartorius. The determined Kp were noticed in table 5.

Table 4
Partition coefficients (Kp)
means of 5 values

pH	1	2	3	5	6
Sartorius lipid barriers		gastric		intestinal	
Cefradine	2,52	4,97	5,29	0,09	0,11
Cefalexine	1,37	3,19	3,98	0,08	0,05

Table 5
Partition coefficients with all the lipid phases.

pH		Lipid phase	Partition coefficient means 5 value s
3	Cefradine	dodecanol	indeterminable
		dodecanol + caprylic acid	indeterminable
		dodecanol + surfactant	5,294
	Cefalexine	dodecanol	
		dodecanol + caprylic acid	
		dodecanol + surfactant	
5	Cefradine	dodecanol + caprylic acid + surfactant	1,265
		dodecanol + surfactant	0,450
		dodecanol + caprylic acid	0,099
	Cefalexine	dodecanol + caprylic acid + surfactant	1,007
		dodecanol + surfactant	0,429
		dodecanol + caprylic acid	0,083
6	Cefradine	dodecanol + caprylic acid + surfactant	0,84
		dodecanol + surfactant	0,297
		dodecanol + caprylic acid	0,116
	Cefalexine	dodecanol + caprylic acid + surfactant	0,393
		dodecanol + surfactant	0,282
		dodecanol + caprylic acid	0,052

Table 6

Ionic species of cephalosporins

Z = Zwitterion ; C = cation and A = anion

pH		1	2	3	5	6
CEFRADINE CEFALEXINE	Z	2,71	22	74	98	88
	C	97	78	26	0,34	0,032
	A	-	-	-	1,29	11,64
CEPHALORIDINE	Z	-	-	-	-	-
	C	28,47	33,39	4,77	0,05	0,009
	A	71,53	66,61	95,23	99,95	99,99

At pH = 3, Kp because indeterminable without the surfactant of the gastric phase.

At pH 5 and 6 when the surfactant was added to the intestinal phase, the values found for Kp became ten times higher than those observed with any tensioactif agent. But if the lipid gastric phase of Sartorius was used at those two pH values, the increases of Kp were weaker.

DISCUSSION

At identical pH results showed that partition coefficients are in accordance with diffusion rate constants for CED and CEX. The absorption increased over a pH range of 1 to 3, then decreased in presence of intestinal phase (pH 5 and 6). Moreover our experiments noticed a maximum absorption at pH = 3. The table 6 showed the

percentage of different ionic species of CEX, CED and CER in aqueous solution according to the pH.
At pH 3 for CEX and CED the percentage as Zwitterion is approximatively 74 % and as cation 26 %.
At pH 5 and 6, 98 % and 88 % of Zwitterion, the most liposoluble species, was found and only 1 and 11 % of anion were present. Many authors, tried to investigate the influence of pH on the intestinal absorption of CED and CEX in situ. They reported a maximum absorption in the pH range (5 and 6) when the Zwitterion concentration of cephalosporins would be at its maximum. (5,6).
About CER which was a weak acid obeying to the "pH partition hypothesis", at pH 6 it was composed of ionized molecules. At pH = 3, 4.77 % of CER were present as unionized form that could explain its best diffusion. The oral way is not used for that main reason. There are a few additional arguments confirming its bad bioavaibility and its use by parenteral way. Callaghan et al. (7) have shown that CEX and CER were not absorbed at the same gastro-intestinal level. Their different chemical structures explained probably these different level absorption.
The molecules with the 7-substituent derivated from α-aminophenylacetic acid (CEX) were better absorbed than compounds in which the 7-substituent was derived from a substitued acetic acid without an α substituent such as CER (Table 1). The nature of the substituent in the 3-position also influences the extent to which a cephalosporin is absorbed orally. At 3-substituent that is small and closely bound such as the methylgroup (CEX) appears to aid absorption. For these authors oral upper smal intestine absorption depended from a good balance between the substi-

tuent in the 3-position and the substituent in the 7-position.

Moreover, Muggleton et al. (8) observed a more important destruction of CER than CEX by microorganisms of intestinal homogenates. Different authors (9, 10) have shown on rat in vivo that a small amount of CER was absorbed from the stomach and the greater part of the CEX and CED absorption took place in the duodenum.

This fact was confirmed with human subjects. Oral cephalosporins (CED, CEX) showed a very good bioavailibity of 80 and 95 %. (11, 12).

In litterature, there are many experimentations using in vitro and in vivo techniques with animals. But a lot of studies were found concerning diffusion of antibiotics with artificial barriers. But none researchs using oral cephalosporins, particularly amphoteric drugs were published with the Sartorius absorption simulator. Many authors (13, 14 and 15) used this simulator especially for acid and basic drugs which are passively transported substances.

In order to explain the difference between our results that is to say, a maximum absorption at pH = 3 and the maximal absorption at pH = 6 observed with animal in vivo, partition coefficients were realized with other lipid phases different from those proposed by Stricker.

Table V shows the importance of the surfactant on the cephalosporins diffusion through artificial lipid barriers. At pH = 3, Kp became indeterminable in presence of dodecanol or docecanol-caprylic acid mixture without any surfactant.

At pH 5; 6 Kp were about ten times higher when surfactant was added to the dodecanol caprylic acid mixture.

The Kd and Kp values were the highest ones at pH =3 when cephalosporins are found at their maximum ionisation.

Then the observed passage type was not a passive diffusion mechanism but a diffusion facilited one by an ionic surfactant which would transport ionized cephalosporin.

In litterature many experimentations describing the type of CED and CEX passages through the gastro-intestinal mucosa either in situ ou ex vivo were published. Tsuji et al. (16) suggested that the mechanism of the intestinal absorption of CED was a simple diffusion with first-order kinetics at high dose and that it was favorised by a specialized transport process following Michaelis-Menten kinetics at low doses. Kimura et al. (5) investigated the absorption mechanisms of CED, CEX, cefadroxil and cefatrizine in rat small intestine. These experiments demonstrated that the absorption rate of the antibiotics was saturable. Recently, Kramer (17) has demonstrated that it exists an interaction between CEX and a membrane protein in the rat small intestine. This protein could be a component of the intestinal system responsible for the uptake of orally effective cephalosporin and dipeptides. This active transport was confirmed by Barcina et al.(18) which investigated the effect of CEX and tetracycline HCl on D-galactose absorption in rat small intestine. Both antibiotics inhibited D-galactose uptake into isolated intestinal mucosa in a dose-dependent mechanism. Furthermore, both drugs reduced mucosa O_2 consumption and inhibited D-galactose absorption when they were perfused in a contiguous loop. Tsuji et al. (19, 20) studied two new oral cephalosporins of third generation, cefixine and FK 089. These antibiotics would be absorbed not only following a simple diffusion mechanism but also by a carrier mediated transport

system similar to those of dipeptides. This system was common to ß-lactamines "cefalexine-like". Transport of a FK 089 was studied with the rat everted small intestine in vitro

CONCLUSION

Because of the very few works about gastro-intestinal passage mechanisms of oral cephalosporins, it has seemed important to determine them by in vitro simulations with artificial membranes. Stricker's resorption apparatus initially commercialized for passive transport has enabled to verify a facilited transport for CED and CEX. Moreover, this device allowed to compare the rate diffusion of two identical orally cephalosporins, which appeared the fundamental parameter for their bioavaibility.

This type of previsional methods has lead towards much more detailled animal studies allowing to identify an active transport as well as the type of membran carrier proteins. The knowing of the passage mechanism has seemed to be compulsory at a time where pharmaceutical research devotes important efforts to the oral cephalosporins field. The apparition of oral cephalosporins from a third generation (cefixime), prodrugs from the second generation (cefuroxime axetil, ester cefotiam) will enlarge the application fields of those antibiotics.

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