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Application of sucrose laurate, a new pharmaceutical excipient, in peroral formulations of cyclosporin A

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Summary

Membrane permeability of sucrose laurate was studied using a regenerated cellulose acetate membrane with an M_w cutoff of 5000. Both the sucrose laurate micelle and even the sucrose laurate monomer were unable to cross the artificial membrane. Sucrose laurate, however, was found to enhance the in vitro absorption of cyclosporin A when normal epithelial tissue and Peyer's patch tissue of guinea pigs were used. Compared to the commercially available drinking solution, absorption was raised by a factor 10. Excess amount of surfactant reduced cyclosporin A absorption. Despite the large excess of sucrose laurate (50 ×), the absorption of cyclosporin A was still superior to the drinking solution. Choleic acid was also found to raise absorption by a factor of 5–6. A comparison of the absorption between normal epithelial and Peyer's patch tissues indicated that the absorption by endocytosis does not contribute significantly to the overall absorption of cyclosporin A. Regarding the high degree of absorption, sucrose laurate may be considered as a new promising excipient for peroral formulations. Preliminary formulation experiments showed that a solid peroral dosage form of cyclosporin A could be made, using sucrose laurate as an excipient. However, further optimization studies must be performed in order to obtain a preparation suited for human application.

Introduction

The preferred and customary route for delivery of drugs is the peroral route. The peroral administration of various drugs, however, may be hindered by a relatively low bioavailability. For poorly water soluble drugs the low bioavailability can be explained by dissolution limited absorption. One of the possibilities to increase the solubility of a poorly water soluble drug, is the use of surface active agents. The generally used water soluble surfactants are all oily liquids or waxy solids, and therefore, less appropriate for use in solid oral dosage forms. Sucrose (mono)laurate, nevertheless, is a crystalline solid (Hahn, 1988; Lerk, 1991). This new promising excipient should be easily processed in solid oral dosage forms like tablets and capsules. The first objective of the present study, therefore, has been to evaluate the

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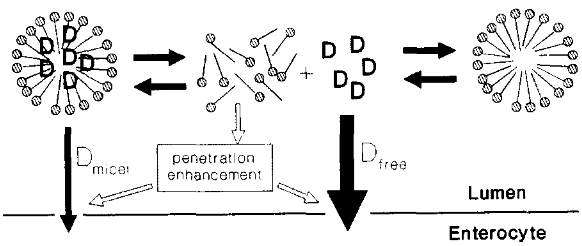

Fig. 1. Schematic representation of micelle mediated transport of a drugsubstance (D) across the boundary layer/mucosa. D_{free} and D_{micel} refer to the diffusion coefficients of the free drug and solubilized drug, respectively. K represents the equilibrium constant between the aqueous phase and micelle.

use of sucrose laurate as an excipient in a peroral formulation. Cyclosporin A was choosen as a test substance because of its very low water solubility (40 ng/ml).

The absorption enhancing or reducing properties of surfactants is generally attributed to their property to modify the mucosal permeability or to their interaction with the drug substance. The surfactant monomer is generally accepted to be the permeability enhancing entity. At surfactant concentrations below the critical micelle concentration, the surface active agent may increase drug absorption. Surfactant concentrations exceeding the critical micelle concentration, on the other hand, may reduce the thermodynamic activity of the drug by micellar solubilization and, as a consequence, reduce absorption rate (Fig. 1). The diffusion coefficient of the free drug is generally much higher as compared to that of the solubilized drug. In the case of a very low water solubility, however, the large capacity of the micelle mediated transport can compensate for a small diffusion coefficient (Table 1).

The effect of sucrose laurate on the gastrointestinal absorption of cyclosporin A is unknown. A second objective of this study, therefore, was to investigate the effect of sucrose laurate on the absorption of cyclosporin A. To include the absorption mechanism by endocytosis, the absorption of cyclosporin A was studied, not only across the epithelia of guinea-pig small intestine, but also across Peyer's patch tissue.

The final intent of these investigations has been to formulate and evaluate at least one solid peroral dosage form.

Materials and Methods

Materials

Sucrose laurate (L-1695) was a gift from Ryoto Co. (Chyoda-Ku, Tokyo, Japan) and was used without further purification. Saccharose, propyl *p*-hydroxybenzoate, Syloid 63FP, Polyplasdone XL, Aerosil and magnesium stearate were either obtained from Merck (Darmstadt, Germany) or Fluka AG (Buchs, Switzerland) and were of pharmaceutical quality. Cyclosporin A was obtained from Sandoz Pharma AG (Basel, Switzerland). Krebs-Henseleit medium (pH 7.4), Protosol 0.5 M (NEN Research Products, Boston, U.S.A.) and Lumagel scintillation liquid (Lumac, Landgraaf, The Netherlands) were all of commercial quality. The regenerated cellulose acetate membrane (M_w cutoff 5000, ϕ 63 mm) was purchased from Diachema AG (Rüschlikon, Switzerland).

Equilibrium dialysis

The equilibrium dialysis experiments were performed using a Dianorm equilibrium dialyzer (Diachema AG, Rüschlikon, Switzerland). One half of the teflon cell (Macro 1) was filled with a 10.00% sucrose laurate solution comprising an excess amount of propyl *p*-hydroxybenzoate. The

TABLE 1

The efficiency of fatty acid transport of a monomer solution compared to a micellar solution (Hofmann, 1976)

	C (mM)	D ($cm^2\ s^{-1}$)	M ($\mu mol\ cm^{-1}\ s^{-1}$)
Fatty acid in monomer solution	0.00001	8.0×10^{-6}	8×10^{-11}
Fatty acid in micellar solution	0.01	1.2×10^{-6}	1200×10^{-11}

C, concentration; D, diffusion coefficient; M, mass transfer ($C \cdot D$)

other half was filled with 6.10% sucrose, which was found to be iso-osmotic with a solution of 10.00% L-1695. The entire unit was immersed in a temperature controlled bath. The temperature was set to 25 or 37°C, the rotation speed was adjusted to 12 rpm. At selected intervals, the amount of propyl *p*-hydroxybenzoate was determined spectrophotometrically (Uvikon 930 spectrophotometer, Kontron Instruments AG, Zürich, Switzerland) at a wavelength of 255.5 nm. Experiments were performed in quadruplicate.

Absorption of cyclosporin A in an intestinal model

The intestinal absorption experiments performed, were based on a new in vitro testing model, developed by Fricker (Sandoz Pharma AG, Basel; department of drug delivery systems (DDS)). Before use, freshly prepared normal epithelia and Peyer's patch tissue of guinea-pig small intestine were incubated in Krebs-Henseleit cell medium (pH 7.4) at 37°C. After 30 min of incubation, the Krebs-Henseleit cell medium was removed and pieces of normal epithelia or Peyer's patch tissue, respectively, were clamped between the donor and acceptor chamber. In the latter case, minimally 75% of the surface area was occupied by Peyer's patch tissue. The diffusion cell which was used is shown schematically in Fig. 2. The acceptor compartment contained cell medium; the donor compartment was filled with about 300 μl of the test solution, spiked with a small amount of radiolabeled cyclosporin A. At distinct periods, the amount of radioactive cyclosporin A in the donor and acceptor compartment and that of radiolabeled drug associated with the tissue were determined. After incubation, the pieces of normal epithelia or Peyer's patch tissue were washed twice with 400 μl of cell medium and dissolved in 1 ml of Protosol 0.5 M. After one night of incubation at 55°C, the suspension was mixed with 10 ml scintillation liquid. The amount of radioactive cyclosporin A in the donor compartment and tissue was determined on a liquid scintillation counter (Packard, Zürich, Switzerland). Absorption was measured, up to 1 h of incubation. Due to the dying off of the epithelial tissue, incubation times of more than 1 h caused excessively large fluctuations.

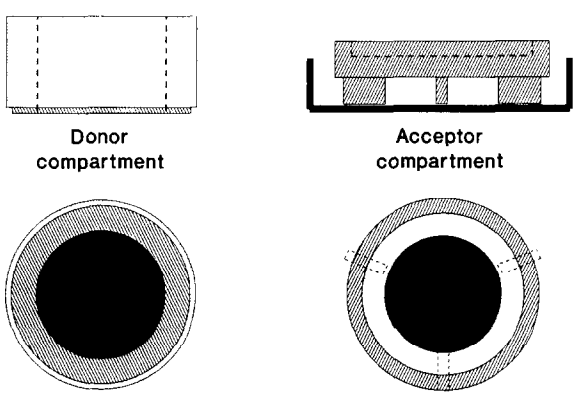

Fig. 2. Schematic representation of the diffusion cell used.

The amount of cyclosporin A in the acceptor phase could not be calculated accurately. Only minimal amounts of drugsubstance crossed the epithelial tissue, which comprised mucosa and muscle tissue.

Results and Discussion

Equilibrium dialysis

The membrane permeability to sucrose laurate micelles was firstly studied, using a regenerated cellulose acetate membrane. The use of an artificial membrane has the advantage that permeation can be studied without the complication of alteration of the permeability of the membrane. The cellulose acetate membrane with a M_w cut-off of 5000 was supposed to be impermeable to the sucrose laurate micelle ($M_w \approx 50\,000$), but permeable to the sucrose laurate monomer ($M_w = 524.6$). Because the identification of trace amounts of sucrose laurate caused some problems, the permeation of sucrose laurate was followed by means of a solubilizate. Therefore, the donor compartment was filled with a solution containing 10% sucrose laurate and an excess amount of propyl *p*-hydroxybenzoate. The acceptor compartment was filled with an iso-osmotic solution comprising 6.10% sucrose. Propyl *p*-hydroxybenzoate, with a molecular weight of 180, was supposed to diffuse freely across the artificial membrane. Due to the extremely low critical micelle concentration, even trace amounts of su-

crose laurate in the acceptor compartment will form micelles, raising the propyl *p*-hydroxybenzoate concentration above its aqueous solubility. The results of these experiments are depicted in Fig. 3. Both curves evidently show that no significant amount of sucrose laurate diffuses across the cellulose acetate membrane, within the observed time period. Control experiments showed that even up to 48 h, no sucrose laurate could be detected. These findings prove that, under the conditions of this investigation, both sucrose laurate micelles and even sucrose laurate monomers are unable to cross the cellulose acetate membrane. From these results it might be assumed that both sucrose laurate micelles and sucrose laurate monomers will also not diffuse through biological membranes.

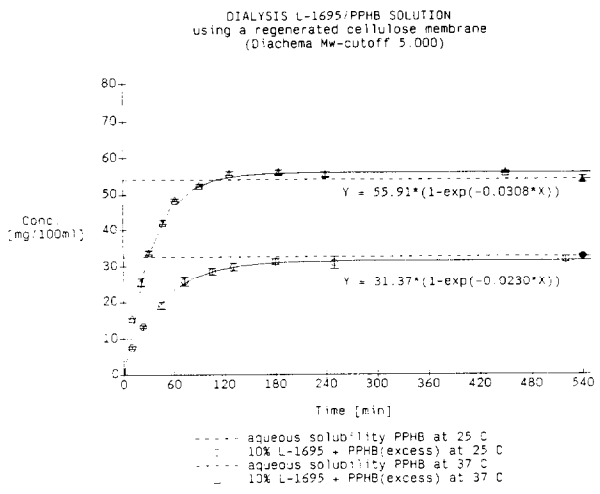

Fig. 3. Diffusion of propyl *p*-hydroxybenzoate across a regenerated cellulose acetate membrane (M_w cutoff 5000), at (○) 25°C and (△) 37°C. The donor compartment was filled with a micellar solution containing 10.00% sucrose laurate and an excess amount of propyl *p*-hydroxybenzoate. The acceptor compartment comprised 6.10% sucrose.

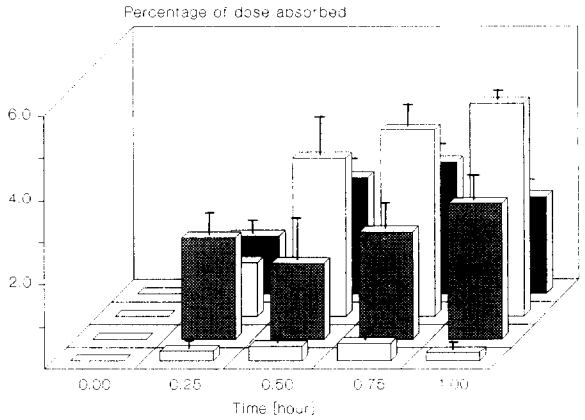

Fig. 4. Mean absorption (±S.D.) of cyclosporin A in normal epithelial tissue, from (⊡) the commercial drinking solution, (▩) bile fluid, (□) 1.0% sucrose laurate and (■) 10.0% sucrose laurate.

Absorption of cyclosporin A in an intestinal model

In the intestinal absorption experiments, four preparations containing 0.3 mg/ml cyclosporin A were compared. The commercial drinking solution and a solution of cyclosporin A in bile fluid were used as reference preparations. The test formulations contained 1.0 and 10.0% sucrose laurate, respectively. The formulation comprising bile fluid was chosen, since bile salts are known to enhance intestinal drug absorption (Kakemi et al., 1970; Schichiri et al., 1978; Moore et al., 1986; Shiga et al., 1987). The micellar solution containing 10% sucrose laurate was used to investigate the effect of a large excess of surfactant.

The results, depicted in Fig. 4, show that the absorption of cyclosporin A from the drinking solution is very low. This finding is in accordance with numerous vivo experiments which all showed a very low bioavailability of the commercial preparation. The sucrose laurate and choleic acid containing solutions exhibit higher absorption percentages. As reported in the publications mentioned previously, the addition of bile fluid increases the absorption of cyclosporin A. Compared to the drinking solution, absorption is raised by a factor 5–6. The solution containing 1.0% sucrose laurate, however, was found to be superior to all three formulations. Differences between the percentages of dose absorbed of both sucrose laurate containing solutions indicate, as expected, that the addition of excess surfactant reduces drug absorption. Despite the large excess of sucrose laurate (50-fold), the absorption of cyclosporin A from the micellar solution is still several times as large as the absorption from the drinking solution. Regarding the high absorptions, sucrose laurate may be considered as a promising new excipient which can also be used

TABLE 2

Absorption of cyclosporin A in normal epithelial tissue and Peyer's patch tissue

Time (min)	Drinking solution		Bile fluid		Sucrose laurate (1.0%)		Sucrose laurate (10.0%)	
	NET	PPT	NET	PPT	NET	PPT	NET	PPT
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.24	0.31	2.39	2.53	1.28	1.55	1.37	2.07
30	0.36	0.35	1.79	2.35	3.75	2.42	2.76	2.36
45	0.43	0.40	2.53	3.24	4.42	4.86	3.12	2.44
60	0.20	0.36	3.21	3.98	5.02	4.99	2.31	–

NET, normal epithelial tissue; PPT, Peyer's patch tissue.

to enhance intestinal absorption. As illustrated in Table 2 no large differences in the absorption of cyclosporin A were found between normal epithelial and Peyer's patch tissues. This finding indicates that absorption by endocytosis does not contribute significantly to the overall absorption of cyclosporin A.

Regarding the intestinal absorption experiments, it should be taken into account that the calculated amount of drug 'absorbed' was composed of (i) the amount adsorbed to the tissue, (ii) the amount which was contained in the intestinal tissue, and (iii) the amount of drug that really passed the tissue. In an in vivo situation, the latter amount of drug is commonly referred to as the real amount of drug absorbed. The vitro model used, however, may be regarded as semi-quantitative. Subsequent in vivo experiments, demonstrated the absorption enhancing properties of sucrose laurate.

Formulation and evaluation of a solid dosage form

The final objective of this part of the study was to illustrate the possibility of the formulation of sucrose laurate into a solid peroral dosage form with cyclosporin A. No efforts were made to optimize the formulation.

The main problem in the processing of sucrose laurate was found to be its hygroscopicity. At a relative humidity of 70%, for example, the processability of a fine powder (500–710 nm), which had previously been dried, was restricted to less than 30 min. Additionally, due to its poor flow characteristics, it was almost impossible to use sucrose laurate for direct compression. The formulation, depicted in Table 3, showed acceptable processability. Syloid 63 FP, with a BET surface area of approx. 800 m^2/g, was used to improve the flow characteristics of the pre-formulation. This formulation was prepared by dissolving cyclosporin A and sucrose laurate in alcohol. After admixing the Syloid 63 FP, the alcohol was removed by vacuum evaporation at 50°C (Buechi, rotary evaporator). The final formulation was prepared by simple mixing. The dissolution profile of the oblong tablets is depicted in Fig. 5. Normally, for drugs with a very low water solubility, a mixture of 0.2% *N,N*-dimethyldodecylamine *N*-oxide (LDAO) in water is used as a dissolution medium. Using sucrose laurate, LDAO can be

TABLE 3

Pre-formulation and formulation of a cyclosporin A containing solid peroral dosage form, using sucrose laurate as an excipient

Pre-formulation	Concentration (mg/dose)	Formulation	Concentration (mg/dose)
Sucrose laurate (L-1695)	312.5	pre-formulation	562.5
Cyclosporin A	50.0	Polyplasdone XL	25.5
Syloid 63 FP	200.0	Aerosil 200	6.0
		magnesium stearate	6.0

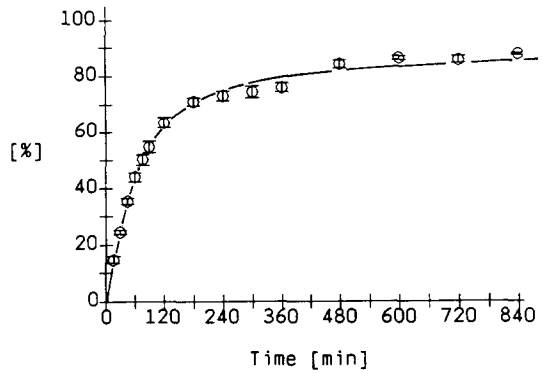

Fig. 5. Dissolution rate profile of a cyclosporin A containing solid peroral dosage form, using sucrose laurate as an excipient.

omitted. According to the solubilization capacity of sucrose laurate (Lerk, 1991), 50 mg of cyclosporin A can be solubilized by 350 mg of sucrose laurate (L-1695). The dissolution of cyclosporin was measured using the USP XXII paddle method at 37°C and 50 rpm.

The finding that only 90% of the initial dose could be recovered could be accounted for by the presence of other competitive non-water soluble excipients. In solubilizing these excipients, the amount of 312.5 mg sucrose laurate becomes insufficient to solubilize the additional 50 mg of cyclosporin A. The low dissolution rate could be raised by replacing Syloid 63 FP with an equal amount of mannose. This substitution increased both dissolution rate and availability; within 2 h, almost 90% was released.

The present results demonstrate that a solid peroral dosage form of cyclosporin A can be made, using sucrose laurate as an excipient. However, the formulation of a preparation for human administration requires further study.

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