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Research paper

In vitro permeation studies comparing bovine nasal mucosa, porcine cornea and artificial membrane: androstenedione in microemulsions and their components

Telse Richter, Sigrid Keipert*

Mathematisch-Naturwissenschaftliche Fakultät I, Humboldt University, Berlin, Germany

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Abstract

The components of a carrier formulation can interact with an added drug as well as with the membrane surface they were applied on. Therefore, they can influence permeability of the membrane and permeation of the drug. The particular membrane structure might lead to different drug permeation out of one and the same carrier formulation. In this study, in vitro permeability of androstenedione (AD) as a highly lipophilic substance was investigated in excised bovine nasal mucosa, porcine cornea and the artificial cellulose membrane Nephrophan®. Two microemulsions (ME) with either the addition of the co-surfactants hydroxypropyl-γ-cyclodextrin (HP-γ-CD; ME-CD) or propylene glycol (PG, ME-PG) were tested in order to be used as carrier systems. Both MEs also consisted of 5% isopropyl myristate (IPM), 20% Cremophor® EL (CrEL), and water. Buffer solution (EBS) with 0.0025% AD served as control solution and was furthermore compared to 0.0025% AD/buffer-solutions containing the ME components HP-γ-CD in different concentrations (0.012, 0.024, 9%) as well as 20% CrEL.

The AD-permeation behaviour through the three tissues was differently influenced by the MEs. The apparent permeability coefficients (P_{app}) of nasal mucosa for both ME systems did not differ from the P_{app} of the AD/buffer solution. In case of the other two barriers (cornea, Nephrophan®), ME-PG as well as ME-CD provoked extended time lags for AD to permeate, so the P_{app} could not be calculated or declined to zero.

P_{app} of AD/buffer solution without any additives resulted for cornea, nasal mucosa and Nephrophan® in a ratio of 1:3:4. CrEL and 9% HP-γ-CD diminished the P_{app}, except HP-γ-CD in molar AD/HP-γ-CD-ratios of 1:1 (0.012%) and 1:2 (0.024%).

It seems that the composition of lipophilic and hydrophilic structures of the carrier systems or the additives had a higher impact on the P_{app} of cornea than on the P_{app} of the other tissues. Structure and character of the different membranes are considered to be mainly responsible for the differentiated permeation behaviour.

Keywords: Androstenedione; Hydroxypropyl-γ-cyclodextrin; Microemulsion; Porcine cornea; Bovine nasal mucosa; In vitro-permeability

1. Introduction

Cornea and nasal mucosa both offer the possibility for simple and comfortable drug administration, but differ in structure: human cornea represents a five-layer barrier (~500 μm thick) consisting of lipophilic epithelium (~50 μm), hydrophilic stroma (~450 μm) and lipophilic endothelium (monolayer) as well as Bowman membrane between epithelium and stroma and Descemet's membrane between stroma and endothelium (both monolayers). In contrast, nasal mucosa possesses a total thickness of only about 100 μm and consists of various cell types, which are ciliated and non-ciliated columnar cells, goblet and basal cells. Both administration sites (conjunctival sack and nasal cavity) are joined by the nasolacrimal duct, where normally lacrimal fluid is able to drain off [1]. But, via this route, drugs for a primarily ophthalmic use may also reach nasal mucosa and will thereby be systemically absorbed.

The amount of drug absorption and binding site could be regulated by varying the carrier composition. Furthermore, galenic formulations can optimize drug solubility or improve membrane permeability and therefore increase the bioavailability of the drug [2–4]. Additionally,

* Corresponding author. Mathematisch-Naturwissenschaftliche Fakultät I, Humboldt University, Prenzlauer Promenade 149-152, Berlin 13189, Germany. Tel.: +49-30-4797-443; fax: +49-30-4797-440.

E-mail address: sigrid.keipert@rz.hu-berlin.de (S. Keipert).

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adjuvants or formulations themselves can have positive effects like wetting the ocular or nasal surface or protect them against environmental influence.

In this regard a lipophilic component in the formulation seems to be advantageous, but especially for ocular use, unwanted side-effects like blurred vision and eye irritations should be considered. In case of nasal administration incompatibilities can be indicated for example by impairment of ciliar movement.

Use of a ME may minimize these side-effects by administering a lipophilic component in a transparent, water-continuous system [5,6]. Microemulsions are multi-component systems, which normally contain a hydrophilic and a lipophilic component together with surfactant and co-surfactant, and represent clear to slightly opalescent and thermodynamically stable systems. In our research group a combination of a cyclodextrin with a microemulsion formulation was developed [14], in which the cyclodextrin replaced the co-surfactant.

Cyclodextrins possess promising advantages in modifying nasal and ocular drug absorption [8,9]. These substances are cyclic oligosaccharides with a hydrophilic outer surface and a hydrophobic inner cavity, so that they are water-soluble and simultaneously able to take up poorly water-soluble, lipophilic molecules or parts of them into their cavities. These inclusion complexes offer various advantages like increasing water solubility or stability of the drug. However, the complex binding is non-covalent and the guest molecule can rapidly dissociate out of the cavity under suitable conditions [10,11].

In order to detect diffusion of a drug out of the carrier formulation as well as influence of the formulation components on different biological membranes, permeation behaviour of a lipophilic drug through porcine cornea as well as bovine nasal mucosa was studied in the presented work. Porcine nasal mucosa only came to use as permeation barrier very recently [15,16], whereas permeation studies using bovine nasal mucosa had been established in our group before [7]. However, use of bovine tissues was restricted and later on not possible to get because of the BSE-crisis. Therefore, porcine cornea was used because of its similar thickness and structure to human cornea.

Androstenedione (AD), the precursor of the male sexual hormone testosterone, was selected as lipophilic model drug component for our permeation studies. This substance may be used for nasal administration in order to achieve systemic effects for healthy ageing in men [17] and also for ocular administration with an indication of dry eye syndromes [18].

For considering bare effects of the preparations and their particular excipients on AD-permeation, permeability of an artificial cellulose membrane, Nephrophan®, was examined under same conditions like the biological tissues. In these studies the AD-permeation was unaffected by influences of biological structures.

2. Materials and methods

2.1. Materials

AD was purchased from Schering AG (Berlin, Germany). HP-γ-CD ($M_{rel} = 1355$, medium substitution grade of 0.6) was a gift from Wacker Chemie GmbH (Burghausen, Germany). Cremophor® EL (Macrogol-1500-glyceroltriricinoleat, CrEL), propylene glycole (PG) and isopropyl myristate (IPM) were purchased from Caesar und Lorentz GmbH (Hilden, Germany). Acetonitrile was used in HPLC grade and purchased from Baker J.T. (Deventer, The Netherlands). All other materials were of analytical grade. Earl's balanced salts (EBS) were used as buffer solution at pH 7.4.

Excised bovine nasal mucosa and porcine cornea were obtained from the slaughterhouse next to the laboratory. The tissues were transported and stored in ice-cold buffer solution and used for permeability studies within at most 2 h.

The artificial membrane, Nephrophan® (Filmfabrik, Wolfen), consists of regenerated cellulose with a pore diameter of 2.4 nm and a total thickness of 14–15 μm. Before each experiment, the membrane was washed for 30 min under fluent water for removing emollients.

2.2. Preparation of microemulsions

Two O/W microemulsions (MEs) were tested, consisting of 5% IPM, 20% CrEL, 66% water and, as co-surfactants, 9% propylene glycol (resulting in the system ME-PG) [6] or 9% HP-γ-CD (resulting in the system ME-CD) [14], respectively. For preparation, CrEL and IPM were mixed under gentle stirring and heating (75 °C). Separately, but under same conditions water and the co-surfactant were put together, respectively. The latter mixture was poured into the IPM/CrEL mixture and stirred on a magnetic stirrer until room temperature and transparency was reached. MEs containing AD (25 μg/ml, m/v) were prepared likewise, dissolving the drug in the water/co-surfactant mixture before combining it with the IPM/CrEL mixture. All formulations represented transparent systems. Characterization of stability and physical-chemical properties of the AD-free ME systems were determined earlier in our group [14,19].

2.3. Preparation of the biological tissues

Porcine corneas were excised from the eye-ball with a small ring of sclera and rinsed with EBS solution, taking care to avoid any damage. The excised tissue was tested by several experiments in our group to be viable at least during the test time period [14,20]. Bovine nosepieces with mucosa were obtained by cutting out a part of the nasal conch along the surrounding bones. After that, mucosa was carefully separated from the connective tissue, as described

previously [21]. Viability of nasal mucosa was guaranteed for 10 h after dissection while storing in ice-cold EBS, saturated with carbogen gas [7].

2.4. Determination of partition coefficients

To simulate the partition behaviour of AD in the ME systems, drug partitioning studies between water and IPM were performed. Two millilitres of an AD/water solution (25 μg/ml) were combined with the same volume of IPM. After mixing vigorously for 2 h followed by centrifugating (5 min, 3500 rpm), 500 μl sample were withdrawn from the water phase and drug concentration determined by HPLC (2.6). Influence of the single excipients on AD partitioning was studied by adding 9% HP-γ-CD or PG to the AD/water solution, respectively.

2.5. In vitro-permeability studies

An Ussing chamber model (diffusion area: 0.5 cm^2; donor and receiver volume: 1.0 ml, respectively) established by our research group [22] was modified [19] to perform permeability studies with curved, thick cornea as well as with plane, thin nasal mucosa. The cornea model receiver half was fitted out with a ring-shaped elevation around the orifice which gripped into a deepening at the donor half to maintain curvature of the cornea. For nasal mucosa, the donor half was plane and the elevation around the receiver orifice was replaced by a sealing ring made of rubber. The tissues were mounted between donor and receiver compartment. First, the receiver chamber was filled with fresh EBS buffer solution bubbled with carbogen gas, a mixture of 95%O_2 and 5%CO_2. Immediately after that, the test formulation was placed in the donor compartment. Before each experiment, the biological tissue was equilibrated in the Ussing chamber with EBS on both sides for 30 min.

The loaded Ussing chamber was placed into a bioincubator (Gesellschaft für Labortechnik mbH, Burgwedel, Germany) at 33 °C ± 0.5 and 100 rpm. Samples (400 μl) were withdrawn on the receiver side at various time intervals, every 15 up to 90 min in case of nasal mucosa and 30, 60 and then every 60 up to 300 min in case of cornea, and replaced by fresh buffer solution, immediately. Studies with Nephrophan® were carried out twice, once within the cornea Ussing chamber and following the time intervals of cornea studies and once using the mucosa time scheme and the mucosa chamber model.

If the biological membrane was pre-incubated with an additive, no former equilibration with EBS was carried out, to not extend duration of the study. The tissues were pre-incubated for 30 min under same conditions as described above. Before refilling the Ussing chamber, both sides were washed out carefully with excess buffer solution.

For all permeability studies, 25 μg/ml AD were dissolved in EBS (reference) or test solutions/ formulations, respectively.

2.6. HPLC method

All samples were analyzed by HPLC as described earlier [7]. In brief, the analyses were carried out by an HPLC instrument equipped with an UV detector (L-4000 Merck-Hitachi, Darmstadt, Germany) and an Intelligent Pump (L-6200, Merck-Hitachi, Darmstadt, Germany). A LiChrospher® 100, RP-18 (5 μm) 125-4 column with a LiChrospher® 100, RP-18 (5 μm) 4-4 guard column (Merck, Darmstadt, Germany) was used. Samples were applied via autosampler (Merck-Hitachi, Darmstadt, Germany) or alternatively with a microliter syringe in a Rheodyne valve (Cotati, California, USA) with a 20 μl loop size. The mobile phase consisted of HPLC-grade acetonitrile and water 60:40, at a flow rate of 1.0 ml/min. The retention time under these conditions for androstenedione was 3.5 min and the detection wave length 240 nm.

2.7. Data analysis

Effective permeability coefficients (P_{app} [cm/s]) were calculated with help of the following equation:

$$P_{app} = \frac{dc}{dt}\,\frac{V}{A \cdot c_0 \cdot 60}$$

where dc/dt is the increase of permeated cumulative drug amount versus time (μg/min), V is the volume of the receiver compartment, A is the surface area of the membrane (0.5 cm^2), c_0 is the initial drug concentration in the donor compartment and 60 is the conversion factor from minute into second. The steady-state flux (dc/dt) was determined from the slope of the linear portion of cumulative permeated drug amount versus time. The lag time was also determined from this graph by extrapolating the linear portion to the x-axis.

Statistical differences between the results were determined by analysis of variance (ANOVA). Differences against EBS as reference were calculated by Dunnett's test, comparison of all values was performed by Student–Newman–Keul's test. The criterion for statistical significance was $P < 0.05$. All statistical calculations were performed with SigmaStat 2.0.

3. Results

Permeability coefficients (P_{app}) of AD through bovine nasal mucosa, porcine cornea and Nephrophan® out of different donor solutions are reported in Table 1. Lag-time, if any, is also listed in Table 1, written in italics beside the corresponding P_{app}.

Statistical comparison of the two differently designed Nephrophan® permeability studies (90 and 300 min) led to no significant differences, except in case of 9% HP-γ-CD. Therefore, they were not combined and listed up due to the test period, respectively (Table 1). P_{app} could only be

Table 1
Results of the in vitro-permeation of androstenedione (25 μg/ml) in presence of different additives and out of two microemulsions

Formulation	P_{app} (10^{-5} cm/s)[a]/lag-time (min)[a]			
	Bovine nasal mucosa	Porcine cornea	Nephrophan®	
			90 min	300 min
EBS (reference)	2.19 ± 0.78/ø	0.73 ± 0.15/*44.9 ± 23.0*	2.82 ± 0.58/ø	3.09 ± 0.84/ø
HP-γ-CD 1:1	2.02 ± 1.34/ø	0.87 ± 0.14/*66.5 ± 16.3*	3.30 ± 0.79/ø	3.00 ± 0.36/ø
HP-γ-CD 1:2	2.01 ± 0.42/ø	0.91 ± 0.10*/*69.2 ± 19.1**	3.32 ± 0.20/ø	3.45 ± 0.21/ø
HP-γ-CD 9%	1.31 ± 0.84*/ø	n.c./*n.c.*	0.93 ± 0.05*/ø	1.15 ± 0.12*/ø
HP-γ-CD pi	1.58 ± 0.43/ø	0.50 ± 0.08*/*63.87 ± 12.2*	2.82 ± 0.73/ø	Not detected
Cremophor® EL 20%	0.50 ± 0.11*/ø	n.c./*n.c.*	n.c./*n.c.*	0.15 ± 0.03*/ø
Cremophor® EL pi	1.61 ± 0.59/ø	0.55 ± 0.12*/*78.3 ± 20.9**	3.31 ± 0.68/ø	Not detected
ME-PG	1.98 ± 0.47/*25.1 ± 12.5*	n.c./*n.c.*	0.00 ± 0*/*n.c.*	0.00 ± 0*/*n.c.*
ME-CD	1.78 ± 0.89/*22.5 ± 15.6*	n.c./*n.c.*	0.61 ± 0.13*/*15.3 ± 9.8*	0.72 ± 0.04*/ø

*, Significantly different ($p < 0.05$) from the EBS value of the concerning tissue.
[a] Means ± SD, $n \geq 5$; pi, the membrane was pre-incubated with the excipient; n.c., not calculable; ø, none.

calculated, if at least three values per chamber were detected, which differed from zero and resulted in a straight line ($R^2 > 0.95$), and if the results of every single chamber of a test series correspond to these requirements. Permeation of AD/EBS solution (reference) through the three different tissues led to an approximate ratio of the P_{app} of 1:3:4 (cornea/nasal mucosa/Nephrophan®).

In our studies, two O/W ME formulations were used as possible carrier systems for nasal and ocular administration. Aqueous continuous MEs are miscible with water and clear, but contain lipophilic regions together with a surfactant component, which may markedly increase solubility of lipophilic drugs. Equilibrium solubility of AD in ME-PG and ME-CD was 4.35 ± 1.3 and 14.01 ± 2.56 mg/ml, respectively, compared to an EBS equilibrium solubility of 0.066 ± 0.002 mg/ml. However, in our permeability studies the AD concentration was kept the same for all test solutions (25 μg/ml) to make possible to compare influences of the single donor system on the permeation behaviour of this drug.

Permeation of AD through the three tissues was differently influenced by the ME formulations (Table 1). In case of nasal mucosa, there was no significant reduction of the AD-P_{app} out of ME-PG and ME-CD. Contrarily, the permeability of Nephrophan® was diminished, in case of ME-CD to about 20% of the EBS value. AD out of ME-PG did not permeate Nephrophan® until 300 min. Permeability of porcine cornea was also drastically reduced in case of both MEs: AD could be detected in the receiver medium only after 240 min (ME-CD) or 300 min (ME-PG). Therefore, P_{app} of AD from both MEs could not be calculated (see above). Fig. 1 shows the cumulative AD-amount after permeation through nasal mucosa (90 min) and cornea (300 min). In case of the MEs the total amount of permeated drug is very different comparing the two tissues as well as EBS and ME value of one membrane, respectively (Fig. 1).

Furthermore, permeability was detected under the influence of two isolated components, the hydroxypropyl derivative of γ-CD and the non-ionic surfactant CrEL. Addition of HP-γ-CD in molar ratios of 1:1 and 1:2 due to AD (1:1 molar addition corresponds to 0.012% and 1:2 to 0.024% of CD) had no influence on permeability of nasal mucosa and Nephrophan®, but the 1:2-ratio significantly increased the permeability of porcine cornea (Table 1).

Fig. 1 also illustrates, that all added excipients decreased the permeated total AD-amount below the corresponding EBS-line, except HP-γ-CD in molar ratios caused it to increase in case of cornea.

If added in a higher concentration of 9% (AD/CD-ratio of 1:764), which is in accordance with the microemulsion composition, all P_{app} values significantly decreased (Table 1) in comparison to the reference EBS. 9% HP-γ-CD reduced permeability of porcine cornea to such a degree, that no P_{app} could be calculated (see above). However, comparison with the nasal mucosa value was possible with the aid of the cumulative drug amounts in Fig. 1. Reduction of the permeated drug amount after 300 min (cornea) compared to the EBS reference was detected to be obviously higher than after 90 min in case of nasal mucosa.

A still higher reduction of the in vitro-permeation of AD occurred under addition of 20% CrEL (Fig. 1), which also corresponds to the concentration in the ME. Again, P_{app} through cornea could not be calculated, because of the extended lag-time (Table 1). For the same reason the permeability coefficient of Nephrophan® after 90 min could also not be specified.

For determining damaging effects of the single excipients to the tissue, the membranes were pre-incubated with 9% HP-γ-CD and 20% CrEL, respectively, followed by permeation of AD out of EBS. If an excipient influences membrane integrity, increasing permeation rates are normally expected. In our studies, no changes of

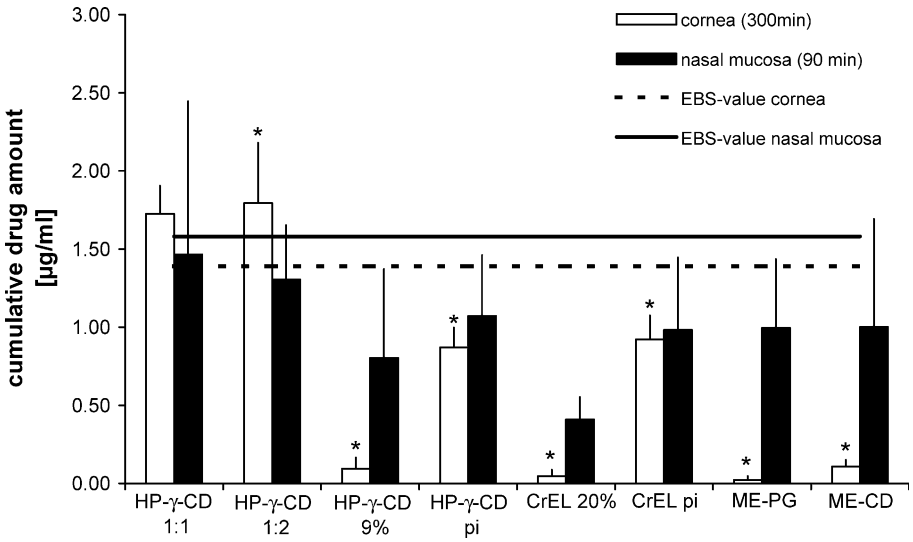

Fig. 1. Cumulative drug amount [µg/ml] of AD through cornea and nasal mucosa in presence of the microemulsions and their isolated components. The horizontal lines represent the EBS values of the two tissues.
*Significantly different ($p < 0.05$) from the EBS value of the concerning tissue.

permeability were detected in case of Nephrophan® and nasal mucosa, whereas P_{app} of AD through porcine cornea was significantly reduced after pre-incubation with both excipients (Table 1). Thus, alteration of the membrane integrity can largely be excluded as a cause of damage. Nephrophan® (90 min) was also pre-incubated, in order to make sure, that the test solutions could completely be washed out of the chambers. There was no significant difference between the apparent permeability coefficients for AD out of EBS, without and after previous incubation of the cellulose membrane (Table 1).

Our studies were performed to characterize effects of the carrier composition only on trans-cellular permeability, so that in this case only tests with the lipophilic AD were carried out, but comparison with a model hydrophilic substance, mainly penetrating the membrane on the paracellular way, will be subject of further work [19].

4. Discussion

Nasal mucosa represents a weak barrier for lipophilic drugs [24,25] as well as for hydrophilic substances [26,27]. Nephrophan® consists of regenerated cellulose without biological structures like cell membranes, ion channels or tight junctions, and limits drug permeation only by pore size and number. In contrast, the considerable thicker cornea shows a five-layer structure with marked lipo- and hydrophilic layers. Therefore, the ranging of P_{app} (cornea < nasal mucosa < Nephrophan® ≈ 1:3:4) regarding AD-permeation out of EBS can mainly be explained by different membrane qualities.

The lag-time represents the time span, that is needed to reach a constant concentration gradient in the membrane, and it is directly proportional to the squared thickness of the barrier [28]. Therefore, we assume that the determined time lags in the cornea studies are caused by the thickness and the structure of the membrane. Corneal AD-permeation out of EBS already showed a 45 min time lag (Table 1), which increased after the addition of excipients and after AD-permeation out of the formulations.

Addition of HP-γ-CD in molar equivalents to AD (1:1 and 1:2) did not cause a significant change in P_{app} (see above) with exception of the cornea values (Table 1). These findings correspond with the results of Masson et al. [11]. They found maximum drug permeability, when just enough CD is added to dissolve the drug completely. Bary et al. [25] revealed that the best ocular bioavailability for hydrocortisone occurs, if it is formulated as an HP-β-CD solution, in which the CD concentration is sufficient to form a complex just with the excess drug. In both cases, the concentration ratio of drug to CD was in accordance to or even lower than the complexation ratio of the inclusion complex. In our experiments a small excess of AD was still available in the solution, because of an inclusion ratio of AD and HP-γ-CD of 1:3, which was calculated by Berndt et al. [14]. In addition, the process of complex formation and dissociation is fast [30].

However, as known from the literature [30,31], high concentration of CDs could decrease permeated drug amount. Therefore, reduction of P_{app} for all tissues was observed as expected under addition of 9% HP-γ-CD (Table 1). Comparing the two biological tissues (Fig. 1), the cumulative permeated drug amount at the end of the permeation time through cornea (300 min) was only 7% compared to 51% through nasal mucosa (90 min) and attended with increased lag time, so that P_{app} could not be calculated (Table 1).

Permeation behaviour of AD from the HP-γ-CD solutions through porcine cornea may be explained by

membrane surface properties. The glycocalyx, which is associated with superficial corneal epithelium cells makes the surface of the epithelium rather hydrophilic [32]. The glycocalyx is anchored within the cell membrane and therefore suggested to be not completely washed off together with the adhesive mucus during equilibration of the tissue. Furthermore, the mucus layer itself may also not be fully washed off, because of its sturdy and stable structure [33]. So, the lipophilic corneal epithelium shows on its surface an hydrophilic character, which represents an additional barrier for the lipophilic drug AD. By inclusion of AD into the HP-γ-CD cavity and therefore giving a more hydrophilic cover to the lipophilic drug molecule, HP-γ-CD may increase contact time and concentration of AD at the epithelium surface.

At higher concentrations of CD, the drug can be complexed repeatedly by many more free CD cavities after dissociation and therefore can be retarded by reaching and penetrating the membrane surface. Since this process takes place in the donor solution, retardation was also detected in case of nasal mucosa and Nephrophan®. Furthermore, this additive may occupy the corneal surface by interaction with the glycocalyx or mucin. Thus, retarded permeation may be associated with an increase of the lag-time. This would also explain the significant decrease of P_{app} after pre-incubation of the cornea with HP-γ-CD.

In contrast, nasal mucus layer is produced by goblet cells located in the mucosal membrane and is moved on the surface by ciliated epithelium cells. Hence, this mucus layer is suggested to be fully washed off during equilibration because of no pronounced adhesion to superficial epithelium cells. Therefore, it seems to have no marked influence on permeability behaviour of AD in these in vitro studies.

As expected reduction of P_{app} was observed for all tissues by addition of CrEL and can be explained in a similar way. In a concentration of 20%, CrEL forms micelles (cmc $< 0.1\%$) and is able to include lipophilic substances out of the solution and to some extend out of the tissue. Furthermore, partial coating of the membrane, especially cornea, is also suggested for CrEL, because of the significant decrease of P_{app} after pre-incubation with the surfactant (Table 1). In comparison, the cyclodextrin is considered to cause retardation only because of presenting hydrophobic cavities to the drug, out of which it can very easily dissociate. However, micelle inclusion is supposed to be a rate limiting factor for AD permeability.

Corbo et al. [24] found nasal mucosa to be primarily lipophilic. They also presumed that the lipid domains of the membrane are important for the permeability of lipophilic drugs, because after lipid extraction the permeability of the lipophilic progesterone was reduced to about 25%. Consecutively, accumulation of lipophilic drugs in the lipophilic epithelium has to be expected in case of cornea. The studies of Bary et al. [29] confirm this assumption as they found after in vitro-permeability studies an approximately six times higher concentration of the lipophilic hydrocortisone in the epithelium of porcine cornea than in the hydrophilic stroma and the endothelium. Hence, the partition coefficient (see below) of a substance plays an important role regarding its permeation behaviour [34,35].

The tested ME-CD represents a mixture of both, HP-γ-CD and CrEL, together with the fatty ester IPM and water. Due to its IPM partition coefficient (see Section 2.4) of 149.7, AD is considered to tend to lipophilic regions of the ME. However, addition of HP-γ-CD (9%) to the aqueous phase reduces IPM partition coefficient of AD from 149.7 to 0.43. For this reason AD is assumed to partially remain in the hydrophilic regions of the HP-γ-CD containing ME-CD and from there to be able to permeate the membrane. Furthermore, the surfactant CrEL is located at the boundary of water and oil regions, probably in a dynamic equilibrium with micelles in the water region, so that interaction with AD in the formulation may be weaker compared to the CrEL-solution.

The second ME system, ME-PG, contains no CD but also CrEL as micelle forming component. So, a complete distribution of AD into hydrophobic domains of the formulation has to be suggested, resulting for Nephrophan® in a loss of permeability and for porcine cornea in a large reduction (Fig. 1, Table 1). Only a very small amount of drug (0.02 ± 0.02 μg/ml) was detected after 300 min and therefore no P_{app} could be calculated. However, it seems that also here tissue properties play an important role, because both MEs showed nearly the same P_{app} through nasal mucosa, which were reduced but not significantly different from the P_{app} of EBS reference. Lipophilic character of nasal mucosa appears to be pronounced, so that diffusion even out of hydrophobic regions of ME-PG seemed to be still possible. However, in case of both MEs a lag-time in the range of 20–25 min was determined, which is possibly caused by the inner structure of these carrier systems.

In summary, the influence of single additives or formulations was definitely more evident for cornea than for nasal mucosa. Small amounts of HP-γ-CD at least tendentiously increased only the corneal P_{app} and cumulative drug amount. On the opposite, high concentration (9%) of this CD-derivative and especially the surfactant CrEL significantly diminished the permeation of AD through the biological tissues as well as the synthetic membrane Nephrophan®.

The MEs may represent innovative carrier systems suitable for ocular or nasal administration. Both tested preparations are water-based, transparent and low viscous, which may minimize blurring of vision as well as restraining of mucociliary clearance, and simultaneously, the fatty component may stabilize the lipolayer of the tear film and protect the nasal mucus. Ocularly administered, ME-CD could be used if drug absorption is desired, ME-PG might rather represent a formulation if local effects are preferred. Furthermore, both MEs offer the possibility of increasing drug solubility. These findings, however, should be confirmed by in vivo-studies, because in vitro-studies do not take physiological functions on the one hand

like pre-corneal loss of drug mainly caused by blinking, tear drainage or non-corneal absorption, and on the other hand like influence of nasal mucus and mucociliary clearance into account. Tests about the physiological compatibility were previously carried out: ME-PG was classified as non irritable by HET-CAM test [6] and the additive HP-γ-CD was also non irritable in histological studies [14].

Furthermore, comparison of the three different tissue barriers brings into account the correlation between lipophilicity of the carrier medium and lipophilic content in the membrane. For nasal mucosa and cornea are different in this content, regulation of drug absorption with carrier composition may be possible.

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