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Short Communication

Fish skin as a model membrane: structure and characteristics

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Abstract

Objectives Synthetic and cell-based membranes are frequently used during drug formulation development for the assessment of drug availability. However, most of the currently used membranes do not mimic mucosal membranes well, especially the aqueous mucous layer of the membranes. In this study we evaluated catfish (*Anarichas lupus* L) skin as a model membrane.

Method Permeation of hydrocortisone, lidocaine hydrochloride, benzocaine, diethylstilbestrol, naproxen, picric acid and sodium nitrate through skin from a freshly caught catfish was determined in Franz diffusion cells.

Key findings Both lipophilic and hydrophilic molecules permeate through catfish skin via hydrated channels or aqueous pores. No correlation was observed between the octanol/water partition coefficient of the permeating molecules and their permeability coefficient through the skin. Permeation through catfish skin was found to be diffusion controlled.

Conclusions The results suggest that permeation through the fish skin proceeds via a diffusion-controlled process, a process that is similar to drug permeation through the aqueous mucous layer of a mucosal membrane. In addition, the fish skin, with its collagen matrix structure, appears to possess similar properties to the eye sclera.

Keywords diffusion; model; mucosa; mucus; permeation

Introduction

The ability of a drug to permeate from a given pharmaceutical formulation through biological membranes is one of the key properties that determines drug bioavailability after, for example, oral, nasal, pulmonary, ocular or dermal application. Current experimental techniques used during high throughput screening of new biologically active compounds,[1] such as the parallel artificial membrane permeability assay and cell-based assays such as Caco-2, are still of limited value during formulation development, even after years of methodological modifications in efforts to overcome limitations of the techniques for, for example, formulations of moderate and high viscosity.[2] Lipophilic membranes such as silicone membranes do, however, mimic lipophilic membrane barriers such as skin and can in some cases be used during formulation development. Most biological membranes have an unstirred or stagnant water layer (UWL) at the surface that acts as a diffusion barrier for rapidly permeating compounds. The thickness of this UWL can vary from a few nanometres to about 8 μm on the eye surface and 30–100 μm in the gastrointestinal tract.[3,4] These relatively thick and viscous UWLs can contribute significantly to the overall barrier function of the membrane.[5,6] Simple lipophilic artificial membranes such as silicone membranes have only very thin UWLs, which make insignificant contributions to the overall membrane barrier. Some membranes completely lack a lipophilic component and consist only of an UWL, as in the case of the eye sclera. The sclera is composed primarily of collagen fibres embedded in a mucopolysaccharide matrix, resembling an aqueous gel structure. The permeability of the sclera has no apparent dependence on the drug lipophilicity (i.e. the octanol/water partition coefficient) but a strong dependence on the molecular weight (MW) of the drug (i.e. the hydrodynamic radius of the permeating drug molecule), the permeability coefficient decreasing with increasing MW.[7,8] Although membrane drug influx and efflux transporters have been located in the eye, the primary route for drug permeation through the sclera is by passive diffusion through an aqueous pathway.[4] Drug permeation though other biomembranes, such as from the gastrointestinal tract, is also predominantly by passive diffusion.

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In general, fish skin consists of three layers (i.e. epidermis, dermis and hypodermis), with an aqueous mucous layer covering the epidermal surface; the hypodermis is the innermost layer closest to the muscle.[9,10] The uppermost layer of the epidermis, the superficial stratum, contains microridges that retain the mucus and antibacterial and antifungal substances secreted at the surface from mucus goblet cells located in the intermediate stratum.[11] The dermis is principally composed of collagenous matrix and the hypodermis is composed of loosely organised collagen and vasculature.[11] Thus, fish skin appears to be structurally similar to mucous membranes in humans and other mammals (e.g. the buccal mucosa), which has generated some interest in using fish skin as a model for various mucous membranes.[12]

A study from 1991 revealed that the mucosal route is important in the excretion of the orally administered drug oxytetracycline in rainbow trout.[13] Further studies revealed that both lipophilic and hydrophilic molecules permeate fish skin via aqueous routes.[14] Catfish (*Anarichas lupus* L) skin does not have any scales. We therefore chose to study catfish skin as a model membrane for mucous membranes and collagen matrix membranes that can be used during drug development.

Materials and Methods

Materials

Hydrocortisone was purchased from INC Biomedicals (Ohio, USA), lidocaine hydrochloride, benzocaine and diethylstilbestrol from Norsk Medisinaldeport (Oslo, Norway), naproxen from Iceland Pharmaceuticals (Reykjavik, Iceland), picric acid and sodium nitrate from Merck (Darmstadt, Germany), β-cyclodextrin from Wacker Chemie (Munich, Germany), and 2-hydroxypropyl-β-cyclodextrin (HPβCD) of molar substitution 0.64 from Roquette (Paris, France). Chromatographic determinations were performed on a Merck–Hitachi AS-2000A autosampler, L-6200A pump and L-4250 l UV detector. All other reagents were of analytical or special reagent grade.

Permeability studies

The catfish skin was obtained within 24 h of the fish being caught. The catfish used weighed from 1.7 to 2.3 kg, which gives us an estimated age of 11 ± 2 years (Marine Research Institute, Reykjavik, Iceland). The skin was removed from the fish by skilled professionals at the fish factory (Hamrafell, Hafnafjörður, Iceland) and used within 2–3 h. A thin layer of the residual muscle tissue was either kept intact or completely removed. A 5 × 5 cm slice was cut from the sub-lateral portion of the catfish skin and placed in a Franz diffusion cell, type FDC 400 15FF (Vangard International Inc., Neptune, NJ, USA), containing 11.9 ml receptor phase. The receptor phase consisted of phosphate-buffered saline, pH 7.4, containing 2.5% (w/v) HPβCD, stirred with a magnetic bar. The donor phase consisted of a solution of the compound to be tested in either pure water or an aqueous HPβCD solution (2–7% w/v), maintaining the aqueous solubility of poorly soluble drugs at about 10 mg/ml, except for measurement of the flux of hydrocortisone through fish skin at 5.5 mg/ml. The diffusion cells were kept at ambient temperature (22–23°C). Two millilitres of donor phase was added to the donor chamber and 100 μl samples withdrawn from the receptor phase at various time points up to 30 h and replaced by fresh receptor phase.

HPLC was used for quantitative determination of the permeating compounds in the receptor phase, except for picric acid, which was determined spectrophotometrically, and nitrate, which was measured using a nitrate-selective electrode.[14] The flux was calculated from the linear part of each permeability profile. The results presented are mean ± SD of three separate experiments. For comparison, the fish skin was replaced by a semi-permeable cellophane membrane with a MW cut-off (MWCO) of 12–14 000, with or without fused 1-octanol collodium membrane.[5] The thickness of the hydrated membranes was estimated by weighing the membranes before and after hydration, using the density of water at room temperature and the area of the membranes. Fish skin thickness was measured with a calipers after placing the fish skin between two thin aluminium sheets. Average thickness was 1.21 ± 0.26 mm for fish skin, and 2.79 ± 0.66 mm for fish skin with residual muscle tissue.

Theory

The total permeation resistance of a given membrane (Figure 1) is the sum of resistance within the UWL (R_{Aq}) and the membrane (R_M), and their relative importance depends on the physicochemical properties of both the permeating compound and the membrane.[6,15,16] The equation describing passive transport through a simple two-layer membrane (i.e. UWL and a lipophilic membrane) is based on Fick's first law:

$$J = P \cdot C_{Aq} = \left(R_{Aq} + R_M\right)^{-1} \cdot C_{Aq} = \left(\frac{1}{P_{Aq}} + \frac{1}{P_M}\right)^{-1} \cdot C_{Aq} \quad (1)$$

where J is the flux through a membrane (mass/area/time), P is the permeability coefficient of the compound through the lipophilic membrane and C_{Aq} is the concentration of dissolved compound in the aqueous donor vehicle. P_M and P_{Aq} are the permeability coefficients through the lipophilic membrane and UWL, respectively. The permeability coefficient is defined as:

$$P = \frac{D \cdot K}{h} \quad (2)$$

where D is the diffusion coefficient of the compound within the membrane or the UWL, K is the partition coefficient of the compound from the UWL into the membrane, and h is the effective thickness of the membrane or UWL.

Finally, the diffusion coefficient can be estimated from the Stokes–Einstein equation:

$$D \approx \frac{R \cdot T}{6\pi \cdot \eta \cdot r \cdot N} \quad (3)$$

where R is the molar gas constant, T is the absolute temperature, η is the apparent viscosity within the UWL or the lipophilic membrane, r is the radius of the permeating molecule, and N is Avogadro's number. Thus, the diffusion constant within the UWL (D_{UWL}) will decrease with

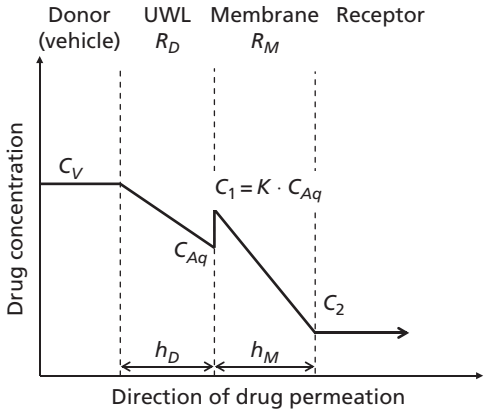

Figure 1 Schematic drawing of drug permeation from a donor (vehicle) through the unstirred water layer (UWL) and then through the membrane into a receptor. C_V, drug concentration in the donor (vehicle); C_{Aq}, drug concentration in the UWL immediate to the membrane surface; C_1, drug concentration within the membrane at the donor side; K, the drug partition coefficient between the UWL and the membrane; h_D, thickness of the UWL on the donor side; h_M, thickness of the membrane. R_D and R_M are the resistances in the UWL at the donor side and within the membrane, respectively.

increasing viscosity of the layer as well as with increasing MW of the permeating molecules. For example, small lipophilic molecules frequently possess a large permeability coefficient through the lipophilic membrane (i.e. large P_M value) and thus may be able to permeate across the lipophilic membrane much faster than they can be transported through the stagnant water layer. Under such conditions, diffusion through the water layer becomes the rate-limiting step in the absorption process.[6] If the membrane consists of a hydrogel-like collagen membrane, then K in equation 2 will equal unity and in Figure 1 $C_{Aq} = C_1$.

Results

Table 1 shows the flux and permeability coefficients of nitrate ions and hydrocortisone through a semi-permeable cellophane membrane with MWCO 12–14 000 and catfish skin. The compounds permeate readily through the cellophane membrane via a diffusion-controlled process. However, permeation of nitrate ions is virtually blocked when a lipophilic octanol collodium membrane is fused to the receptor side of the artificial membrane, while the lipophilic hydrocortisone molecules are still able to permeate the membrane. In this case, permeation of the hydrophilic nitrate ions becomes membrane controlled (i.e. $P_{Aq} >> P_M$), while introduction of the lipophilic membrane does not change the barrier function towards hydrocortisone (i.e. $P_M >> P_{Aq}$). Both the hydrophilic nitrate ions and the lipophilic hydrocortisone molecules readily permeate the fish skin, indicating that permeation proceeds via hydrated channels or aqueous pores; leaving a thin layer of the muscle tissue on the skin did not affect the permeation mechanism. Slightly lower flux through the muscle-containing skin could be due to the greater effective thickness of the permeation barrier (i.e. h in equation 2). There were no significant differences between groups in Table 1 (Kruskal–Wallis one-way analysis of variance).

Table 2 shows the permeability coefficient (P) of various compounds through catfish skin. No correlation between the octanol/water partition coefficient ($K_{O/W}$ (K in equation 2)) and P was observed, indicating that the lipophilic membrane does not create any permeation resistance ($R_M \approx 0$). In contrast, there appears to be a correlation between the calculated diffusion coefficients in water (D_W or D in equation 2) and P. This again indicates that permeation through the catfish skin is diffusion controlled.

Discussion

Skin, as well as most other biological membrane barriers, in mammals consists of both an aqueous diffusion barrier and a lipophilic membrane barrier. Under such conditions, maximum flux is obtained when the permeating compound has some optimum lipophilicity, as measured by the logarithm of the octanol/water partition coefficient ($logK_{O/W}$). For example, the optimum $logK_{O/W}$ is 3.3 for permeation of steroids through human epidermis, 5.5 for permeation of undissociated bases through human buccal mucosa, and 2.8 for permeation of steroids through rabbit cornea.[20] In the case of catfish skin, such optimum $logK_{O/W}$ does not appear to exist. Instead, the catfish skin is mainly a diffusion barrier where the flux is proportional to the diffusion coefficient of the permeating molecule in pure water (i.e. D_W or D in equation 2)

Table 1 The flux and permeability coefficient of sodium nitrate (molecular weight (MW) 85.0) and hydrocortisone (MW 362.5) through semi-permeable cellophane membrane with MW cut-off 12–14 000, with and without fused octanol collodium membrane, and fish skin, with and without a thin layer of attached muscle tissue, at ambient temperature (22–23°C)

Membrane and its thickness	Flux (μmol cm per h)		Permeability coefficient (cm/h)	
	Nitrate	Hydrocortisone	Nitrate	Hydrocortisone
Cellophane (230 μm)	9.81 ± 0.47	0.40 ± 0.05	$83.4 \pm 4.0 \times 10^{-3}$	$14.5 \pm 1.8 \times 10^{-3}$
Cellophane with octanol collodium (350 μm)[a]	0.003 ± 0.006	0.42 ± 0.05	$0.03 \pm 0.05 \times 10^{-3}$	$15.2 \pm 1.8 \times 10^{-3}$
Fish skin (1400 μm)	5.95 ± 0.11	0.25 ± 0.02	$92.0 \pm 1.7 \times 10^{-3}$	$16.4 \pm 1.3 \times 10^{-3}$
Fish skin with tissue (2700 μm)	5.66 ± 0.14	0.09 ± 0.03	$87.5 \pm 2.2 \times 10^{-3}$	$7.4 \pm 2.5 \times 10^{-3}$

The donor phase consisted of aqueous 5% (w/v) 2-hydroxypropyl-β-cyclodextrin (HPβCD) solution (cellophane membrane) or 2.5% (w/v) HPβCD (fish skin). The concentration of sodium nitrate and hydrocortisone in the donor phase was 10 mg/ml (cellophane) or 5.5 mg/ml (fish skin).
[a]Estimated unstirred water layer 180 μm.
Values are means ± SD from three separate experiments.

Table 2 Permeability coefficient of selected compounds through fish skin (without muscle tissue) at ambient temperature

Compound	MW (Dalton)	*pKa*	$logK_{O/W}$[c]	D_W[d] (cm^2/s)	[HPβCD] (% w/v)	Permeability coefficient (cm/h)
Sodium nitrate[a]	85.0			17.5	5	$92.0 \pm 1.7 \times 10^{-3}$
Benzocaine	165.2	2.5	1.9	7.08	7	$16.3 \pm 3.8 \times 10^{-3}$
Picric acid[a]	229.1	0.3	–0.9	7.08	2	$33.3 \pm 5.1 \times 10^{-3}$
Naproxen[a]	230.3	4.2	–1.1	5.84	7	$19.4 \pm 0.5 \times 10^{-3}$
Diethylstilbestrol	268.4		5.1	5.14	7	$14.7 \pm 0.3 \times 10^{-3}$
Lidocaine HCl monohydrate[b]	288.8	7.9	1.7	5.02	2	$23.7 \pm 2.0 \times 10^{-3}$
Hydrocortisone	362.5		1.6	4.48	5	$16.4 \pm 1.3 \times 10^{-3}$
β-Cyclodextrin	1135.1		< –3	2.56	0	$1.1 \pm 0.0 \times 10^{-3}$

MW, molecular weight; *pKa,* ionisation constant of the acid or protonised form of the base; $logK_{O/W}$, logarithm of the partition coefficient between 1-octanol and water at neutral pH; D_W, calculated diffusion constant in dilute aqueous solution; [HPβCD], concentration of 2-hydroxypropyl-β-cyclodextrin in the donor phase.
Data are means ± SD of three separate experiments.
[a]Ionised at neutral pH.
[b]Partly ionised at neutral pH.
[c]From refs 17 and 18.
[d]Calculated according to ref. 19.

or inversely proportional to the hydrodynamic radius of the molecule (i.e. r in equation 3). In that way, fish skin, which is a membrane barrier, appears to be similar to the eye sclera where the drug permeation coefficient has no apparent dependence on $logK_{O/W}$, but a strong dependence on the hydrodynamic radius (D_W) of the permeating drug molecule.

Conclusions

Permeation of both lipophilic and hydrophilic molecules through catfish skin is diffusion controlled and most likely proceeds through hydrated channels or aqueous pores. The skin appears to mimic the mucous layer of mucosal membranes and thus can be used as a model membrane when the mucus (i.e. the UWL) is the rate-limiting barrier. However, it is not a good model of mucosal membranes where the lipophilic membrane itself is the rate-limiting barrier. In addition, catfish skin can be used to mimic collagen matrix membranes such as the eye sclera.

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Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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