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Newborn Pig Skin as Model Membrane in In Vitro Drug Permeation Studies: A Technical Note

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INTRODUCTION

The in vitro study of drug skin permeability plays an essential role in the selection of candidates for the development of transdermal dosage forms. Such experiments are generally performed by using a diffusion cell whose donor and receiver compartments are separated by a membrane. Human skin, either excised from surgical reduction or obtained from a cadaver, is the membrane of choice, but it is not readily available. Thus, in the last decades many efforts have been made to individuate a suitable alternative using synthetic and biological membranes. The former cannot be used to replace human skin in an experimental context. In the setting of animal models for transdermal permeation studies, the characteristics of excised skin from mice, rats, rabbits, and pigs were thoroughly investigated and widely used. However, animal skin is different from human skin in several features. Indeed, the main barrier to drug permeation through skin is the stratum corneum, which has been reported to differ in terms of lipid composition, water content, and morphological characteristics (thickness, number of pores, and follicles) on the basis of species. Pig stratum corneum is the most similar to human stratum corneum in terms of lipid composition, but it presents a marked difference in terms of thickness.[1] On the other hand, the thickness of newborn pig stratum corneum is considerably thinner than that of adult pig and more similar to that of human skin, even if the number of hair follicles is higher than that of human or adult pig skin. However, even if this membrane could represent a favorable alternative to rat and mouse skins, only a few studies have used the newborn pig skin in ex vivo skin permeability studies.[2-8] Moreover, a limited number of investigations aiming to compare the performances of newborn pig skin with respect to excised human epidermis are available in literature.[3] Songkro et al pointed out that newborn pig skin gave rise to similar fluxes when the permeant was a lipophilic molecule, namely, propranolol hydrochloride.[3]

The present work is aimed at confirming the suitability of newborn pig skin in preliminary skin permeation screenings. With this purpose, the permeability through newborn pig skin of a series of benzoxazinones (Figure 1) was determined, and the results were compared with the data through human epidermis (SCE) reported in the literature.[9] This set of molecules, which differed in substituent groups and their positions on the aromatic ring, was selected as it presents a quite narrow range of the relevant physicochemical parameters to the diffusion through human skin. Moreover, it enabled evaluation of the influence of different regioisomers with the same substituents (-OCH_3, -Cl) in 2 different positions.

MATERIALS AND METHODS

The 7 benzoxazinones reported in Figure 1 were kindly donated by Italfarmaco Research Centre (Milan, Italy). All substances and chemicals were used as received.

The in vitro skin permeation studies were performed in triplicate (at least 3 times in order to achieve statistical significance) by means of vertical Franz diffusion cells with an effective diffusion area of 0.636 cm^2. The experiments were performed by using the skin of 2 newborn Goland-Pietrain hybrid pigs. The pigs (~1.2 kg) died of natural causes a few hours after birth, and they were provided to us by a local slaughterhouse. The subcutaneous fat was carefully removed, and the skin was cut into 3×3 cm^2 samples and randomized. The skin samples were stored at −18°C and placed at 4°C the day before the experiments. Two hours before the beginning of the experiments, the skin was pre-equilibrated in phosphate buffer solution (PBS) at 25°C. A circular specimen of the skin was sandwiched securely between the 2 halves of the Franz diffusion cell, with the stratum corneum side facing the donor compartment. The receiver compartment (5.5 mL) was filled with 0.9% NaCl solution:polyethylene glycol 400 (80:20, %vol:vol) solution, containing 100 μg/mL streptomycin as preservative, thermostated at 37°C ± 1°C, and magnetically stirred.

At the beginning of the experiment, 1 mL of water:polyethylene glycol 400 (80:20, vol:vol) drug-saturated solution was placed in the donor compartment.

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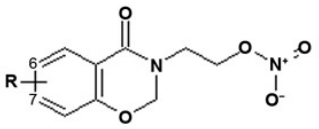

Compound	*R*	Mw (Da)	Mp (°C)	S (μg/mL)	Log P
a	H	238.2	55.5-56.5	2604 ± 18	1.68 ± 0.02
d	6-methyl	252.2	77-78	669 ± 5	2.41 ± 0.05
e	6-hydroxy	254.2	80-82	3477 ± 23	1.63 ± 0.00
f	6-methoxy	268.2	62	1490 ± 128	2.07 ± 0.08
g	6-chloro	272.6	98-99	134 ± 1	2.30 ± 0.13
m	7-methoxy	268.2	101-102	227 ± 2	0.56 ± 0.14
n	7-chloro	272.6	86.88	226 ± 1	1.62 ± 0.26

Figure 1. Compound list and substituent and their main physicochemical characteristics. Mw indicates molecular weight; Mp, melting point; S, solubility.

After elapsed times of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 24 hours, 0.2 mL of the receiving solution was withdrawn and replaced with an equal volume of prethermostated fresh medium. Sink conditions were maintained throughout the experiment.

The concentrations of each compound in the receiver medium were determined by high-performance liquid chromatography (HPLC) assay as previously described.[9] The apparatus used was a Liquid Chromatograph Alliance 2690 HPLC System (Waters Corp, Milford, MA), equipped with a photodiode array detector and a computer integrating apparatus (Millennium 32, Waters). The column was a Nova-Pack C18 (60Å 4 μm, 3.9×150 mm, Waters). Samples were injected using an autosampler.

The cumulative amount that permeated through the newborn pig skin per unit area was calculated from the concentration of each substance in the receiving medium and plotted as a function of time. The flux (J) was determined as the slope of the linear portion of the plot. The permeability coefficient was calculated according to Fick's first law of diffusion (Equation 1):

$$K_p = \frac{J_{\max}}{S} \qquad (1)$$

where K_p (cm/h) is the permeability coefficient, $J_{\max}$ (μg/cm^2 per hour) is the flux obtained with the saturated solution, and S is the drug donor concentration (μg/cm^3), corresponding to the drug solubility in the vehicle at 32°C. The solubility values were taken from literature.[9]

Results and Discussion

Skin permeation profiles of benzoxaziones from newborn pig skin are represented in Figure 2. Compound a (Figure 1), which presents the lowest molecular weight, showed the highest flux. As far as regioisomers are concerned, the compounds substituted in position 6 of the benzoxazinone, compounds f and g, evidenced fluxes higher than those of the molecules with the same substituent in position 7, compounds m and n (Figure 1). The decrease of log P caused by the presence of the hydroxylic group (compound e; Figure 1), determined the lowest coefficient of permeability (Table 1). These experimental evidences were consistent with the data obtained in the case of skin permeation studies performed using human epidermis.[9] Also, in the present set of experiments, the lag phase was negligible (<1 hour) as already reported in the permeation studies performed through human skin.[9] This evidence significantly differed with respect to the data reported by some authors using adult pig skin, where an extended lag time was described.[10]

The J_{max} obtained by the present set of benzoxazinones were compared with the human skin permeability data[9] by means of the factor of difference value (FoD) described by Dick and Scott[11] (Equation 2):

$$FoD = \frac{J_{\max,\,p}}{J_{\max,\,h}} \qquad (2)$$

where $J_{max,p}$ denotes J_{max} through newborn pig skin and $J_{max,h}$ denotes J_{max} through human epidermis. The study suggested that an animal model represents a significant prediction for the human skin behavior if its associated FoD value is less than 3.[11]

The FoD of this set of experiments ranged from 0.48 to 1.91, indicating that the J_{max} determined using newborn pig skin was in the same order of magnitude as that of benzoxazinones associated with human epidermis (Table 1). Moreover, the FoD values did not appear to be related to any physicochemical properties of the set of molecules reported in Figure 1: this suggests that the differences between the

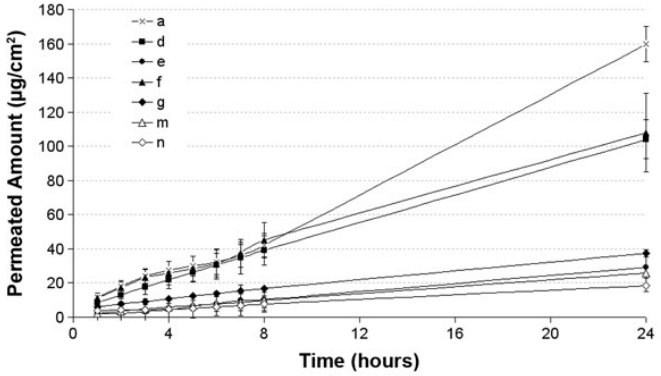

Figure 2. Skin permeation profiles of benzoxazinones from newborn pig skin.

Table 1. Maximum Flux (J) and Permeability Coefficient (K_p) of Benzoxazinones Determined by Using Newborn Pig Skin and Human Epidermis and FoD

	Newborn Pig Skin		Human Epidermis*		
Compound	J ($\mu g/cm^2$ per hour)	$K_p \times 10^3$ (cm/h)	J ($\mu g/cm^2$ per hour)	$K_p \times 10^3$ (cm/h)	FoD
a	6.64 ± 1.45	2.55 ± 0.55	9.91 ± 1.50	3.81 ± 0.58	0.67
d	4.34 ± 1.20	6.49 ± 1.79	3.86 ± 0.76	5.78 ± 1.09	1.12
e	1.21 ± 0.29	0.35 ± 0.08	1.05 ± 0.33	0.30 ± 0.09	1.15
f	4.38 ± 1.13	2.94 ± 0.75	5.49 ± 1.66	3.68 ± 1.11	0.80
g	1.56 ± 0.09	11.64 ± 0.67	0.90 ± 0.34	6.73 ± 2.52	1.73
m	1.07 ± 0.50	4.71 ± 2.20	0.56 ± 0.14	2.49 ± 0.60	1.91
n	0.78 ± 0.17	3.45 ± 0.75	1.62 ± 0.26	7.17 ± 1.17	0.48

*Data from Minghetti et al.[9]

2 membranes; namely, human epidermis and newborn pig skin, have no direct implications concerning the diffusion of benzoxazinones, a fact which confirms the validity of the model in the present study.

A good correlation was found by plotting the $J_{max,p}$ as a function of $J_{max,h}$ (Equation 3).

$$J_{\max,\,p} = 1.44 \pm 0.19 J_{\max,\,h} - 0.79 \pm 0.70 \qquad (3)$$

$$r^2 = 9.915;\ F = 54.11;\ p = 0.0007 \qquad (4)$$

The low value of the intercept and its high standard deviation is consistent with the lack of the lag phase in the permeation process. Considering that the intercept is negligible, the coefficient 1.44 in Equation 3 can be regarded as the average FoD value. It turned out to be very close to the value reported in the literature for adult pig ear skin,[12] so it is possible that the use of newborn pig skin during in vitro permeation studies may overestimate the human percutaneous absorption. However, taking into account the discussion above together with the FoD range (Table 1), this animal model can be regarded as predictive of human skin permeability.

Summary and Conclusions

The suitability of newborn pig skin as an alternative to human epidermis in in vitro permeation studies was investigated. A set of 7 benzoxazinones was used to perform in vitro experiments by using a modified Franz diffusion cell and excised newborn pig skin as a membrane. The maximum flux through newborn pig skin ($J_{max,p}$) was compared with the maximum flux through excised human epidermis ($J_{max,h}$), available from the literature, by means of the factor of difference value FoD = $J_{max,p}/J_{max,h}$. The FoD values ranged from 0.48 to 1.91, indicating that $J_{max,p}$ and $J_{max,h}$ were in the same order of magnitude.

This result confirmed the suitability of this membrane to assess the permeability of not completely freely water soluble drugs, such as the set of benzoxazinones used in the present study and propranolol hydrochloride.[3] Considering that the skin was withdrawn from animals that died of natural causes, the ethical problems connected with the use of animal skin in preliminary permeation screenings can be bypassed.

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