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In vitro permeation of progesterone from a gel through the shed skin of three different snake species

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Abstract

The in vitro diffusion of progesterone from a gel formulation using the European Pharmacopoeia method for transdermal dosage forms is described. The membranes used were the dorsal and ventral portions of the shed skin of three different species of snake. Considerable differences are apparent between the dorsal and ventral sites and between the different species of snake. The dorsal area shows better permeability for progesterone and the permeability order for the different species is python > cobra > viper. These differences may be due to the thickness of the skin and the hinge:scale ratio. The results indicate that shed snake skin is not a model membrane for human skin.

Keywords: Shed Snake Skin; Progesterone; In vitro diffusion

1. Introduction

Recently there has been considerable interest (Itoh et al., 1990; Rigg and Barry, 1990; Harada et al., 1993; Takahashi et al., 1993; Craane-van Hinsberg et al., 1995; Kuramoto et al., 1996) in the use of shed snake skin as a model membrane for in vitro diffusion studies monitoring the release of a number of drugs from semisolid formulations. Shed snake skin has also been suggested as a model membrane for the study of the effects of a number of penetration enhancers (Fleeker et al., 1989; Wong et al., 1989; Bhatt et al., 1991; Hirvonen et al., 1991; Bhattachar et al., 1992; Fu Lu et al., 1992; Buyuktimkin et al., 1993; Hirvonen et al., 1993; Turunen et al., 1993; Suh and Jun, 1996). Although shed snake skin is not a mammalian integument, it has been reported that some compounds penetrate snake skin and human stratum corneum at similar rates (Itoh et al., 1990; Rigg and Barry, 1990; Harada et al., 1993; Takahashi et al., 1993).

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In some countries it is particularly difficult to obtain human skin for in vitro experimentation and it is therefore important to have alternate biological or synthetic membranes which mimic human skin membranes for diffusion experiments. In South Africa, shed snake skin is easily obtainable from the many snake parks present in the country. Since snakes moult periodically, a single animal can provide repeated sheds, thus reducing inter-individual variability. Skins can be obtained without injury to the animal and do not have to be subjected to chemical or heat stress prior to use. The epidermis is shed as a large intact sheet, thus a single snake skin can provide multiple samples. Shed snake skin is not a living tissue, can be stored for long periods at room temperature and is easily transported. Stored and fresh snake skins appear to show no differences in permeability (Haigh, J.M., Smith, E.W., unpublished results, 1997). Since snake skin lacks hair follicles, the problems associated with the transfollicular route of penetration, which may be significant in mammalian skins, can be avoided.

Snakes shed their skin every two to four months, depending upon the species. Skins are shed in different ways. One of us (J.M.H.) has observed this shedding in a South African snake park. Two of the species reported on here (cobra and viper) rub their noses against a hard surface, normally a rock, to break the old skin. They then, with very gentle muscular contractions, loosen the whole skin and ease their bodies out of the hole in front, leaving behind a complete shed skin. The python, on the other hand, tends to rub its body against the rocks, thus the skin comes away in fragments. It seems reasonable to suggest, on the basis of this observation, that the cobra and viper skins are likely to have better integrity than python skin.

Shed snake skin is composed of two very different regions; scales which are separated by hinges. The scales are rigid, whereas the hinge region is elastic. The scales on the dorsal surface are much smaller than the scales on the ventral surface and the size of the scales varies considerably between species. The lipid composition of shed snake skins has previously been described (Itoh et al., 1990; Rigg and Barry, 1990).

A survey of reported studies indicates that different snake species have been used as well as both dorsal and ventral skin. Some studies report which species of snake was utilised and whether dorsal or ventral skin was used in the experiments. Some studies, however, did not report whether dorsal or ventral skin was used (Fleeker et al., 1989; Wong et al., 1989; Bhatt et al., 1991; Buyuktimkin et al., 1993; Harada et al., 1993; Takahashi et al., 1993; Suh and Jun, 1996) and two studies did not indicate which species was used (Bhattachar et al., 1992; Fu Lu et al., 1992).

This study was designed to evaluate the diffusion characteristics of three different species of snake skin and to investigate the differences, if any, between the dorsal and ventral skin sites.

2. Materials and methods

2.1. Diffusion experiments

The in vitro permeation of progesterone from a commercially available gel formulation (Crinone® 4%, Columbia Laboratories, United Kingdom) was monitored over a period of 72 h. The diffusion cell of the European Pharmacopoeia (1997) was utilised with a 32 mm diameter diffusion surface. The receptor solution was 1 l of 0.5% sodium lauryl sulphate in water maintained at 37°C in a waterbath and stirred at 100 rpm. Sodium lauryl sulphate was chosen as the solute as it increases the solubility of the progesterone in the receptor solution without interfering with the analytical procedure. A temperature of 37°C was chosen as this formulation was designed for vaginal use. 1 g of the gel was used in each diffusion cell. 1 ml samples were withdrawn for analysis at 2, 4, 8, 24, 48 and 72 h after commencement of the experiment. The receptor solution was not replaced after each withdrawal. Both dorsal and ventral skin was utilised, six replicates being performed for each skin site. At the end of the diffusion experiment the skins were intact and no

Table 1
Release constants, lag times, r^2 values and thickness measurements for shed snake skin (mean $\pm$ S.D., $n = 6$)

Membrane	Apparent release constant (μg/cm^2 per h$^{1/2}$)	Lag time (h$^{1/2}$)	r^2 (95%)	Thickness (μm)	
				Scale	Hinge
Python dorsal	24.9 (2.0)	1.1 (0.1)	0.945	14 (1)	9 (3)
Python ventral	19.2 (1.3)	1.2 (0.1)	0.957	16 (1)	11 (3)
Cobra dorsal	17.9 (2.4)	1.3 (0.1)	0.931	16 (1)	5 (3)
Cobra ventral	17.8 (1.3)	1.4 (0.1)	0.931	42 (4)	6 (3)
Viper dorsal	7.7 (0.6)	0.2 (0.1)	0.965	24 (2)	8 (4)
Viper ventral	6.1 (0.7)	0.0 (0.0)	0.972	35 (2)	13 (4)

stretching was observed, indicating no diffusion of the receptor fluid through the membrane into the gel.

2.2. Snake skins

The shed skins of three different species (*Python sebae natalensis* (African rock python), *Naja melanoleuca* (forest cobra) and *Bitis nasicornis* (rhinoceros viper)) of snake were used. The shed snake skins were freshly moulted samples obtained from the Port Elizabeth Museum Snake Park, Port Elizabeth, South Africa. The skins were cut to size and hydrated by allowing them to soak overnight in water in a covered petri dish. The outer surface of the skin was placed in contact with the progesterone gel formulation.

2.3. Analytical procedure

Progesterone in the receptor fluid was determined by HPLC analysis. The analytical procedure developed was based on a previously published report (Das Gupta, 1982) with some modifications. The method was validated in our laboratories for reproducibility, specificity, linearity and accuracy. The detection limit for progesterone is 0.2 μg/ml.

2.4. Electron microscopy

A scanning electron microscope (JEOL JSM 840) was used to determine the integrity, surface morphology and thickness of the snake skins. For the surface topography micrographs, samples were sputter-coated with a thin layer of gold. For the transverse sections, the samples were freeze fractured before the coating process.

2.5. Calculation of constants

Linear regression analysis of the data was performed by plotting the cumulative amount of progesterone determined in the receptor solution versus the square root of time in hours. From these plots, the apparent release constant (corresponding to the slope) and the lag time (corresponding to the intercept on the square root of time axis) were determined for each of the membranes used (Table 1).

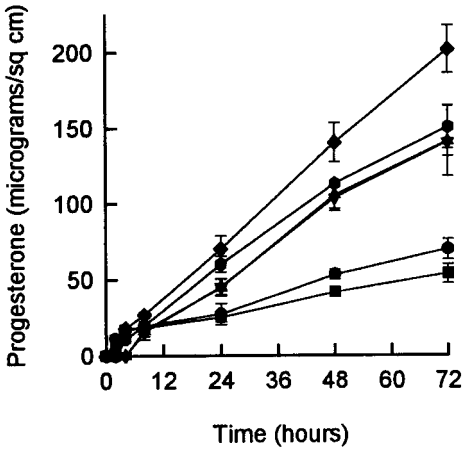

Fig. 1. Progesterone diffusion from a gel through python dorsal (◆), python ventral (●), cobra dorsal (▲), cobra ventral (▼), viper dorsal (●) and viper ventral (■) skins (mean $\pm$ S.D., $n = 6$).

3. Results and discussion

It can be seen from Fig. 1 that all three species of snake produce shed skins which display different characteristics as diffusion membranes. Most permeable is python skin with dorsal skin being significantly more permeable than ventral skin. Cobra skin displays intermediate permeability with dorsal and ventral skin producing identical results. Viper skin is the least permeable with dorsal skin being more permeable than ventral skin. The apparent release constants (Table 1), obtained from regression analysis of the diffusion curves, display the same trend. The lag time is very similar for python and cobra skins but virtually zero for viper skin. Of interest is that while dorsal and ventral skins of a single species appear to have different diffusion characteristics, the lag times are almost identical. There has been one previous report (Rigg and Barry, 1990) which showed that the dorsal skin of the python (*Python molurus*, a different species to that reported on here) was more permeable to radioactive 5-fluorouracil than the ventral skin, depending on the pretreatment method. Pretreatment of the shed python skin with propylene glycol produced ventral skin which was more permeable to 5-fluorouracil than dorsal skin. Pretreatment with five other penetration enhancers showed the reverse trend.

The thickness of the skins (Table 1) shows some interesting trends. The ventral skin of all three species is always thicker than the dorsal skin both in the scale and hinge regions. This is certainly due to the fact that when the snake moves, the ventral surface is in contact with the ground. It would therefore have to be more robust than the dorsal surface. The hinge region is always considerably thinner than the scale region, especially in the cobra and viper. The thicknesses of the scale and hinge areas of the snake skins were determined from the electron micrographs of the freeze fractured specimens. Typical electron micrographs are illustrated in Figs. 2 and 3 (cobra). The electron micrographs of the skins of the other species showed the same type of structure for the hinge and scale regions. The ventral scales are very much larger than the dorsal scales in all three snake species therefore there is a much larger hinge:scale ratio for dorsal skin than there is for ventral skin. Since permeation appears to be more facile through dorsal skin, it is tempting to suggest that diffusion occurs mainly through the hinge region of the skin.

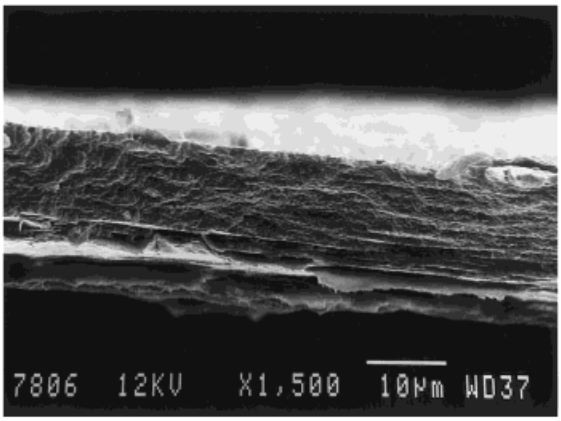

Fig. 2. Electron micrograph of transverse section of dorsal cobra scale.

The difference between species is more difficult to explain. The size of the scales may be a factor. The scales of both the dorsal and ventral surface of the python are much smaller than those of the cobra and viper, therefore more hinge area would be in contact with the formulation. This may account for the higher permeability of the python skin. It may also be due to a reduction in integrity

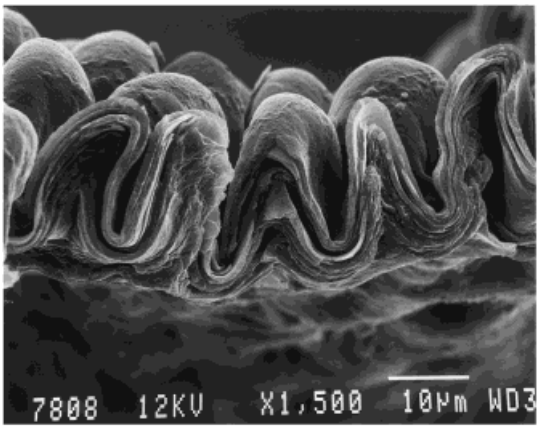

Fig. 3. Electron micrograph of transverse section of dorsal cobra hinge.

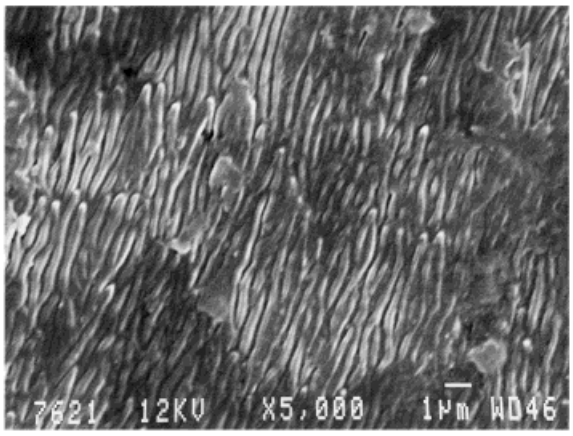

Fig. 4. Electron micrograph of dorsal python scale (outer surface).

because of the rough manner in which this snake sheds its skin, although the replicate determinations showed good reproducibility as can be seen in Fig. 1. As the electron micrographs show, the surface topography of the snake skins of each species is completely different. Figs. 4–6 show the surface topography of the dorsal scale for each of the three species. This difference in the structure of the snake skins may also be a reason for the different diffusion characteristics. The dorsal hinge, ventral scale and ventral hinge areas also display differences in surface structure. High magnification electron microscopic examination of larger areas of the dorsal and ventral scale and hinge areas of the

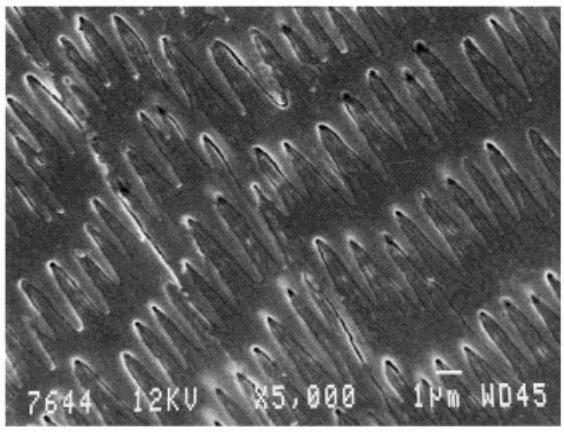

Fig. 5. Electron micrograph of dorsal cobra scale (outer surface).

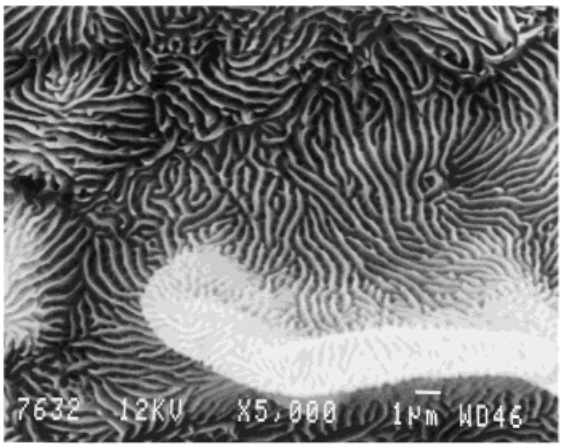

Fig. 6. Electron micrograph of dorsal viper scale (outer surface).

inner and outer surfaces of the skins of all three species indicated that there are no pores present.

Previous reports have described shed snake skin as a 'model' membrane, i.e. a membrane which shows similar permeability to human stratum corneum (Itoh et al., 1990; Harada et al., 1993; Takahashi et al., 1993). The results reported here show clearly that, for progesterone, the three species of snake produce shed skin with completely different diffusion characteristics when all other conditions are identical. As can be seen from Fig. 1, python skin is approximately three times more permeable to progesterone than viper skin. It may well be that there is one particular species of snake which produces shed skin of identical permeability to human stratum corneum, but to describe shed snake skin in general as a model membrane seems to be an erroneous conclusion.

4. Conclusions

The results of this study indicate that shed snake skin may be useful as a membrane for in vitro studies of this nature, but since each species displays different permeation characteristics, it is difficult to see how shed snake skin may be considered to be a 'model' membrane. It is important that if shed snake skin is used as a membrane, the species and skin site should be reported. The integrity of

shed snake skin, as verified by electron microscopy, indicates that it behaves as a diffusion membrane. It appears that shed snake skin may well be a useful membrane for comparing the diffusion of specific drugs from different formulations or the effects of different enhancers but care must be exercised when extrapolating to the in vivo situation.

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