ELSEVIER

European Journal of Pharmaceutical Sciences 13 (2001) 169–177

EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES

www.elsevier.nl/locate/ejps

A comparative study of an in situ adapted diffusion cell and an in vitro Franz diffusion cell method for transdermal absorption of doxylamine

Johannes P. Venter, Douw G. Müller*, Jeanetta du Plessis, Colleen Goosen

Department of Pharmaceutics, School of Pharmacy, Potchefstroom University for Christian Higher Education, Private Bag X6001, Potchefstroom 2520, South Africa

Received 15 May 2000; received in revised form 20 November 2000; accepted 6 December 2000

Abstract

In order to determine whether a drug shows the potential for percutaneous absorption, both in situ and in vitro studies are used. In vitro studies are good indicators of transdermal drug delivery, but the possibility exists that anatomical changes in excised skin can influence drug delivery. The aim of this study was to compare the in vitro Franz diffusion cell method with an in situ adapted diffusion cell method. A saturated aqueous solution of doxylamine succinate was used as model drug and the receptor phase was an isotonic Sörensen buffered solution. The in vitro permeation studies were conducted using vertical Franz diffusion cells with nude mice skin. For in situ studies, a diffusion cell was implanted under the dorsal skin of a nude mouse, simulating the in vitro method. Both in situ and in vitro experiments were conducted over a period of 12 h during which samples were collected every 90 min. The mean steady-state flux from Franz diffusion cells was 0.164 ± 0.045 $\mu g/cm^2/h$ and flux determined by the in situ method was 0.113 ± 0.034 $\mu g/cm^2/h$. A statistical significant difference existed between the permeation results of the in vitro and in situ experimental methods. A subjective, semi-quantitative assessment of histological changes to excised nude mouse skin was done using light microscopy. This showed that excised skin undergoes sub-lethal injury (necrosis) during in vitro experiments, which may lead to increased permeability of the drug. It was noticed that in vitro and in situ permeation results showed very close correlation until approximately 4.5 h after commencement of experiments, after which, the permeation through excised skin increased. It was assumed that cell necrosis occurred to such an extent after approximately 4.5 h, that the barrier function of the stratum corneum decreased and permeation of the drug increased.

Keywords: Transdermal; Absorption; Doxylamine; In vitro; In situ

1. Introduction

The skin is very effective as a selective penetration barrier. The epidermis provides the major control element — most small water-soluble non-electrolytes diffuse into the capillary system a thousand times more rapidly when the epidermis is absent, damaged, or diseased (Barry, 1988). Percutaneous absorption is a complex physico-chemical and biological process and many in vitro and in vivo experimental methods for determining transdermal absorption have been used to understand and/or predict the delivery of drugs from the skin surface into the body of living animals or humans (Barry, 1990; Bronaugh and Stewart, 1985; Grissom, 1990; Panchagnula and Ritschel, 1991; Ridout et al., 1990; Roberts et al., 1997; Toddywala and Chien, 1991). In situ and in vivo techniques uses the skin of living humans or experimental animals. In vitro methods used to determine percutaneous absorption utilizes excised human/animal skin to simulate the living skin in vivo. The reason for possible unsuccessful simulations lies in the frequently uncritical use of a favourite in vitro model to simulate the in vivo process. Model and reality then do not correspond, or what is even more dangerous, may accidentally correspond and then lead to general predictions. It is important to remember that in vitro experiments make use of excised skin as simulation for living skin and that possible differences may develop between excised and in situ/in vivo skin.

The rationale for measuring percutaneous absorption with in vitro techniques is based on the fact that absorption rates are determined by passive diffusion through the non-living stratum corneum. The stratum corneum is attached to the viable epidermis, which is metabolically active and able to undergo degradation after excision, and

*Corresponding author. Tel.: +27-18-299-2272; fax: +27-18-299-2248.

E-mail address: fmsdgm@puknet.puk.ac.za (D.G. Müller).

0928-0987/01/$ – see front matter
PII: S0928-0987(01)00110-5

therefore the stratum corneum is possibly also subject to anatomical changes. These changes may occur as a result of a lack of blood and lymph flow to the epidermis during long-term in vitro experiments. The lack of proper blood supply and nutritional deficiencies may lead to cell injury and death. Cellular death has been defined as a process during which the cell loses its integrity as a functional unit. Death of tissue in the living body is known as necrosis. Necrosis includes subsequent degeneration of a dead cell in the living body by hydrolytic reactions, which convert the cell to a mass of debris or complete dissolution to simple inorganic compounds. In part, this decomposition results from the release of hydrolases from lysosomes, a process of self-digestion or autolysis. Products of putrefactive bacteria result in further decomposition after somatic death. Not all tissues require the same amount of blood, but the minimum must be met or the tissue will die. The immediately critical requirement is that of oxygen. Lack of essential nutrients will lead to death of cells by reducing available metabolites, or indirectly through such mechanisms as anoxia (lack of oxygen) (Jones and Hunt, 1983). Therefore the rate determining barrier could be partially absent during the in vitro technique and as a result the drug release from the vehicle becomes rate determining.

The main advantages of in vitro experiments are, the experiments are easier to perform, it is less expensive and it is easier to control the environmental factors, such as temperature. Accurate absorption rates can be determined, since sampling can be done frequently directly beneath the skin (Bronaugh et al., 1990). The main disadvantages of in vitro experiments are: (1) it does not take into account cutaneous metabolism; (2) the transport of molecules of low aqueous solubility is not possible (unless a solvent such as ethanol is added to increase aqueous solubility) (Cooper and Berner, 1985); (3) it does not take into account the effect of the possible separation and degradation of the stratum corneum from the rest of the epidermis and (4) it is inevitable that physiological and anatomical changes will take place after excision and removal of the cutaneous bloodflow.

In vivo percutaneous absorption methods described in literature have certain disadvantages. Most do not provide a method to measure the permeability of a substance directly at the site of application and distribution and metabolism of the substance occurs before permeability is measured. The molecules are distributed in various organs in the body and performing a mass balance of the applied test sample is complicated.

Due to the possibility of certain anatomical changes in the stratum corneum after excision of the skin for use in in vitro experiments, the in situ adapted diffusion cell method was developed for transdermal permeation studies. The purpose of the development of this in situ method was to determine the absorption of drugs through living skin using a relative simple experimental design, comparable to the in vitro Franz diffusion cell method.

The emphasis in this study rested on a preliminary comparative study between the in vitro Franz diffusion cell method and the in situ adapted diffusion cell method, and also to determine possible anatomical damage to excised nude mice skin by histopathological comparison. Nude mice were used in both in situ and in vitro models since the skin over the dorsal and abdominal surface is loose and nonadhering to the viscera, making it possible to remove skin membranes of uniform thickness (Michniak, 1983) and to implant the in situ adapted diffusion cell under the skin. Doxylamine succinate is an ethanolamine derivative (Reynolds, 1993) and it is the best documented anti-emetic for use against morning sickness in pregnancy (Pray, 1994). It is well absorbed after peroral or parenteral administration, but hepatic biotransformation occurs (USP DI, 1994). Based on the physicochemical characteristics of doxylamine succinate e.g. low melting point (100–104°C), high aqueous solubility (0.99 g/ml), high lipophilicity ($\log P = 3.51$) and a low molecular weight (388) made it a feasible drug of choice for transdermal delivery. The advantage of the in situ adapted diffusion cell is that the permeability of a substance can be measured directly underneath the site of application and intact skin, microcirculation and metabolism are present, providing a realistic image of the transdermal absorption of a drug.

2. Materials and methods

2.1. Chemicals

Doxylamine succinate was supplied by Iropharm Ltd. (Arklow, Co. Wicklow, Ireland) and mepyramine maleate (internal standard) was supplied by Sigma (Jetpark, South Africa). HPLC analytical grade acetonitrile and methanol were used, as well as potassium dihydrogen phosphate, triethylamine and glacial acetic acid, all supplied by Merck Co. (Midrand, South Africa). Sodium chloride anhydrous, di-sodium hydrogen orthophosphate and sodium dihydrogen orthophosphate hydrate were also obtained from Merck Co. (Midrand, South Africa) (ISO 9001 certified). Double distilled deionized water was prepared by a Milli-Q Academic water purification system (Millipore, Milford, USA).

2.2. Chromatography

A high performance liquid chromatography (HPLC) assay was used to quantitatively determine the doxylamine succinate, which penetrated through nude mouse skin mounted in the diffusion cells had. The HPLC system consisted of a SpectraSYSTEM P1000 pump and a SpectraSYSTEM UV variable wavelength UV-detector (Thermo Separation Products (TSP), California, USA). A SpectraSYSTEM AS 3000 Autosampler (TSP, California, USA) was used to inject samples (200 μl) onto a Waters Nova-

Pak® CN HP 60-Å 4-μm (4.6×250 mm) column (Waters, Milford, USA). A Waters Guard-Pak™ Inserts Nova-Pak® CN HP (Waters, Milford, USA) was used to remove soluble biopolymers eluted from skin. The mobile phase comprised of potassium dihydrogen phosphate buffer (5 mM)/acetonitrile/methanol (75:20:5, v/v) and 72 μl triethylamine per 100 ml mobile phase. The pH of the mobile phase was adjusted to 5.2 with 0.1 M glacial acetic acid. The flow rate was 1.75 ml/min and the detection wavelength 262 nm. Quantitation of the amounts in the samples was performed using a SpectraSYSTEM SN 4000 that was attached to a PC 1000 software integrater (TSP, California, USA).

2.3. Laboratory animals

In order to compare the in situ adapted diffusion cell method and the in vitro Franz diffusion cell method, fluxes ($\mu g/cm^2/h$) of doxylamine were obtained through nude mice skin. Twelve male Athymic nude MF 1 nu/nu mice, between the ages of 16–18 weeks and weighing between 20 and 25 g were used for both the in vitro and in situ methods. The experiments were carried out under the approval of the Ethical Committee of the Potchefstroom University for CHE (Potchefstroom, RSA).

2.4. Anaesthesia apparatus for in situ adapted diffusion cell

The anaesthesia apparatus consisted of a 1- and a 5-l plastic bag, connected to a three-way valve. The two plastic bags were connected to two exits of the three-way valve and the mouse was connected to the third exit. The plastic bags were filled with oxygen and halothane, 4% for induction and 2% for maintenance, respectively. The exhaled carbon dioxide was absorbed by 10 g soda lime, which was placed in both bags. While insuring easy breathing, the 5-l plastic bag containing medicinal oxygen and 2% halothane was connected via the three-way valve to the head of the mouse with a latex rubber sheath. The level of anaesthesia was determined by the breathing rate or by the reflex reaction when the toes of the animal were pinched. The 4% halothane bag was used to control the level of anaesthesia, when necessary (see Fig. 1).

2.5. In situ adapted diffusion cell

A cut was made in the dorsal skin of the anaesthetized mouse, without damaging or stretching the skin. The skin was separated from the body by blunt dissection in such a way that no visible bleeding occurred, indicating limited alteration in the blood supply to the specific skin area. An adapted receptor diffusion cell was then implanted under the dorsal skin of the animal. The diffusion cell consisted of a receptor chamber, with a volume of 0.13 cm^3, that was implanted under the skin, and a donor cell which was

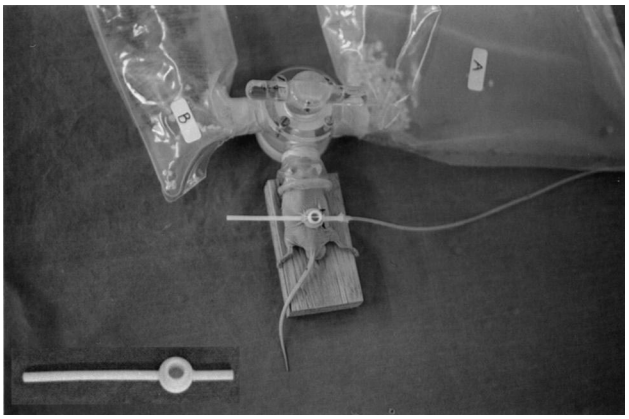

Fig. 1. In situ adapted diffusion cell method (Insert: diffusion cell).

placed on top of the receiver cell on the outside of the skin. The skin acted as a membrane-seal between the two half-cells, preventing possible leakage. To ensure the correct position on top of the receiver cell and to prevent leakage of the fluid from the donor cell, the donor cell was tied to the inlets and outlets of the receiver cell with a piece of string. The donor cell had a height of 4.5 mm and a radius of 7 mm, exposing 0.32 cm^2 of skin to the applied drug (Fig. 2). A freshly prepared saturated solution (750 μl) of doxylamine succinate in water (0.99 g/ml) was applied as a liquid in the donor cell and an isotonic Sörensen buffer solution (0.9% NaCl buffered at pH 7.4) was send through the receptor cell at a rate of 5 ml/h. The experiment ran over a period of 12 h during which samples were collected every 90 min from the receiver chamber-outlet for HPLC analysis of doxylamine. To prevent a drop in body temperature, the in situ experiments were all performed in an incubator with a temperature of 32°C.

2.6. In vitro Franz diffusion cell

In vitro permeation studies were conducted with vertical Franz diffusion cells with a 6.5-ml capacity receptor compartment and a 1.79 cm^2 diffusion area. The nude mice were euthanased by injecting 1 ml of a 200 mg/ml solution of Euthapent® (sodium pentobarbitone). The dorsal skin of the mouse was then removed by surgical procedure. A circular piece of dorsal skin was then carefully mounted onto the receiver compartment of the diffusion cells with the stratum corneum facing in the direction of the donor compartment. When the donor compartment was fastened to the receptor compartment with a clamp, the skin acted as a seal between the two half-cells. The receptor medium consisted of Sörensen buffer solution (pH 7.4) and care was taken to prevent air bubbles in the receiver compartment. This medium was maintained at 32°C by circulating water through the jacket of the lower compartment, and constantly stirring with a teflon-coated magnetic bar. A saturated solution (750 μl) of doxylamine succinate in water (0.99 g/ml) was added

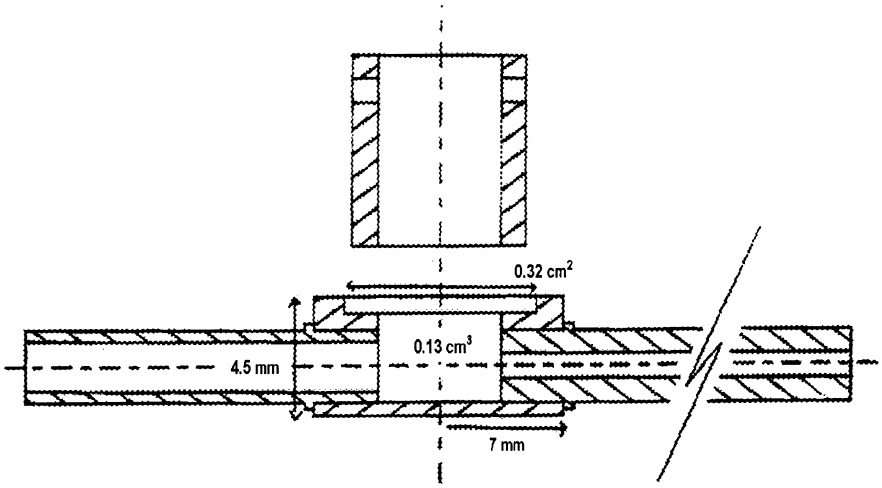

Fig. 2. Schematic illustration of the in situ adapted diffusion cell.

into the donor compartment of each cell. At eight time intervals corresponding to those used with the in situ method, the entire receptor volume was withdrawn and replaced with fresh 32°C, isotonic Sörensen buffer solution. This was done to ensure that sink conditions existed throughout the experiment. The concentration of doxylamine succinate in each receptor volume was then determined by HPLC. The duration of the typical skin permeation experiments was 12 h.

2.7. Preparation and administration of doxylamine succinate solution

Numerous transdermal absorption studies were conducted using a saturated aqueous solution of the drug for determination of the flux ($\mu g/cm^2/h$) (Diez-Sales et al., 1991; Clarys et al., 1998). A saturated solution is used in order to achieve maximum thermodynamic activity, for it is the thermodynamic activity of a solid drug in a formulation, which establishes its maximum practical driving force for permeation. Therefore, in this study a saturated aqueous solution of doxylamine succinate was used as model drug to compare the in situ adapted diffusion cell method and the in vitro Franz diffusion cell method. The saturated solubility of doxylamine succinate in the vehicle used for delivery (0.99 g/ml) was obtained by equilibrating excess amounts of the drug with analytical-grade water (32°C). The solutions were vigorously and continuously stirred for 24 h using teflon-coated magnetic stirring bars. On each and every occasion, an excess of solute was present in the solutions. Preliminary work indicated that under the conditions used, saturation of the aqueous phase was achieved well within 1 day. Therefore, samples were taken after 24 h of vigorous mixing. These samples were filtered through filters (0.45 μm cellulose nitrate membrane filter, Millipore, Milford, USA) preconditioned to the experimental temperature. Each filtrate was diluted with analytical-grade water prior to its assay. Drug concentrations were determined by HPLC. Drug solutions were freshly prepared on each and every occasion before adding the drug solution in the diffusion cells.

2.8. Histopathology

Due to the possibility of changes to excised skin during extended in vitro experiments, light microscopy was used to determine possible anatomical changes in nude mice skin. During the in vitro experiments, a portion of skin (1 cm^2) was collected from the proximal and distal dorsal side of each mouse. The proximal dorsal skin was marked A, and immediately stored in a 10% buffered formaline solution. The distal dorsal skin was marked C and kept in isotonic saline solution at 32°C for a 12-h period, after which it was stored in a 10% buffered formalin solution. The skin patch in contact with doxylamine succinate was removed from the Franz cells after the 12-h experimental period and a 1-cm^2 disc of skin cut from the centre. This portion of skin was marked B and also stored in 10% buffered formaline solution. The skin samples A and C were used as controls for the experimental skin sample (B). The portion of skin (1 cm^2) in contact with doxylamine succinate during the in situ adapted diffusion cell method, was also removed after completion of each experiment and marked D before being stored in 10% buffered formaline solution. Twelve mice were used in each of the experimental designs. A subjective, semi-quantitative assessment of the histological changes to each of the 48 skin samples was done with light microscopy and correlated with fluxes determined experimentally. Sample A was used as control for normal living skin. Samples B and D were histopathologically compared as examples of the in vitro and in situ methods, respectively. Sample C acted as a control to represent the in vitro experiment without the presence of doxylamine succinate, to determine the possible influence of the drug on the skin.

2.9. Data analysis

Diez-Sales et al. (1991) developed an equation (Eq. (1)) to describe the amount of drug crossing a membrane at a given time:

$$Q(t) = AKhC\left[D\frac{t}{h^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(\frac{-Dn^2\pi^2 t}{h^2}\right)\right] \quad (1)$$

where $Q(t)$ is the quantity passed through the membrane at a given time (t), A is the actual surface diffusion area, K is the partition coefficient between the membrane and donor vehicle, h is the membrane thickness, D is the diffusion coefficient of the permeant in the membrane and C is the concentration in the donor solution. As t approaches infinity the exponential terms become negligible and the linear steady-state expression is given by Eq. (2):

$$Q(t) = AKhC\left[D\frac{t}{h^2} - \frac{1}{6}\right] \quad (2)$$

Using the latter equation, the cumulative concentration per unit area ($\mu g/cm^2$) was plotted as a function of time (h) for each of the experimental cells (in situ and in vitro techniques). Since K, D and h are unknown, the products Kh and D/h^2 can be calculated by curve-fitting the permeation data. The curve fitting of data on Easyplot for Windows provided calculated values for Kh and D/h^2.

The product $Kh \times D/h^2$ is equal to the permeability coefficient (k_p). Kh and D/h^2 values were calculated for every individual diffusion experiment. The flux ($\mu g/cm^2/h$) for doxylamine succinate through each of the experiments was then calculated by using Eq. (3):

$$\text{Flux}\ (\mu g/cm^2/h) = k_p \times \text{Saturated solubility}\ (0.99\ g/ml) \quad (3)$$

3. Results

3.1. In situ and in vitro permeation

A bar plot of the mean steady-state fluxes of doxylamine succinate from the in situ and in vitro methods can be seen in Fig. 3. The permeation data ($n = 12$) were plotted as the cumulative amount of drug penetrated through skin as a function of time. The steady-state flux was determined from the slope of the linear portion of the cumulative amount versus time plot. Fig. 4a and b shows an example of the curve-fit from Easyplot for Windows of the mean cumulative amount permeated through nude mouse skin versus time profiles for both the in situ and in vitro methods, respectively. All correlation coefficients for each individual diffusion cell (in situ and in vitro) were greater than 0.99.

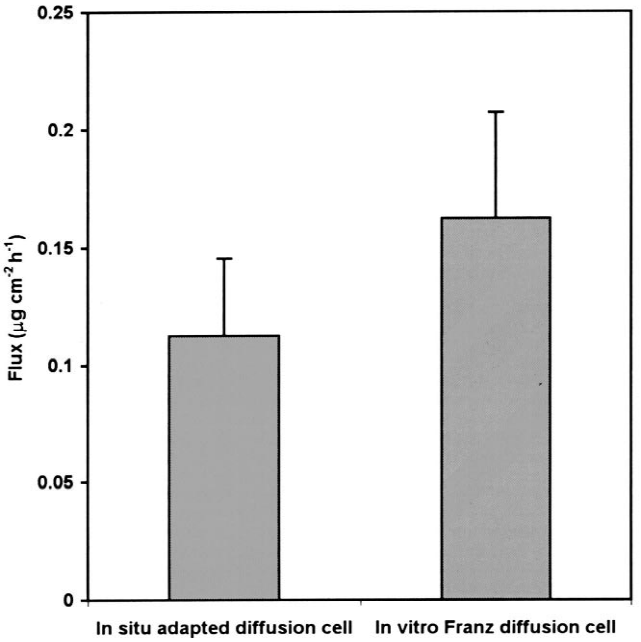

Fig. 3. A bar plot of the mean steady-state fluxes of doxylamine succinate from the in situ adapted diffusion cell and in vitro Franz diffusion cell methods.

3.2. Histopathology

A subjective, semi-quantitative assessment was done using light microscopy on samples identified as A (normal, healthy nude mouse skin), B (in vitro experiment: nude mouse skin in contact with doxylamine succinate for 12 h, C (in vitro experiment: nude mouse skin in contact with normal saline solution for 12 h, and D (in situ experiment: nude mouse skin in contact with doxylamine succinate for 12 h. Fig. 5 shows the light microscopy micrograph ($\times 200$) of normal, healthy epidermis and dermis (a) and normal panniculus muscle (b) from nude mouse skin. As was expected no histological abnormalities could be found in the skin sections of the control group (A).

3.2.1. Changes to the epidermis

In the skin sections from group B (contact with doxylamine succinate, in vitro) there were consistent changes in the epidermis. These consisted of moderate acute swelling (intracellular oedema) of the epidermal cells, seen as spongiosis of the cellular layers, especially those in the stratum basale (acute cell swelling is indicative of sub-lethal injury to the cell) (Fig. 6a). In many places (i.e. multifocal sites) the basal epidermal cells had been so badly injured that they had undergone necrosis (cell death) (Fig. 6b). All that remained of these necrotic basal cells were traces of an amorphous bluish material (admixed cytoplasmic and nuclear debris from dead cells) contained in clefts at the base of the epidermis. Identical changes, but of different degree, was also present in the samples from group C (12 h in saline) and group D (contact with

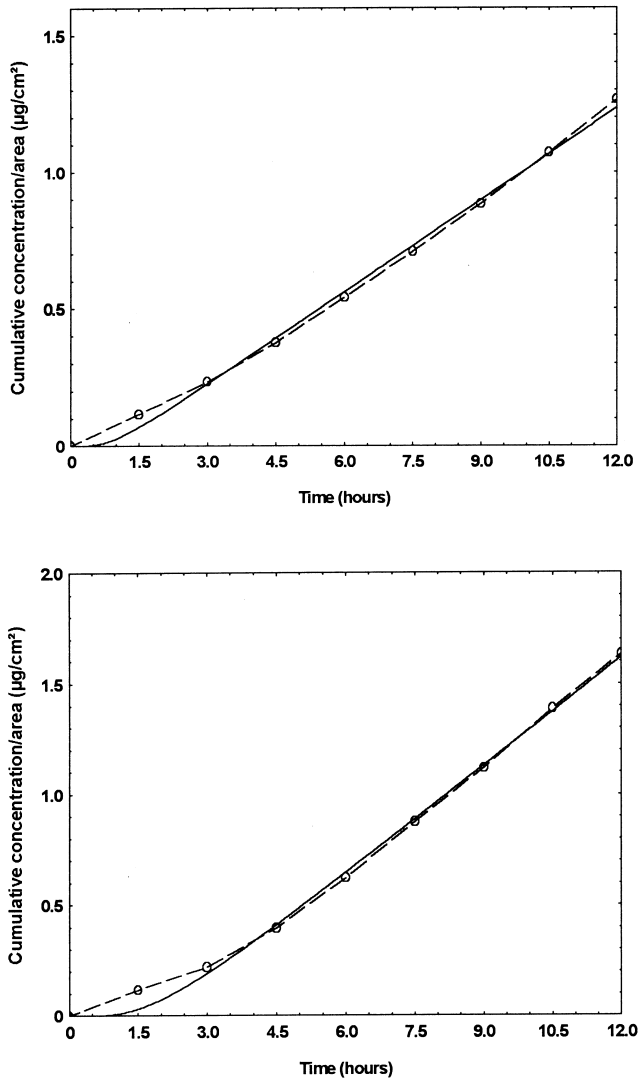

Fig. 4. An example of the curve-fit from Easyplot for Windows for the average permeation data ($n=12$) of doxylamine succinate through nude mouse skin on in situ adapted diffusion cells ($Kh=66.1\times10^{-6}$, $D/h^2=0.170$ and $R^2=0.997$) (a) and on in vitro Franz diffusion cells ($Kh=1.97\times10^{-6}$, $D/h^2=0.0824$ and $R^2=0.996$). (b) All individual diffusion experiments (in situ and in vitro) had standard deviations less than 30%.

doxylamine succinate, in situ), respectively. The epidermal changes were scored as moderate in group B, mild in group C, and very mild in group D (scored as absent in group A).

3.2.2. Changes to the dermis

Necrosis of cells in the collagen matrix of the superficial dermis, as well as degradation ('injury') of the collagen in this same location of group B was found (Fig. 6c). In addition muscle cells in the panniculus muscle (deep dermis) showed acute cell swelling, which was evidenced as irregular areas of intracellular oedema (Fig. 6d). In group C sections necrosis of cells in the collagen matrix was also present but fewer cells were affected, and they were in an even more superficial location than those in

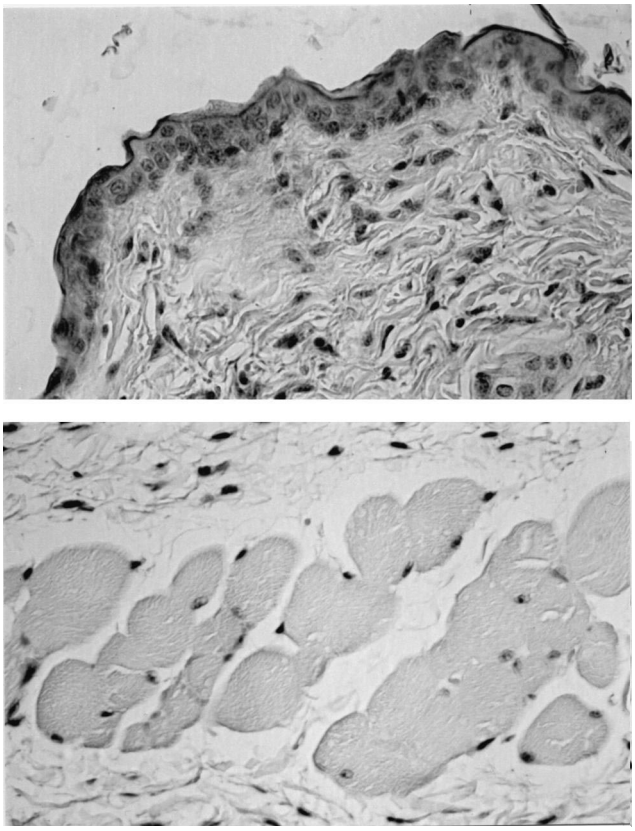

Fig. 5. Light microscopy micrograph (×200) of freshly excised nude mouse skin prior to examination, showing normal epidermis and dermis (a) and normal panniculus muscle (b).

group B. The collagen matrix itself, as well as the muscle cells, appeared to be unaffected. As can be seen from Fig. 7a and b, samples from group D (in situ) showed none of the dermal changes seen in groups B and C (in vitro).

3.2.3. Statistical analysis

Statistical analysis of doxylamine succinate permeation data ($n=12$) was done to establish whether a statistical significant difference exist between the in situ and in vitro methods. ANOVA (analysis of variation) was applied to the mean flux values ($\mu g/cm^2/h$) using the Tukey's Studentized range (HSD) test. The flux values of the methods were compared with each other and a P value of <0.05 were found. This indicated that statistically significant differences with a confidence interval of 95% ($P<0.05$) were found for the parameter, flux, between the two experimental methods studied.

4. Discussion

The hypothesis is that excision of skin for in vitro transdermal absorption studies could lead to possible anatomical changes that could influence the barrier function of the stratum corneum, since the stratum corneum is

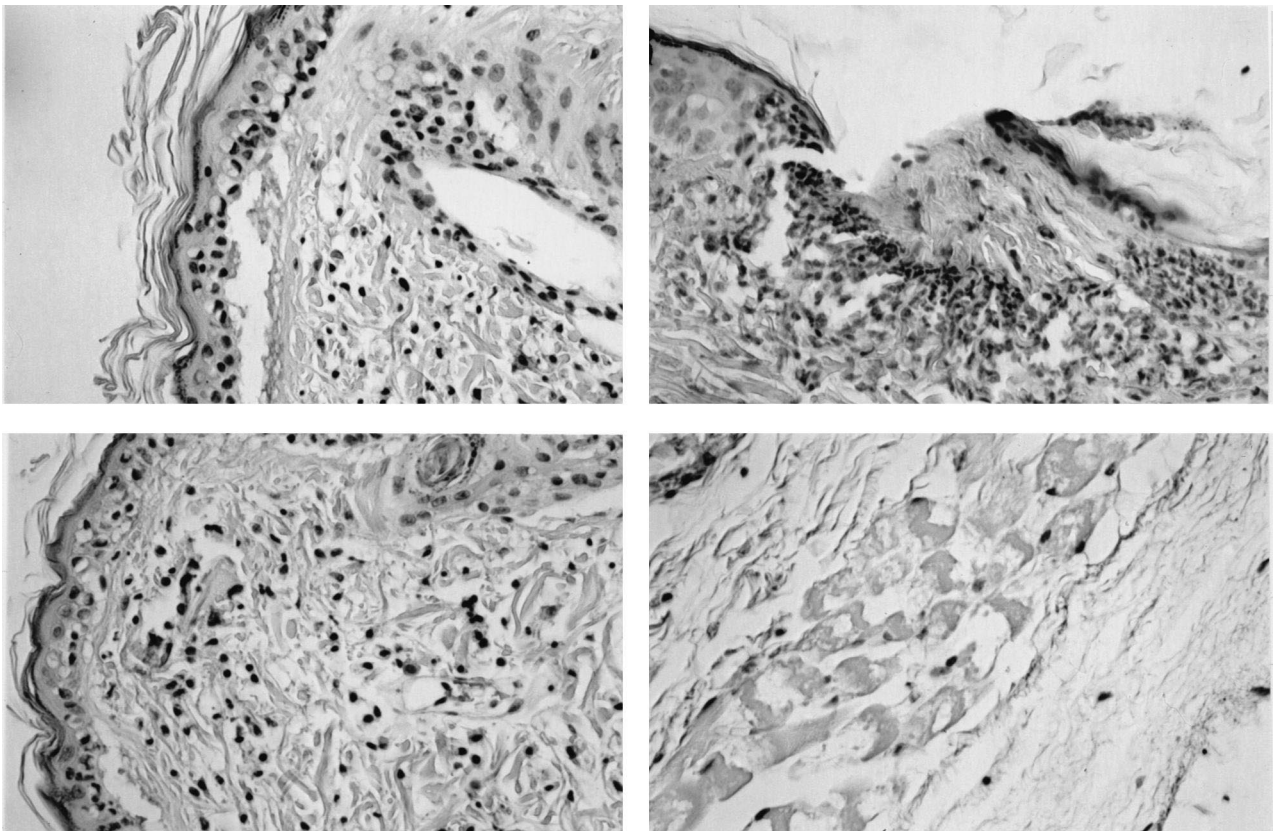

Fig. 6. Light microscopy micrograph (×200) of excised nude mouse skin after the in vitro experiment for 12 h, showing spongiosis of the epidermis (a), necrosis of the epidermis (b), degradation of the dermis (c), and necrosis of the panniculus muscle (d).

attached to the viable epidermis. The question rose: 'if such changes were to occur in the stratum corneum, to what extend would the flux of drugs be influenced/affected?'

During the comparison of the in situ adapted diffusion cell method and the in vitro Franz diffusion cell method, the permeation results through nude mouse skin were expected to be approximately identical, since environmental factors were closely controlled to be the same during both experimental methods. If the assumption was made that no anatomical damage (cell necrosis) occurred to excised skin, the in situ method could possibly show slightly higher permeation results, because microcirculation was intact which resulted in a optimum concentration gradient.

It is apparent that the rate of absorption of doxylamine is higher in nude mouse skin with in vitro Franz diffusion cells (0.1623 ± 0.045 $\mu g/cm^2/h$) than in mouse skin with in situ adapted diffusion cells (0.1124 ± 0.033 $\mu g/cm^2/h$) (Fig. 3). The significant difference in the permeation results between the in situ and in vitro mouse-models could be indicative of anatomical changes to the structure of excised nude mouse skin. It was shown that there are visible anatomical changes to excised skin during in vitro transdermal experiments over a period of 12 h. The degradation of the epidermis is seen in cell death (necrosis) and separation of the cells from each other, which may alter the permeability of the skin. In the dermis there was necrosis of cells in the collagen matrix of the superficial dermis, as well as degradation of the collagen in this same location. In addition muscle cells in the panniculus muscle (deep dermis) showed acute cell swelling, which was evidenced as irregular areas of intracellular oedema. It was noticed that in vitro and in situ permeation results showed a very close correlation until approximately 4.5 h after commencement of experiments, after which, the permeation through excised skin increased. Since the stratum corneum is the rate-determining barrier for transdermal absorption, the permeability of the drug may be enhanced as seen during the in vitro Franz diffusion cell experiments. It was assumed that cell necrosis occurred to such an extent after approximately 4.5 h, that the barrier function of the stratum corneum decreased and permeation of the drug increased.

5. Conclusion

One of the ultimate aims of the in vitro permeability tests on membranes is to approximate the in situ absorption processes through specific membranes. It is difficult to closely imitate the in situ situation of transdermal absorp-

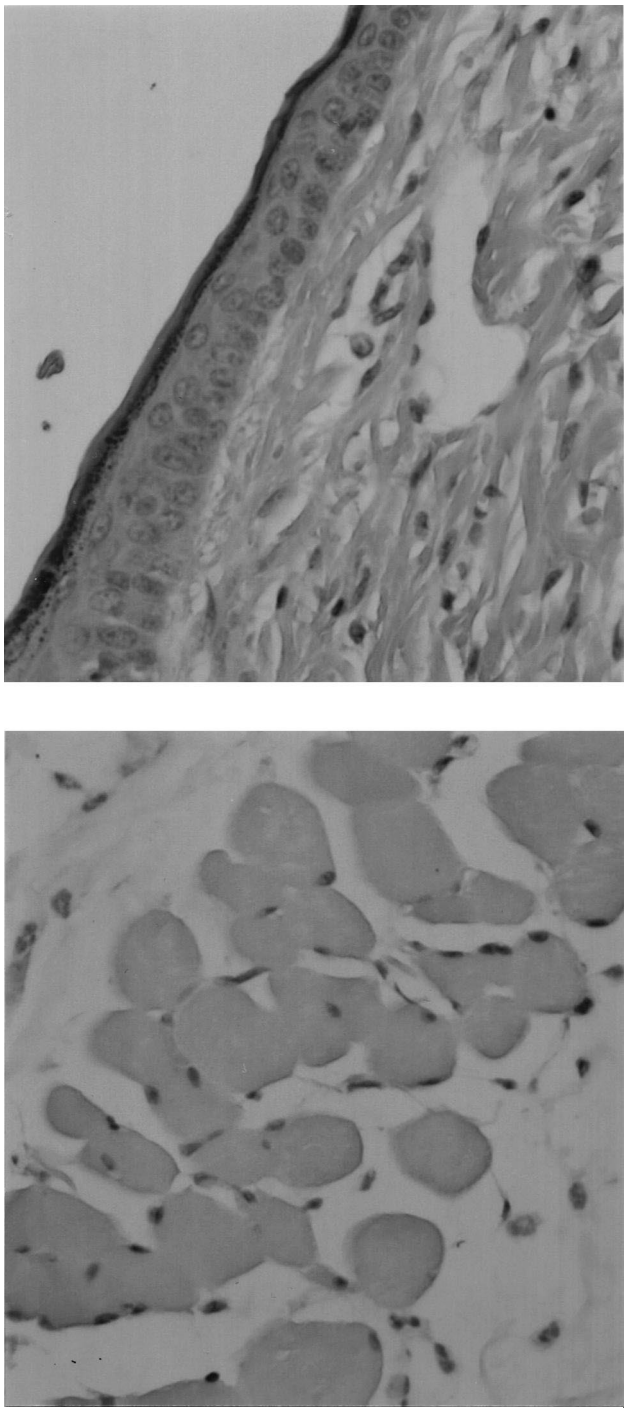

Fig. 7. Light microscopy micrograph (×200) of excised nude mouse skin after the in situ experiment for 12 h, comparing well with the control group and showing none of the dermal (a) and panniculus muscle (b) changes seen with the in vitro experiment.

tion in a laboratory and therefore new methods are constantly developed, tested and compared with existing methods.

It was shown in this study that excised nude mice skin is susceptible to anatomical degradation, which could lead to an increased permeability of drugs through the stratum corneum. The in situ adapted diffusion cell, developed to mimic the in vitro Franz diffusion cell, showed promise as an alternative method for transdermal absorption studies.

Although the mean steady-state flux obtained for the in vitro Franz diffusion cell method and the in situ adapted diffusion cell method with nude mouse skin, indicated a statistical significant difference ($P<0.05$), this difference (approximately 0.05 $\mu g/cm^2/h$) is omissibly small when considering transdermal absorption results. The coefficient of variance in transdermal absorption studies is usually high, even if skin from the same donor and donor site is compared. Site-to-site and donor-to-donor differences can further increase the coefficient of variance. Therefore, for all practical purposes, there exists a limited difference between these two methods over a period of 12 h. This compels one to consider the advantages of both methods when choosing a method to study percutaneous absorption. The main advantage of the in situ adapted diffusion cell compared to the in vitro Franz diffusion cell, is the presence of intact skin and microcirculation, which could have an influence on permeation results during extended transdermal studies. The main advantage of the in vitro Franz diffusion cell is the ease of experiments as well as a high amount of experiments can be performed, since it is not necessary to keep the mice anaesthetised during the experimental period. A further advantage of the in vitro method is the possibility to reduce the use of nude mice and make use of human skin for transdermal experiments. As it is not possible to use the in situ adapted diffusion cell on human volunteers, this method is restricted for use in animals.

Considering these advantages and the results obtained from this study, it can be concluded that the in vitro Franz diffusion cell is the method of choice for experiments on percutaneous absorption, but care must be taken when performing studies over extended periods, since it was shown that the degradation of excised skin occurs. The in situ adapted diffusion cell is very promising as an alternative method for transdermal diffusion studies, but it may become difficult to perform the experiments.

References

Barry, B.W., 1990. Some problems in predicting human percutaneous absorption via in vitro animal models. In: Scott, R.C., Guy, R.H., Hadgraft, J. (Eds.), Predictions of Percutaneous Penetration. Billing, Worcester, pp. 204–211.

Barry, B.W., 1988. Topical preparations. In: Aulton, M.E. (Ed.), Pharmaceutics: The Science of Dosage Form Design. Churchhill Livingstone, New York, pp. 381–411.

Bronaugh, R.L., Stewart, R.F., 1985. Methods for in vitro percutaneous absorption studies. IV. The flow-through diffusion cell. J. Pharm. Sci. 74, 64–67.

Bronaugh, R.L., Collier, S.W., Storm, J.E., Nathan, D.L., Stewart, R.F., 1990. In vitro absorption/metabolism studies in human and animal skin. In: Scott, R.C., Guy, R.H., Hadgraft, J. (Eds.), Prediction of

Percutaneous Penetration. IBC Technical Services, London, pp. 58–66.

Clarys, P., Alewaeters, K., Jadoul, A., Barel, A., Manadas, R.O., Préat, V., 1998. In vitro percutaneous penetration through hairless rat skin: influence of temperature, vehicle and penetration enhancers. Eur. J. Pharm. Biopharm. 46, 279–283.

Cooper, E.R., Berner, B., 1985. Skin permeability. In: Skerrow, C.J., Skerrow, D. (Eds.), Methods of Skin Research. Wiley, New York, pp. 408–431.

Diez-Sales, O., Copovi, A., Casabo, V.G., 1991. A modelistic approach showing the importance of the stagnant aqueous layers in in vitro diffusion studies and in vitro–in situ correlations. Int. J. Pharm. 77, 1–11.

Grissom, R.E., 1990. In vivo and in vitro penetration of topically applied compounds. In: Scott, R.C., Guy, R.H., Hadgraft, J. (Eds.), Predictions of Percutaneous Penetration. Billing, Worcester, pp. 73–83.

Jones, T.C., Hunt, R.D., 1983. Veterinary Pathology. Lea & Febiger, Philadelphia.

Michniak, B.B., 1983. Advances in topical corticosteroid therapy. Int. J. Cosmet. Sci. 5, 277–291.

Panchagnula, R., Ritschel, W.A., 1991. Development and evaluation of an intracutaneous depot formulation of corticosteroids using Transcutol as a cosolvent: in vitro, ex vivo and in vivo rat studies. J. Pharm. Pharmacol. 43, 609–614.

Pray, W.S., 1994. Health problems during pregnancy. US Pharmacist 19, 22–30.

Reynolds, J.E.F. (Ed.), 1993. Martindale: The Extra Pharmacopoeia, 30th Edition. The Pharmaceutical Press, London, p. 2363.

Ridout, G., Hadgraft, J., Guy, R.H., 1990. Model membranes in predicting percutaneous absorption. In: Scott, R.C., Guy, R.H., Hadgraft, J. (Eds.), Predictions of Percutaneous Penetration. Billing, Worcester, pp. 84–92.

Roberts, M.S., Lai, P.M., Cross, S.E., Yoshida, N.H., 1997. Solute structure as a determinant of iontophoretic transport. In: Potts, R.O., Guy, R.H. (Eds.), Mechanisms of Transdermal Drug Delivery. Marcel Dekker, New York, pp. 291–349.

Toddywala, R., Chien, Y.W., 1991. Evaluation of silicone-based pressure-sensitive adhesives for transdermal drug delivery. II. Effect of penetrant lipophilicity. Drug Dev. Ind. Pharm. 17, 245–269.

USP DI, 1994. 14th Edition. United States Pharmacopeial Convention, Vol. I. USP DI, Rockville, MD, 3351 pp.