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Note

Release of rooperol tetra-acetate from topical bases: in vitro studies using silicone membrane

S.C. Pefile [a], E.W. Smith [a,*], C.F. Albrecht [b], P.B. Kruger [b]

[a] *School of Pharmaceutical Sciences, Rhodes University, Grahamstown, 6140, South Africa*
[b] *Department of Medicine, Stellenbosch University, Tygerberg, 7505, South Africa*

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Abstract

The influence of the vehicle on the delivery of rooperol tetra-acetate, a lipophilic drug, from a gel, oil-in-water, water-in-oil and ointment vehicle was investigated using an in vitro membrane permeation system. Diffusion studies were performed under occluded and unoccluded conditions using polydimethylsiloxane membrane. The results obtained showed that after 8 h of diffusion, the occluded and unoccluded gel systems, followed by the occluded and unoccluded oil-in-water systems most effectively promoted the delivery of rooperol tetra-acetate. The mean calculated flux rate was 22.50 ± 4.65 mg/cm^2 per h from the occluded gel vehicles compared to 8.23 ± 3.23 mg/cm^2 per h from the occluded oil-in-water vehicle. The remaining two systems studied exhibited lower flux rates with the ointment base showing the least drug release. The data obtained from the in vitro permeation studies performed here can be used as a predictive model for the release characteristics of rooperol tetra-acetate from topical vehicles. © 1998 Elsevier Science B.V.

Keywords: Rooperol tetra-acetate; Polydimethylsiloxane; Gel; Oil-in-water; Water-in-oil; Ointment

1. Introduction

Topical drug diffusion can be enhanced or retarded by altering the vehicle in which the active ingredient is formulated. To achieve optimal drug penetration into the skin it is necessary to determine the release characteristics of the drug in relation to the physico-chemical properties of the vehicle (Rougier et al., 1989). Rooperol tetra-acetate is a lipophilic drug of high molecular weight (Fig. 1) and the hypoxoside of rooperol is a cytotoxic agent with a potential for use in the treatment of solar keratosis (Albrecht et al.,

* Corresponding author.

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1995a,b; Smit et al., 1995). To obtain the release of rooperol tetra-acetate into the skin for this purpose, an effective delivery system needs to be developed. In an attempt to optimise delivery vehicle composition, in vitro permeation studies were performed to assess the delivery of rooperol tetra-acetate from a gel, oil-in-water, water-in-oil and ointment formulations across polydimethylsiloxane membrane mounted in Franz diffusion cells.

The use of artificial membranes is common in laboratory percutaneous absorption studies. Synthetic membranes are not intended to mimic the barrier properties or the heterogeneous nature of the skin but, instead, to serve as predictive models for topical drug release (Twist and Zatz, 1989). They are useful in preformulation studies designed to determine the leaving potential of an active drug from its vehicle and in ensuring batch-to-batch uniformity of the formulated topical vehicles (ECETOC, 1993). Furthermore, the stability and batch-to-batch uniformity of synthetic membranes makes them desirable for use in diffusion experiments (Hadgraft et al., 1987). Polydimethylsiloxane membrane is ideal in that it comprises a simple homogenous monolayer which allows for the dissolution and subsequent passage of drug molecules through its matrix system (Barry, 1983).

The objective of this investigation was to compare the permeation of rooperol tetra-acetate from four topical bases, each possessing different physico-chemical properties. The assessment also involved a comparison of the diffusion properties of the drug under occlusive and non-occlusive conditions. High performance liquid chromatographic techniques were utilised to determine the rate and amount of drug diffusing from each topical vehicle into an ethanol/water receptor phase.

2. Materials and method

2.1. Materials

Rooperol tetra-acetate was obtained from Stellenbosch University, South Africa and prazepam was donated by Parke Davis, South Africa. The following chemicals were used in the formulation of the topical vehicles: sodium carboxyl methylcellulose, propylene glycol, mineral oil, liquid paraffin, white soft paraffin, hard paraffin, white beeswax, cetomacrogol and cetostearyl alcohol. All chemicals utilised were pharmaceutical grade and were obtained from local suppliers. The receptor phase comprised of HPLC grade ethanol (BDH, Poole, UK) and water in the ratio 60:40. All water used in the study was purified by processing through an Elgacan system (Elga, Buckinghamshire, UK). A mobile phase of water and HPLC grade acetonitrile (Burdick and Jackson, Muskegon, USA) was used for analytical evaluation of the receptor phase. The polydimethylsiloxane (ref. 7458) was 0.12 mm thick and was obtained from Atos, Horby, Sweden.

2.2. Preparation of vehicles

Three individual batches of each vehicle type were manufactured. All topical bases were formulated to contain 0.1% rooperol tetra-acetate. Cetomacrogol Emulsifying Cream B.P. and Simple Ointment B.P. were selected for the formulation of the oil-in-water and ointment preparations respectively and were prepared according to formulation procedures published in the British Pharmacopoeia (1988). The water-in-oil vehicle was an extemporaneous preparation containing 12% cetomacrogol 1000, 10% cetostearyl alcohol, 16% white soft paraffin, 12% light mineral oil,

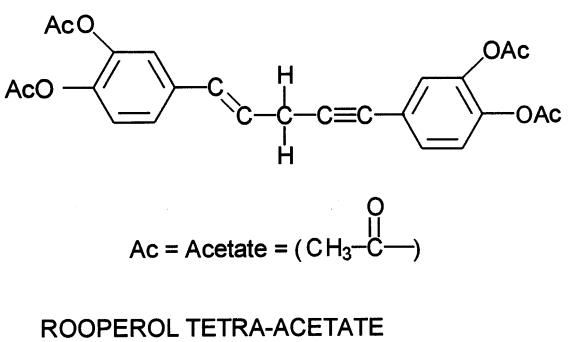

Fig. 1. The chemical structure of rooperol tetra-acetate (M_w = 450.45 g/mol).

25% propylene glycol and water. The gel formulation was manufactured by mixing 25% propylene glycol with 2% sodium carboxyl methylcellulose and adding water to form a gel matrix.

2.3. Permeation studies

An assembly of three Franz diffusion cells (Crown Glass, Somerville, NY) with a diffusional area of 1.76 cm^2 was utilised in the diffusion analyses. Three samples of each batch of topical formulation compounded were assessed for drug release potential. The polydimethylsiloxane membrane was soaked in the receptor fluid for an hour before the start of the diffusion trials.

Prior to each permeation study, approximately 0.25 g of the test vehicle was measured and evenly distributed over the entire surface of the membrane in the donor compartments using a spatula, thus creating a dosing of approximately 0.14 mg/cm^2. In the unoccluded studies, the donor compartment of the diffusion cell was exposed to ambient laboratory conditions. Occluded conditions were achieved by covering the donor cell of each diffusion cell with parafilm for the duration of the test. The receptor compartment of each cell was filled with 12 ml of a 60:40 ethanol/water mixture. The receptor phase was agitated by means of a star-head magnetic stirrer and the temperature of the cells was maintained at 30°C. Following application of the test vehicle to the membrane in the donor compartment, 500 μl aliquots of the receptor phase were withdrawn at designated time intervals. The receptor compartment was replenished with 500 μl of fresh receptor phase immediately after each sample collection.

2.4. Analytical method

Analysis of the receptor phase was accomplished by a modified high performance liquid chromatographic technique originally developed in this laboratory (Pefile et al., 1997; Smith and Haigh, 1989). A solvent pump, (model SP8810, Spectra-Physics, CA, USA) was connected to a manual injection valve (Model 7126, Rheodyne, CA, USA) equipped with a 20 μl injection loop. A variable U.V. detector (Linear 200 Model, Spectra-Physics, CA, USA) was connected to a datajet integrator (Model SP4600, Spectra-Physics, CA, USA) and set at a wavelength of 260 nm and a sensitivity of 0.002 A.U.F.S. A 10 μm C18 analytical column custom packed with octadecylsilane was used. The solutions consisted a 0.5 μg/ml prazepam solution containing rooperol tetra-acetate in the concentration range of 0.5–1 μg/ml in 85:15 acetonitrile:water solvent.

2.5. Data analysis

Fick's second law represents the change in concentration with time at a specific location within a membrane during mass transport of a drug. Ideally, the law needs to consider the rate of change of diffusant concentration at a particular point in the system. Fick's second law takes into account the non-steady state of flow and therefore provides a fundamental mathematical statement of diffusion in a form most useful for resolving diffusional problems (Guy and Hadgraft, 1989). In the normal experimental situation in which diffusion is unidirectional, Fick's second law can be expressed as:

$$\frac{dC}{dt} = D\frac{d^2C}{dx^2} \tag{1}$$

where D is the diffusion coefficient, C the concentration of a drug diffusing in the single direction x over time t. To determine the total amount of drug that has diffused over a specified time, Fick's second law is expanded to give the following expression:

$$M = \frac{DC_0t}{h} - \frac{hC_0}{6} - \frac{2hC_0}{\Pi^2}\left[\frac{(-1)^n}{n^2}\exp\left(\frac{-Dn^2\Pi^2t}{h^2}\right)\right] \tag{2}$$

Eq. (2) considers the diffusion of a cumulative mass of diffusant M of concentration C through a unit area of membrane with thickness h in time t. This equation takes into account the lag time where non-steady state conditions occur. After the lag time, steady state is achieved and linear diffusion is observed. A straight line equation can be derived by modifying Eq. (2).

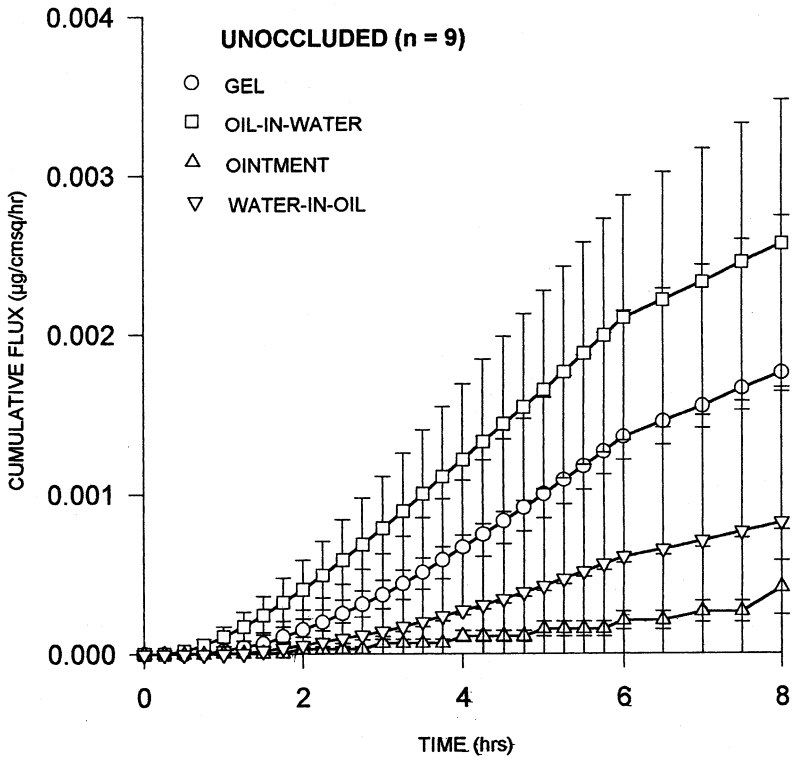

Fig. 2. Graph showing the cumulative drug permeation for each topical vehicle under unoccluded conditions.

$$M = \frac{DC_0}{h}\left[t - \frac{h^2}{6D}\right] \quad (3)$$

Extrapolation of the steady state portion of the straight line to the time axis results in an intersection where $M = 0$. This intersection is the lag time (L) which can be measured from the graph. D can be calculated by using Eq. (4), provided that the membrane thickness is known.

$$L = \frac{h^2}{6D} \quad (4)$$

Steady state data can be further used to determine the partition coefficient of the drug between the membrane and vehicle, provided that the concentration in the donor cell (C_0) is known. Differentiation and substitution of Eq. (3) yields the following:

$$\frac{dM}{dt} = \frac{DC_0K}{h} \quad (5)$$

Having measured the drug flux (J_s) from the diffusion experiments, the above equations were used to calculate the permeation parameters for rooperol tetra-acetate from the different bases.

3. Results

Figs. 2 and 3 depict composite diffusion profiles for rooperol tetra-acetate from each of three batches of the four topical vehicles analysed under unoccluded and occluded conditions respectively. Each point represents the mean $\pm$S.D. of nine determinations. The steady-state flux rates obtained experimentally are listed in Table 1 for the unoccluded and occluded state. The lag time listed in Table 1 was obtained by applying linear regression analysis to extrapolate the linear steady-state portion of the permeation profiles of each batch of formulation to the time axis. In all cases the calculated correlation coefficient (r) was not less

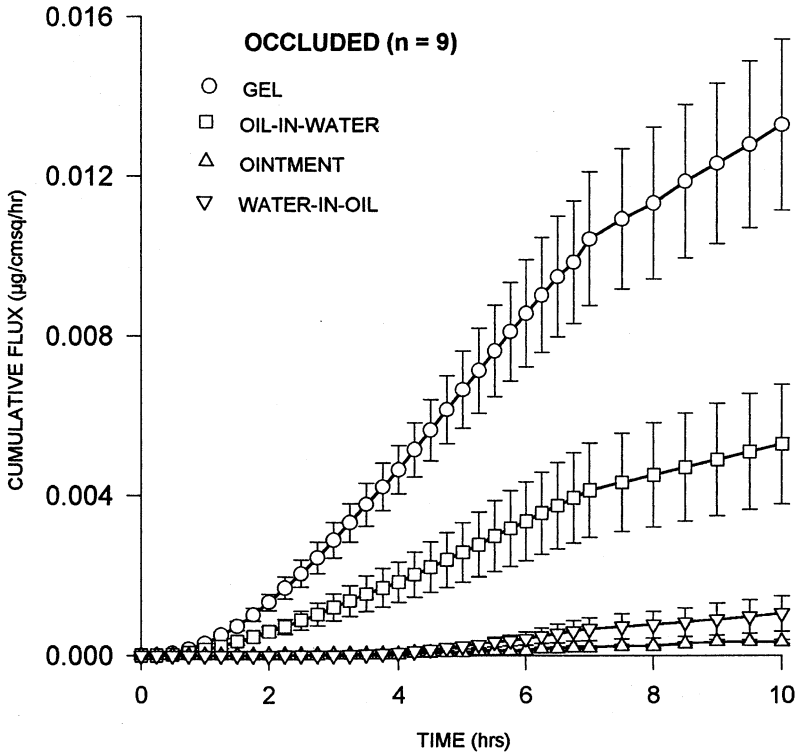

Fig. 3. Graph showing the cumulative drug permeation for each topical vehicle under occluded conditions.

than 0.98. The diffusion coefficients, which are a function of the lag time and membrane thickness, were thereafter calculated using Eq. (4). The gradient of the linear region of the plots of mass of permeant in the receptor fluid against time gave the flux rates (J_s) presented in Table 1. Once the permeability and diffusion coefficients had been determined, it was possible to calculate the partition coefficient. The calculated partition and permeability coefficients for rooperol tetra-acetate from each vehicle are shown in Table 1. It must be noted that the partition coefficients were calculated from data obtained from the occluded diffusion experiments as greater control over experimental conditions was possible and therefore, the influence of external variables capable of altering drug release was minimised.

Typical diffusion results of a lipophilic drug formulated in bases of different physico-chemical properties are displayed in Fig. 3 for occluded delivery conditions. The greatest drug release occurred from the more hydrophillic vehicles and as the lipophilic content of the vehicle was increased, drug penetration decreased. The standard deviation bars plotted in Fig. 3 suggest that there are distinct differences in the drug delivery of each base analysed. The *t*-distribution test, which was applied to the results obtained from each base, indicated that at a 95% confidence level all vehicles are significantly different except for the ointment and water-in-oil bases. This is the rank order of diffusion that one would expect based on thermodynamic leaving potential principles. The results presented in Fig. 2 deviate slightly from the theoretically expected diffusion profiles due to unocclusion of the donor compartment. Greater release of rooperol tetra-acetate is noted from the oil-in-water preparation than from the gel vehicle. The ointment and water-in-oil plots in Fig. 2 show a similar diffusion profile as that seen in Fig. 3. The wide standard deviation bars seen in Fig. 2 suggest that there are no significant differ-

Table 1
Mean permeation parameters for all vehicles under unoccluded and occluded conditions ($n = 9$)

Formulation	J_s (mg/cm^2 per h)	L (h)	D (cm/h $\times 10^5$)	P (cm/h $\times 10^2$)	K
Unoccluded					
Gel	3.64 ± 2.02	1.96 ± 0.27	1.25 ± 0.16	0.20 ± 0.11	1.87 ± 0.87
Oil-in-water	5.04 ± 1.81	1.29 ± 0.26	1.93 ± 0.35	0.28 ± 0.10	1.67 ± 0.45
Ointment	0.55 ± 0.11	1.82 ± 0.53	1.42 ± 0.35	0.03 ± 0.01	0.26 ± 0.05
Water-in-oil	1.72 ± 0.76	2.59 ± 1.00	1.04 ± 0.30	0.09 ± 0.04	1.00 ± 0.27
Occluded					
Gel	22.50 ± 4.65	1.49 ± 0.32	1.67 ± 0.30	1.02 ± 0.21	7.57 ± 1.91
Oil-in-water	8.23 ± 3.23	1.44 ± 0.29	1.95 ± 0.29	0.37 0.15	2.35 ± 0.99
Ointment	0.61 ± 0.15	2.66 ± 0.59	0.95 ± 0.21	0.03 ± 0.01	0.37 ± 0.14
Water-in-oil	2.31 ± 1.06	3.66 ± 0.46	0.62 ± 0.24	0.10 ± 0.05	2.07 ± 0.92

ences in the vehicles tested. Application of the t-distribution test to the results presented in Fig. 2 showed that at a 95% confidence level only the drug delivery from the gel and oil-in-water bases were not significantly different.

4. Discussion

Drug penetration is dependent upon the influence of the vehicle on the thermodynamic activity of the active ingredient. Rooperol tetra-acetate is a lipophilic drug, hence its release is favoured from hydrophilic bases such as the gel and the oil-in-water formulations which are poor solvents for the drug. Consequently, there are fewer drug-vehicle interactions leading to improved partitioning of the drug into the membrane. Figs. 2 and 3 clearly demonstrate the superior drug release from the hydrophilic vehicles when subjected to unoccluded and occluded conditions. Furthermore, the diffusion coefficients of the gel and oil-in-water bases shown in Table 1 are notably higher than those of the hydrophobic bases. The lower thermodynamic activity and therefore slower release of rooperol tetra-acetate from the ointment and water-in-oil bases is a result of the enhanced affinity between the drug and the lipophilic vehicles. The longer lag time values of the ointment and water-in-oil vehicles (Table 1) give further support to the hypothesis that lipophilic drugs are preferentially retained by hydrophobic bases. In addition, the membrane–vehicle partition coefficients provide an indication of the extent to which drug–vehicle interactions influence drug release. A high partition coefficient suggests that the drug favours the membrane above the vehicle resulting in enhanced penetration. In these experiments, the greatest partition coefficient was calculated for the drug in the gel/membrane system under occluded conditions. This corroborates the high thermodynamic leaving potential proposed for this system and is exemplified by the very high flux rates measured from the gel vehicle.

An equally important factor which influences drug partitioning from a topical vehicle into a membrane, especially biological tissues, is the degree of hydration. Maintaining adequate membrane hydration is essential to the dissolution of the drug into and subsequent diffusion through the polymer matrix. During the diffusion experiments, the receptor fluid was in continuous contact with the membrane resulting in complete hydration of the membrane. Further hydration was provided through the application of external occlusion which prevented the evaporation of water and volatile constituents from the formulations. Moreover, the composition of the vehicles in the occluded mode remained relatively constant throughout the delivery period. In contrast, immediately after the application of a topical base onto a membrane under unoccluded conditions, the base begins to undergo physical changes which alter the composition of the vehicle. In the unoccluded mode, evaporation of the aqueous and other volatile components of the base takes

place throughout the diffusion process. Consequently, the thermodynamic leaving potential of the drug is constantly changing resulting in a gradual increase in the lipophilicity of the vehicles, giving rise to conditions which do not favour the delivery of rooperol tetra-acetate. It is apparent from comparing Figs. 2 and 3 that the unoccluded hydrophilic bases, especially the gel formulation, are more affected by this steady change in vehicle composition. The inability to control the evaporation process of the volatile components from the vehicles resulted in erratic drug release from the unoccluded bases with marked variability in drug release rates (Fig. 2).

In vitro diffusion experiments of this type are useful in that they provide valuable insight into the performance of potential therapeutic formulations. The studies performed adequately demonstrated batch-to-batch uniformity of the topical vehicles manufactured and tested in the occluded state. It is clear from these results that quality control tests for rooperol tetra-acetate are best performed in the occluded diffusion mode. The use of polydimethylsiloxane membrane allowed for controlled research into the physico-chemical factors that influence rooperol tetra-acetate release from hydrophilic and lipophilic bases. The results obtained can be used to elucidate the mechanisms by which the release of rooperol tetra-acetate from various topical bases occurs and thus predict the ideal conditions required for optimum percutaneous delivery of the drug. However, clinical administration of products is more likely in the unoccluded mode and, hence, the final formulation developed for this therapeutic agent will need to maximise delivery potential in spite of evaporation of volatile components.

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References

Albrecht, C.F., Kruger, P.B., Smit, B.J., Freestone, M., Gouws, L., Miller, R., van Jaarsveld, P.P., 1995a. The pharmacokinetic behaviour of hypoxoside taken orally by patients with lung cancer in a phase I trial. S. Afr. Med. J. 85, 861–865.

Albrecht, C.F., Theron, E.J., Kruger, P.B., 1995b. Morphological characterisation of the cell-growth inhibitory activity of rooperol and pharmacokinetic aspects of hypoxoside as an oral pro-drug for cancer therapy. S. Afr. Med. J. 85, 853–860.

Barry, B.W., 1993. Dermatological Formulations: Percutaneous Absorption. Marcel Dekker, New York.

British Pharmacopoeia, 1988. Office of the British Pharmacopoeial Commission, London, pp. 653, 713.

ECETOC, 1993. (European Centre for Ecotoxicology and Toxicology of Chemicals), Percutaneous Absorption, Monogram 20, Brussels, p. 20.

Guy, R.H., Hadgraft, J., 1989. Mathematical models of percutaneous absorption. In: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous Absorption: Mechanisms-Methodology-Drug Delivery. Marcel Dekker, New York, pp. 13–17.

Hadgraft, J., Ridout, G., 1987. Development of model membranes for percutaneous absorption measurements. I. Isopropyl myristate. Int. J. Pharm. 39, 149–156.

Pefile, S.C., Smith, E.W., Kruger, P.B., Albrecht, C.F., 1997. The validation of a new stability-indicating HPLC technique developed for the quantitative analysis of rooperol tetra-acetate. J. Liq. Chrom. 20, 3061–3072.

Rougier, A., Dupuis, D., Lotte, C., Maibach, H.I., 1989. In: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous Absorption: Mechanisms-Methodology-Drug Delivery. Marcel Dekker, New York, p. 425.

Smit, B.J., Albrecht, C.F., Liebenberg, R.W., Kruger, P.B., Freestone, M., Gouws, L., Theron, E., Bouic, P.J.D., Etsebeth, S., van Jaarsveld, P.P., 1995. A phase I trial of hypoxoside as an oral pro-drug for cancer therapy-absence of toxicity. S. Afr. Med. J. 85, 865–870.

Smith, E.W., Haigh, J.M., 1989. In vitro diffusion cell design and validation. I. A stability- indicating high-performance liquid chromatographic assay for betamethasone 17-valerate in purified isopropyl myristate receptor phase. Pharm. Res. 6, 431–435.

Twist, J.N., Zatz, J.L., 1989. Interaction of vehicles with model skin membranes in permeation process. In: Bronaugh, R.L. and Maibach, H.I. (Eds.), Percutaneous Absorption: Mechanisms-Methodology-Drug Delivery, Marcel Dekker, New York, pp. 147–148.

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