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In vitro dermal and transdermal delivery of doxycycline from ethanol/migliol 840 vehicles

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Abstract

This work investigated the feasibility of dermal and transdermal delivery of doxycycline from vehicles containing Migliol 840 (M840) and ethanol. Delivery of the drug via the skin would provide a useful alternative to oral delivery, which has many undesirable side-effects, such as oesophageal ulceration and disturbance of the normal gut flora. Potential applications include malaria prophylaxis, and the treatment of acne vulgaris, Lyme disease and Reiter syndrome. Experiments were performed to determine the permeation of doxycycline across excised full-thickness human skin and heat-separated epidermal membranes from saturated solutions in ethanol, 1:1 and 2:1 ethanol/M840. Unusual burst behaviour was observed using an ethanol vehicle, possibly as a result of the formation of dimers at saturation. Doxycycline permeated to a higher degree from ethanolic vehicles when M840 is present, suggesting that M840 is capable of enhancing the permeation of doxycycline. The flux across full-thickness skin was highest from a 2:1 ethanol:M840 vehicle (2.41 $\mu g\ cm^{-2}\ h^{-1}$), sufficient to deliver 282 $\mu g\ l^{-1}$ using an area of application of 30 cm^2. The data also produced unexpected results in that permeability across heat separated skin was an order of magnitude greater than across full-thickness skin (28.75 $\mu g\ cm^{-2}\ h^{-1}$ for the 2:1 ethanol:M840 vehicle). Depth profiling indicated that the drug distributed quite evenly throughout the epidermis. The mean amount of doxycycline recovered from the epidermis at the end of a permeation experiment was 458.4 $\mu g\ ml^{-1}$. This was far higher than the volume of extractable lipid present in the same unit area, approximately 52.3 $\mu g\ ml^{-1}$ and indicated that a large proportion of the drug must have been located within the proteinaceous domain. The data therefore suggest (1) significant amounts of doxycycline can be administered into and across the skin; (2) M840 is a potentially useful enhancing vehicle; and (3) the transcellular route was of significance.

Keywords: Doxycycline; Migliol 840; Lyme disease; Malaria; Acne vulgaris; Reiter syndrome; Dermal delivery; Transdermal delivery

1. Introduction

Doxycycline is a semi-synthetic tetracycline antibiotic, possessing a broad spectrum of activity and is indicated in the treatment of a wide range of bacterial and protozoal diseases (Finch, 1996).

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It is active against a variety of Gram positive and Gram negative bacteria, for example *chlamydiae*, *mycoplasmas*, *spirochaetes*, *rickettsiae* and some mycobacteria. It has also demonstrated potential in prophylaxis of malaria (Ohrt et al., 1997). Doxycycline is generally more active than tetracycline, a property partially attributed to its greater lipophilicity and more widespread tissue distribution (Finch, 1996). A significant advantage of doxycycline is its activity against tetracycline-resistant *S. aureus* and *enterococci*. At present, doxycycline is administered orally but this gives rise to side-effects such as oesophageal ulceration (Tanaka et al., 1997), photosensitivity (Foster and Sylvia, 1994) and disturbance of the normal gut flora. It is anticipated that these side-effects could be overcome by the delivery of doxycycline via the skin (Schaefer and Redelmeier, 1996).

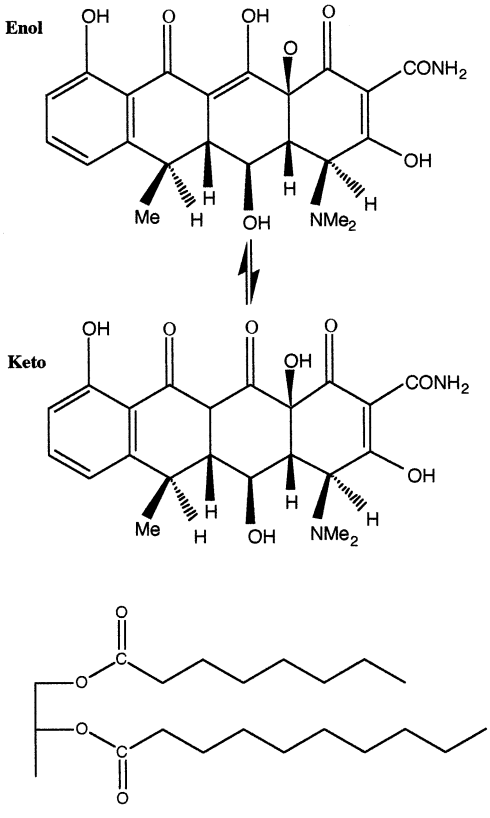

Fig. 1. Chemical structure of doxycycline tautomers and representative structure of Migliol 840.

There is a paucity of literature investigations into the delivery of doxycycline via the skin, which may in part be explained by the low lipophilicity of the drug, particularly as the hydrochloride salt (Fig. 1). However, Langston et al. (1998) found that significant amounts of doxycycline HCl could indeed permeate full-thickness excised human skin from propylene glycol/ethanol vehicles. Dermal and transdermal delivery of doxycycline could have several potentially useful applications.

1.1. Anti-malarial

Doxycycline has anti-malarial action against the most pathogenic species of malarial parasite, *Plasmodium falciparum*. It is currently recommended for the chemoprophylaxis of malaria in areas such as South-East Asia and Oceania, where strains resistant to the commonly used anti-malarials, mefloquine and chloroquine are prevalent (Mehta, 1998).

1.2. Acne vulgaris

Doxycycline is used orally for the systemic treatment of acne. A topical formulation would have the advantage of delivering the drug more directly to the site of action and minimising systemic side effects. There do not appear to have been any investigations made into the dermal delivery of doxycycline for this application, although topically applied tetracycline was found to be effective (Wechsler et al., 1978).

1.3. Lyme disease

Lyme disease is a multisystem disorder caused by the spirochete *Borrelia burgdorferi*, introduced to the patient by tick bites (Steere, 1989). Doxycycline is the antibiotic of choice as it achieves higher tissue levels than tetracycline and has a longer duration of action (Schreiner and Digranes, 1985). Specific advantages of the delivery of this drug via the skin include direct application to an infected area, thereby delivering the drug to areas where it is most needed. A secondary effect, given sufficient permeability, is the antibiosis of

plasma-borne spirochetes. Occlusion of the device may help circumvent photosensitivity problems (Layton and Cunliffe, 1993).

1.4. Reiter syndrome

Reiter syndrome has been linked to many causative organisms and doxycycline HCl is the agent of choice in most cases (Rothe and Kerdel, 1991).

The current study investigated the in vitro delivery of doxycycline from vehicles containing Migliol 840 (M840) — a diester of propylene glycol and either caprylic, C8, or capric, C10, fatty acids (Fig. 1). Mayorga et al. (1996) reported a 19-fold increase in the in vitro flux of primaquine free base across rat skin from M840, relative to other glyceride-type vehicles. More recently, Morris et al. (1998) reported high permeation of primaquine from a M840 vehicle across human skin. The results were rationalised in terms of solubility parameter (Fedors, 1974), in that both primaquine and M840 have solubility parameters of approximately 10 (cal mol^{-3})$^{0.5}$. This was significant in that the solubility parameter of the skin's barrier function has also been determined to have this value (Liron and Cohen, 1984), hence each component has maximum miscibility. Interestingly, vehicles with SP other than ten gave much lower permeation rates, indicating that it is the parity in solubility parameter of the skin and the vehicle that is of significance. As M840 is regarded as non-toxic and is already approved by the FDA, the pursuance of its use as a vehicle with penetration enhancing effects is worthwhile. As a result of the low solubility of doxycycline in M840, a co-solvent of ethanol was employed (Barry, 1983). Bommannan et al. (1991) suggested that ethanol may affect permeation by extracting skin lipids, therefore removing the barrier.

The purpose of this study was to determine the permeation of doxycycline across full-thickness and heat separated excised skin, in addition to determining the localisation of doxycycline HCl within the epidermis, with a view to potentially addressing the aforementioned disease states. We also aimed to demonstrate the potential of M840 as a suitable enhancing vehicle for this permeant.

2. Experimental

2.1. Materials

2.1.1. Chemicals

Doxycycline hydrochloride was obtained from Sigma, UK. Migliol 840 was a gift from Chondea Chemie. Ethanol, methanol and acetonitrile were HPLC-grade from Fisher Scientific, UK. Sodium oxalate was supplied by Aldrich Chemicals. The buffer components were all analytical grade and obtained from Fisher Scientific, UK. Phosphate buffer saline (PBS) pH 7.4 was used as a receptor providing an effective sink for this (hydrophilic) permeant. The solution was filtered and degassed prior to use.

2.1.2. Full-thickness skin membanes

Human cadaver skin specimens (male and female, age range 45–75) were obtained from the midline incision during autopsy. The subcutaneous fat was carefully removed using a combination of scissors and a razor blade. The skin was cut into pieces of approximately 1 inch square and stored at −20°C until the time of use.

2.1.3. Heat separated epidermis

The skin was prepared utilising a similar technique to that described above, but immediately prior to freezing, the skin specimens were immersed in de-ionised water at 60°C for 60 s, enabling the epidermis to be peeled away from the dermis using surgical forceps (Touitou et al., 1998). The epidermis was allowed to float on the water, stratum corneum uppermost, so that it could be readily taken up onto a filter paper (Whatman No 5) support in a flat, crease-free state.

2.2. Saturated solutions

Saturated donor solutions of doxycycline HCl were prepared at 32°C in 20 ml ethanol, 2:1 and1:1 ethanol:M840 by adding excess drug to the liquid in screw cap vials, which were stirred overnight in an oven. Solutions were filtered through a pre-warmed 0.2 μm pore size filter before use. Using a pre-warmed pipette tip, 1 cm^3

aliquots of each filtered solution were diluted with ethanol and used to determine the saturated solubility of doxycycline.

2.3. In vitro permeation of doxycycline across human skin

2.3.1. Full-thickness skin

Skin specimens were mounted in Franz diffusion cells, with pre-greased flanges, then clamped. The receptor compartments were filled with PBS and small magnetic fleas added. The whole assemblies were placed on a submersible magnetic stirrer in a water bath, such that the receptor compartments were immersed in the water. Constant agitation of the receptor fluids limited the stagnant layer effects and enabled representative samples of the receptor solution to be taken from the sampling arms. The water bath was set at 37°C to represent the core temperature in vivo and maintain the surface temperature of the skin at 32 ± 1°C. After 30 min, 1 cm^3 of donor solution, at 32°C, was added to the donor compartment of each cell using a pre-warmed syringe. This procedure ensured that the state of saturation was not affected by changes in temperature. The sampling arms and donor compartments were occluded to prevent evaporation and therefore changes in concentration. At specific time points, a 200 μl aliquot of the receptor fluid was taken from each cell and replaced with PBS pH 7.4 maintained at 32°C. The samples were stored in autosampler vials at -20°C prior to analysis. In accordance with the FDA guidelines and as a result of the high extent of variability in skin permeation, 12 replicates were run for each donor solution (Skelly et al., 1987).

2.3.2. Heat separated epidermis

The procedure used was identical to that described above, although only vehicles of 1:1 and 2:1 ethanol:M840 were used. The experiments were terminated at 48 h (upon attainment of steady state flux), the cells dismantled and the membranes recovered for use in 2.4.

2.4. Localisation of doxycycline within epidermis

2.4.1. Depth profiling

For dermal delivery, a knowledge of the localisation of the drug within the different layers of the skin is important. For example, in Lyme disease, the causative spirochete accumulate at the dermo-epidermal junction, as well as being found superficially in the erythematous border (Van Mierlo, 1993). Five epidermal membranes recovered from 2.3.2 after 48 h, i.e. attainment of steady state flux, were washed three times with deionised water and the filter paper supports carefully removed from the lower surface of the skin using forceps. The skin was then pulled flat across the donor aperture using a cotton bud and immobilised onto the surface of a clean rigid PVC board using cyanoacrylate adhesive. When the adhesive had set (< 1 min) excess drug on the surface of the skin was removed using cotton buds immersed in methanol. To ensure that stripping occurred consistently, the diffused skin areas, complete with plastic backing, were punched out using an 8 mm diameter cork borer. The discs were then immobilised on the board, again using cyanoacrylate adhesive and Scotch tape was used to remove successive layers of the skin. Using 3 cm lengths, the tape was firmly pressed onto the skin surface by thumb and the tape jerked away, complete with the stripped material. The first four strips were discarded as they could be seen to contain surface deposits of doxycycline. Each subsequent strip was placed in a 15 ml screw-capped vial. A total of 3 ml of methanol was then added to each vial such that the tape was completely immersed in the liquid and the vials shaken for 24 h on a shaking plate. Solutions were then decanted into glass tubes and the methanol evaporated in a vacuum oven overnight. The residues were taken up in 500 μl of methanol and mixed on a vortex mixer, operated for 3×10 s per tube. These solutions were then decanted into Eppendorf vials and centrifuged at 14 000 rpm for 5 min. Finally, 200 μl aliquots of the supernatant were transferred to HPLC autosampler vials.

Table 1
Solubility of doxycycline HCl in three vehicles

Vehicle	Saturated solubility (mg ml^{-1})	SE
Ethanol	27.84	0.94
2:1 ethanol:M840	11.38	0.42
1:1 ethanol:M840	4.90	0.51

2.4.2. Total absorbed doxycycline

Four skin specimens recovered from 2.3.2. were used to determine the total amount of drug in the epidermis after 48 h. The surface of the epidermis was washed and tape-stripped twice, the tape being discarded. The diffused areas were punched out using the 8 mm diameter borer, placed in screw-capped vials containing 15 ml of methanol and macerated for 10 min using a homogeniser (PowerGen 700, Fisher Scientific, UK). The solutions were left for 18 h on a rotary blood cell mixer, sonicated for 1 h and filtered through Whatman filter paper. The residue and filter were then returned to the screw-capped vials together with 10 ml of methanol and again sonicated for 1 h with heat. The filtering, extraction and sonication process was repeated three times (no measurable doxycycline was obtained by further extraction). The portions of methanol from successive washings were pooled in round-bottomed flasks and the solvent removed using a rotary evaporator. The residues were taken up in 5 ml of methanol, of which 0.5 ml aliquots were transferred to HPLC autosampler vials.

2.5. HPLC analysis

Samples were analysed by reverse phase high performance liquid chromatography (HPLC), using a 4.6 × 150 mm Phenomenex Kingsorb C18 5 μm ODS column fitted to a Hewlett Packard 1100 automated isocratic system, with UV detection at 346 nm. The mobile phase was 40:60 acetonitrile: 5 g l^{-1} sodium oxalate in deionised water. Before use, the solution was degassed and filtered through a 0.2 μm nylon filter. The injection volume was 40 μl and the flow rate was set at 1.0 ml min^{-1}. Under these conditions a retention time of approximately 3.5 min was obtained. Standard solutions of doxycycline HCl were prepared in the range 10–70 μg ml^{-1} and analysed to obtain a calibration curve. The detection limit was determined to be ~50 ng ml^{-1}.

2.6. Data processing

The flux of the drug in each cell at steady state was determined and the cumulative amount penetrated plotted against time for each cell. The gradient of the plot at steady state provided the flux (J) for each cell. Cricket graph software on an Apple Macintosh computer was used for data processing and graphing. Cumulative permeation and standard error were plotted against time and the permeability coefficient (P) was then calculated from Ficks first law (Eq. (1)):

$$J/C_i = P \quad (1)$$

Where, J = flux at steady state, C_i = initial concentration.

3. Results

3.1. Saturated solubility

Of the vehicles examined, doxycycline HCl was found to be most soluble in ethanol. Increasing the proportion of M840 lead to substantially reduced solubility (Table 1). Doxycycline HCl was found to be practically insoluble in M840 alone.

3.2. Skin permeation

3.2.1. Permeation across full-thickness skin-ethanol vehicle

In order to determine the enhancing effects of M840, the flux from a saturated solution of doxycycline HCl in ethanol was first investigated. The permeation behaviour was unconventional in that there was a very high initial flux followed by a marked reduction after 3 h (Fig. 2). Beyond this point the flux rate was very low: 0.13 ± 0.10 μg cm^{-2} h^{-1}. Upon dismantling the cells, blistering was observed on the surface of two of the skin samples. The amount of doxycycline that had permeated through these cells at 48 h was signifi-

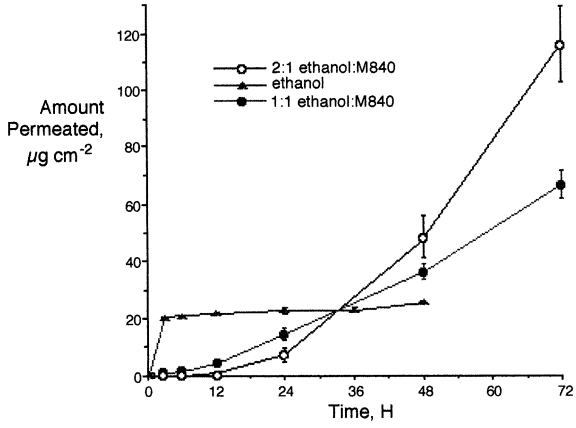

Fig. 2. Permeation of doxycycline across full-thickness excised human skin from ethanol, 1:1 and 2:1 ethanol/M840.

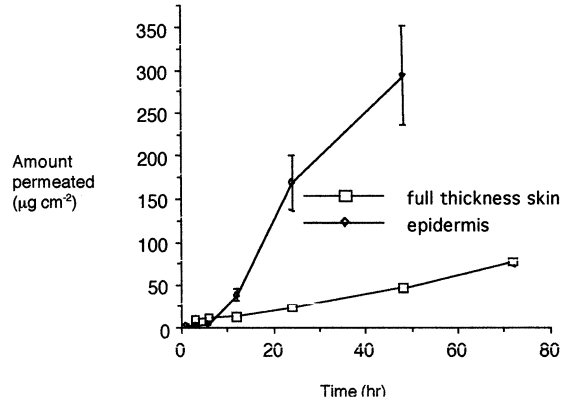

Fig. 3. Permeation of doxycycline 1:1 ethanol/M840 across heat-separated epidermis and full-thickness skin.

cantly higher than the other cells, indicating barrier breakdown, and so the values obtained were omitted from subsequent calculations.

3.2.2. Permeation across full-thickness skin — 2:1 ethanol:M840 vehicle

Using a vehicle of 2:1 ethanol:M840 a typical permeation profile was observed (Fig. 2). The mean flux was 2.41 ± 0.37 μg cm^{-2} h^{-1} and the mean permeability coefficient was $2.11 \times 10^{-4} \pm 0.33 \times 10^{-4}$ cm h^{-1}. Blistering was observed with two of the twelve skin samples, and the data from those cells discluded. At 48 h, the amount permeated from this vehicle was approximately twice that seen when M840 was absent.

3.2.3. Permeation across full-thickness skin — 1:1 ethanol:M840 vehicle

A vehicle with a higher proportion of M840 was used to investigate whether flux could be increased even further. A typical permeation profile was observed (Fig. 2), the mean flux was half that using 2:1 ethanol/M840 (1.30 ± 0.25 μg cm^{-2} h^{-1}); the mean permeability coefficient was $2.66 \times 10^{-4} \pm 0.52 \times 10^{-4}$ cm hr^{-1}. No blistering was observed on any of the skin samples.

3.2.4. Permeation across heat separated epidermis — 1:1 ethanol:M840 vehicle

A typical permeation profile was seen for the permeation of doxycycline HCl through an epidermal membrane (Fig. 3). The mean flux through the epidermis was 12.309 ± 0.844 μg cm^{-2} h^{-1}, some order of magnitude greater than across full-thickness skin (Table 2). P was $2.52 \times 10^{-3} \pm 0.17 \times 10^{-3}$ cm h^{-1}.

Table 2
Summary of skin permeability data for permeation of doxycycline HCl across full-thickness (F/T) skin and heat-separated epidermis from ethanol/Migliol 840 vehicles

Membrane type	Vehicle	Mean Flux (μg cm^{-2} h^{-1})	SE	Mean P (cm h^{-1})	SE
F/T	Ethanol[a]	0.13	0.01	4.77×10^{-6}	0.45×10^{-6}
F/T	2:1 ethanol:M840	2.41	0.37	2.11×10^{-4}	0.33×10^{-4}
F/T	1:1 ethanol:M840	1.30	0.25	2.66×10^{-4}	0.52×10^{-4}
Epidermis	2:1 ethanol:M840	28.75	0.99	2.53×10^{-3}	0.09×10^{-3}
Epidermis	1:1 ethanol:M840	12.31	0.84	2.52×10^{-3}	0.17×10^{-3}

[a] Discluding initial burst.

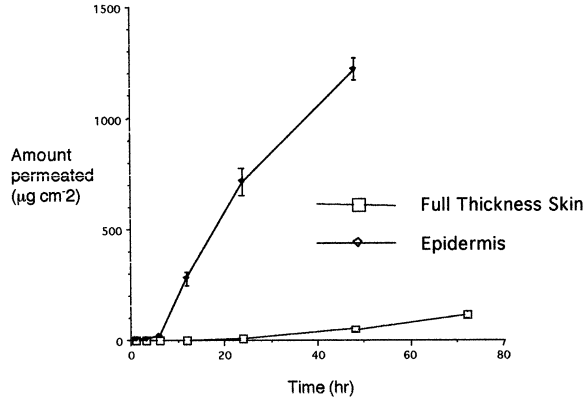

Fig. 4. Permeation of doxycycline 2:1 ethanol/M840 across heat-separated epidermis and full-thickness skin.

3.2.5. Permeation across heat separated epidermis — 2:1 ethanol:M840 vehicle

A typical permeation profile through the epidermal membrane was again observed. The mean flux was 28.750 ± 0.986 μg cm^{-2} h^{-1} which was over double that observed with the 1:1 vehicle and again 10-fold greater than across full-thickness skin (Fig. 4, Table 2). P was $2.53 \times 10^{-3} \pm 0.09 \times 10^{-3}$ cm h^{-1} and similar to the value determined for the 1:1 vehicle.

3.3. Depth profiling

3.3.1. Localisation of doxycycline within epidermis

The mean doxycycline extracted for each tape stripping was plotted against the strip number in order to construct a profile illustrating the amount of drug present versus relative depth (Fig. 5). The plot indicated that a larger amount of doxycycline is located in the outer layers of the epidermis but the levels do not then drop significantly, as might be predicted. The amount of drug after approximately the tenth strip decreased slightly.

3.3.2. Total absorbed doxycycline

At the end of the diffusion period, the mean concentration of drug within the diffusional area was calculated to be 0.46 ± 0.03 mg cm^{-2}. The differential between the mean total amount in all the tape strips for a skin sample (0.29 ± 0.011 mg cm^{-2}) and the mean amount in the epidermis was a result of incomplete stripping.

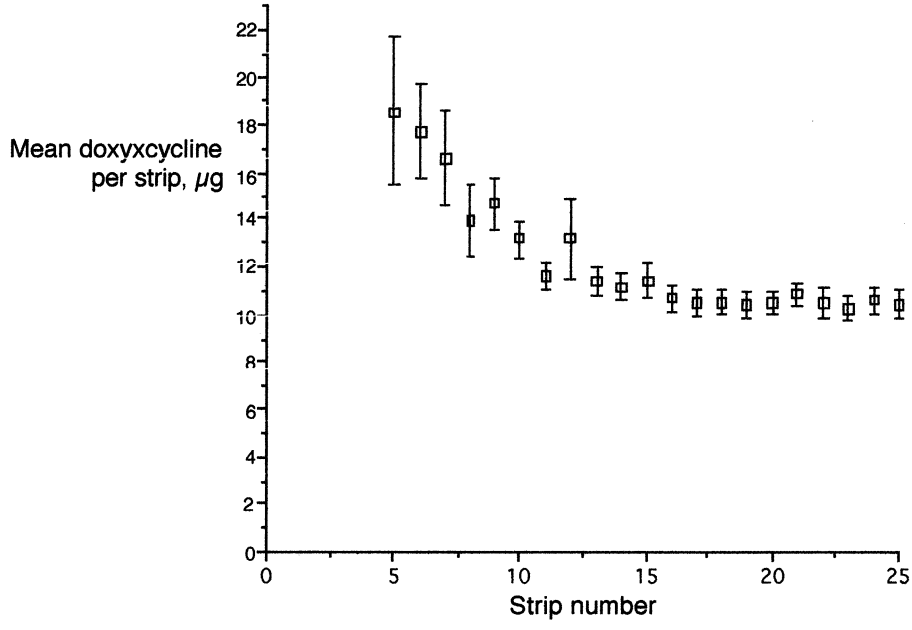

Fig. 5. Depth profile of doxycycline within heat separated epidermal membranes from 1:1 ethanol:M840 ($n = 5$, $\pm$SE).

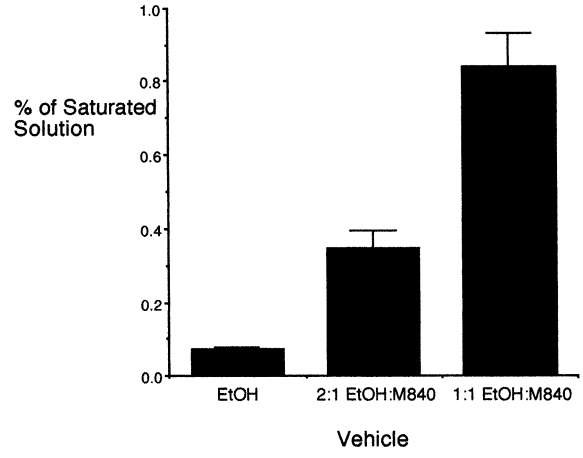

Fig. 6. Percentage of applied dose of doxycycline permeated across full-thickness human skin from three vehicles after 48 h.

3.4. Pharmacokinetic considerations

To determine whether a therapeutic dose can be delivered transdermally, Eq. (2) was used:

$$J_{max} \times \text{Area of application} = \text{Clearance} \times \text{Plasma Concentration} \quad (2)$$

The clearance of doxycycline is 3.06 l h^{-1} and the maximum area of application is generally considered to be about 30 cm^2. J_{max} for the experiments performed was 28.75 and so the plasma level that this would deliver, using an area of application of 30 cm^2 is 282 μg l^{-1}.

4. Discussion

On consideration of the physicochemical properties of doxycycline HCl (e.g. $\log P - 0.2$, solubility parameter 17.2 (cal mol^{-3})$^{0.5}$ it would not have been predicted that such a large amount of the drug could have localised within and permeated across the skin. The results therefore do not fit widely accepted skin permeability theory, although the unusual chemistry of the tetracyclines may go some way towards providing a rationalisation.

It is known that the tetracyclines can exist as either keto or enol tautomers and that, at saturation in alcoholic solutions, the enol form of tetracyclines predominates (Naidong et al., 1981). The existence of two tautomeric forms (Fig. 1) may complicate the permeation through skin, as the two forms will differ in terms of polarity and solubility parameter. Of perhaps greater significance is the fact that doxycycline has been shown to self-associate when at a concentration nearing saturation (Bogardus and Blackwood, 1979). The site of association is the β-diketone moiety of the enol form and would cause the shielding of polar groups and a subsequent increase in net lipophilicity (Roberts et al., 1996). This could explain the highly unusual burst effect observed from the ethanol vehicle, that showed parallels to previous results (Langston et al., 1998). As the solution became increasingly sub-saturated the concentration of doxycycline in the dimeric form decreased, causing a marked decrease in the rate of drug permeating. The tightness of the error bars discludes the possibility of damaged membranes. The lag time observed using the 1:1 ethanol:M840 vehicle was unexpectedly shorter than the 2:1 vehicle, given the subsequent permeation rates. This may also have been the result of the higher relative ethanol content giving rise to dimers and consequent (but much-reduced) burst. Such bursts may be of therapeutic value where the administration of a dose is required rapidly.

The percentage of donor solution that had permeated by the end of the diffusional period was 0.07% from the ethanol vehicle, 0.35% from the 2:1 ethanol/M840 vehicle and 0.84% from the 1:1 ethanol/M840 vehicle (Fig. 6). Therefore, although the concentration of doxycycline HCl was lowest in the vehicle with the highest proportion of M840, the percentage of drug that permeated from it was the highest. This confirmed that the presence of M840 exerted an enhancing effect, although the relative abundance of the chemical forms of doxycycline may have contributed. Nevertheless, enhancement diminished with increasing M840 concentration. Blistering was less apparent as the proportion of M840 increased, suggesting that the presence of the ethanol was responsible for this effect and that M840 is relatively benign in this respect.

Each of the donor solutions used were saturated, therefore they all had a thermodynamic activity of 1. As chemical potential is the main driving force behind passive diffusion, then unless the vehicle exerted an effect on the membrane, the fluxes should have been equal in all of the experiments. The flux was not equal but the permeability coefficient, which is representative of the steady state flux independent of concentration, was equal for the two full thickness skin experiments and the two epidermal membrane experiments. This verified the soundness of the experiments. As it is the stratum corneum that is widely held to be the rate-limiting step, the fluxes obtained for epidermal membranes and full thickness skin should have been equal. The observed 10-fold increase in permeation across epidermal membranes relative to full-thickness skin was unexpected, and indicated that the dermis was the rate limiting step for diffusion rather than the stratum corneum. This is usually only expected for highly lipophilic compounds which rapidly traverse the lipid domains of the stratum corneum and then are retarded by the relatively hydrophilic nature of the dermis. Although an explanation for the anomalous behaviour of doxycycline is unclear, it was indicative of a transcellular route and may again have been a manifestation of the complex chemical properties of the compound.

Tape stripping revealed that doxycycline was fairly evenly distributed throughout the stratum corneum and viable epidermis in relatively high concentrations. This has implications in the dermal treatment of Lyme disease where the majority of the population of causative organism is located at the dermal-epidermal junction and superficially in the erythematous border. It is not possible to speculate upon the potential use for the treatment of acne, although it has been suggested that tetracycline may penetrate the skin via the appendages (Barry, 1983). A knowledge of the minimum inhibitory concentrations for the organisms is required in order to fully determine the efficacy of the drug delivered dermally. A daily oral dose of 100 mg has been used for malaria prophylaxis and is reported to deliver a plasma concentration of 600 μg l^{-1} (Kotecka et al., 1996), although minimum inhibitory concentration data were not stated. However, even on the basis of these figures, the application of 2×30 cm^2 patches could achieve malaria prophylaxis.

Although the most predominant route across the statum corneum is widely held to be intercellular, it has also been contended that the pathway is too narrow and tortuous to account for the observed fluxes across the stratum corneum (Elias and Friend, 1975). The mean amount of doxycycline HCl recovered from the skin following a diffusion experiment was 458.4 ± 27.6, which was far higher than the volume of extractable lipid present in the same unit area-approximately 52.3 μg ml^{-1} (Abrams et al., 1993). This again indicates that a large proportion of the drug must be located within the proteinaceous domain. The transcellular pathway involves sequential passage through hydrophilic corneocytes and hydrophobic lipid bilayers. It is commonly thought that the multiple partitioning processes required to traverse the stratum corneum in such a manner make it an unlikely route. However, evidence exists to support the partitioning of drugs into corneocytes where binding to protein can take place, leading to the formation of a reservoir from which the drug can be slowly released (Hasiguchi et al., 1998). Favourable conditions such as a high chemical potential gradient may provide a sufficient driving force to allow the drug to partition and diffuse through the protein and lipid domains of the stratum corneum.

To summarise, the data suggest (1) significant amounts of doxycycline can be administered into and across the skin, and could provide alternative therapies for a number of conditions; (2) M840 is a potentially useful enhancing vehicle; and (3) the transcellular route was of significance in the permeation process.

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