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A therapeutic dose of primaquine can be delivered across excised human skin from simple transdermal patches

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Abstract

This work investigated the permeation of primaquine across full-thickness excised human skin from two acrylate transdermal adhesives. Primaquine base was formulated with National Starch 387-2516 and 387-2287 to provide aluminium foil-backed 1-cm diameter patches, each loaded with 10 mg drug. Other patches were prepared that included Migliol 840 as a potential penetration enhancer. The patches were applied to cadaver skin in Franz-type diffusion cells and the permeation of primaquine determined over a 24-h period. Relatively high fluxes were found, the highest being from those formulations lacking the Migliol 840: $5.68 \pm 0.30 \times 10^{-2}$ mg cm^{-2} h^{-1} from 387-2516; $4.94 \pm 0.20 \times 10^{-2}$ mg cm^{-2} h^{-1} from 387-2287. It was determined that a simple patch with a diameter of ≈ 13 cm^2 could deliver a therapeutic in vivo dose, with possibilities for the treatment and prophylaxis of *Plasmodium vivax*, *P. ovale* and *P. falciparum* forms of malaria. The presence of Migliol 840 failed to produce the anticipated enhancing effect: flux rates that were approximately halved. These results could to a certain extent be rationalised in terms of thermodynamic activity. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Primaquine; Skin; Malaria; Transdermal delivery; Acrylate adhesive

1. Introduction

Malaria is an ancient disease that continues to be a major cause of fatality in tropical and sub-tropical regions. The WHO estimates of malarial incidence in the world currently stands at 300–500 million clinical cases annually with some 1.5–2.7 million dying of malaria each year. The situation has recently become even more acute due to the increase in resistance to the drugs normally used to combat the parasites that cause the disease. Poor compliance, e.g. the inconvenience of frequent oral dosing, has been a major contributory factor in this. Immunisation would be ideal, but despite much research in this area, an effective vaccine has yet to be developed. Also, serious side effects have been a cause of concern in recent years, particularly the neurological and psychological disturbances which have been reported with mefloquine, an otherwise effective prophylactic drug. New anti-malarial regimens are urgently required and one possibility involves delivery across the skin. There are several distinct

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advantages of the transdermal delivery over other forms of administration, including improved patient compliance due to less frequent dosing.

Primaquine is a tissue schizonticide and is used to eradicate the pre-erythrocytic liver stage of malaria caused by *Plasmodium vivax* and *P. ovale*. It is also effective against the primary exoerythrocytic stage as well as being a gametocytocidal and sporontocidal agent. It is the only 8-amino-quinoline which is still widely employed as an anti-malarial. It is the only anti-malarial effective in treating the liver stages of the parasite. There have also been several recent papers describing the potential of primaquine in the prophylaxis of malaria caused by *P. falciparum* and *P. vivax*. Fryauff et al. (1995) found that primaquine was well-tolerated and effective when administered daily for 1 year to men with normal G-6-PD activity. In a 15-week randomized, double-blind, placebo-controlled field study primaquine had an efficacy and toxicity competitive with those of standard agents for prophylaxis against *P. falciparum* malaria (Soto et al., 1998). Baird et al. (1995) found that primaquine offered better prophylaxis for both *P. falciparum* and *P. vivax* than chloroquine. Soto et al. (1998) claimed a further advantage of primaquine is that prophylaxis may be discontinued only 1 week after the recipient has left the endemic area, although incidences of mild to severe gastrointestinal distress were also detailed. However, this and other dose-dependent side-effects associated with primaquine (Clyde, 1981) may be circumvented by transdermal delivery.

Primaquine has already been the subject of work aimed at the development of a transdermal system (Mayorga et al., 1998). Thassu and Vyas (1993) reported significant permeation across cadaver skin from occluded formulation based upon ethylene vinyl acetate copolymer. In a liquid formulation experiment, Mayorga et al. (1996) found the highest primaquine permeation rates across hairless rat skin were obtained from a Migliol 840 (M840) vehicle. A similar investigation by Morris et al. (1998) found that highest permeation rates across excised human skin were also obtained from a vehicle of M840—a diglyceride with C8–C10 alkyl chains. The results were attributed to an enhancement effect, rationalised in terms of the solubility parameter (Fedors, 1974) of primaquine and M840 both being approximately 10. This was of particular significance as the lipoidal barrier function of the skin has also been determined to have a solubility parameter of 10 (Liron and Cohen, 1984), hence each component is mutually soluble. In this work, we examined the permeation of primaquine across excised human skin from two simple drug-loaded acrylate transdermal adhesive matrices and whether the enhanced permeation of primaquine observed using M840 from liquid vehicles would be reflected in such devices.

2. Materials and methods

2.1. Materials

Primaquine diphosphate was obtained from Sigma (UK). Primaquine free base was liberated by neutralisation of the salt with 4 N NaOH followed by solvent extraction. The product was a viscous, dark-red liquid. Migliol 840 was supplied by Condea, Germany. Duro-tak® 387-2287 and 387-2516 transdermal adhesives were gifts from National Starch and Chemical, Holland. Acetonitrile and methanol were obtained from Fisher and were both HPLC-grade. Perchloric acid (60% w/w solution) and buffer salts were obtained from Aldrich. Male and female cadaver skin samples were obtained from a local mortuary. The subcutaneous fatty layer was removed by blunt dissection to give full-thickness skin samples of approximately 2.5 cm^2. Hair from male samples was clipped using scissors.

2.2. Preparation of transdermal patches

The chemical nature of a transdermal adhesive can affect the solubility of the drug and previous work demonstrated that adhesives containing acidic side-chains are not compatible with the basic form of primaquine (Jeans et al., 1998). However, adhesives containing OH functionality were found to produce homogenous and stable blends, possibly due to hydrogen bond formation,

that provided good rates of primaquine release across Visking membrane. Adhesives with alcohol functionality (National Starch and Chemical, Duro-tak® 387-2287 and 387-2516) were consequently used in this project (Table 1).

The drug was dissolved in solvent and added to the adhesive (Jeans et al., 1998). Chloroform was the solvent of choice as it was fully miscible with the adhesive system and primaquine. The aim was to prepare a 1-cm diameter aluminium-backed adhesive film of $\approx$1-mm thickness that contained 10 mg of primaquine. Loading >10 mg primaquine produced unstable matrices. A custom-made aluminium former (15×2 cm, area 30 cm^2) was lined with aluminium foil and the ends blanked off with tightly fitting cork blocks. To obtain a 1-cm diameter device (0.785 cm^2) that would contain 10 mg of primaquine, an adhesive/drug solution was prepared by dissolving 382 mg of primaquine in 3 ml of chloroform in a 20-ml snap top vial. When M840 was included in the formulation 2.5 ml of chloroform was used with 0.5 ml of M840, to give a concentration of 10%. To this 4.5 ml of the adhesive was added. The mixture was then inverted several times, carefully avoiding the introduction of bubbles and poured into the former, covered and left overnight to allow the solvent to evaporate. The product was a film of $\approx$1 mm depth.

2.3. Skin permeation experiments

When required for use discs were cut from the adhesive/drug film using a 1-cm diameter ($\approx$0.79 cm^2) cork-bore and then firmly pressed onto the centre of a fully thawed skin specimen. Once adhesion to the skin surface had been confirmed the skin was quickly mounted on the pre-greased flange of a Franz-type diffusion cell receptor compartment (nominal volume 2.5 ml), such that the patch was situated precisely over the flange aperture. The donor compartment was then placed in position and the two halves of the cell clamped together. To represent physiological pH the receptor phase used was isotonic phosphate buffer at pH 7.4, which was degassed by filtration before use. Although primaquine base is lipophilic, this receptor medium has previously proven effective (Morris et al., 1998)—the drug ionises either within the skin or at the receptor compartment interface. The receptor compartment was filled carefully to avoid air bubble formation on the underside of the skin. The complete cells were placed on a magnetic stirrer submerged in a water bath maintained at 37°C, thereby maintaining an approximate surface skin temperature of 32°C. Sampling arms and donor apertures were occluded. At 1, 3, 6, 12, 24, 48 and 72 h time points 200-μl aliquots of receptor phase was transferred to 500-μl plastic autosampler vials which were sealed and stored at -20°C prior to analysis. Receptor phases were replenished with pre-equilibrated buffer. In this work, a total of four experimental conditions were employed (Table 1) involving 12 replicates and four donor skin samples per run.

Table 1
Patch constituents[a]

Experiment	Patch matrix (1 cm diameter, 0.79 cm^2)
1	Primaquine (10 mg) in NSD 387-2516
2	Primaquine (10 mg) in NSD 387-2516+ M840 10%
3	Primaquine (10 mg) in NSD 387-2287
4	Primaquine (10 mg) in NSD 387-2287+ M840 10%

[a] NSD, National Starch Duro-tak; M840, Migliol 840.

2.4. HPLC analysis

Analysis was performed using a Milton Roy HPLC system including a CM4000 solvent delivery system, CM4000 UV detector, Marathon autosampler and Phenomenex Kingsorb ODS C-18 (2.6×250 mm) column. Data were acquired using Jones Chromatography JCL6000 software running on an Azteq Pentium 100 PC. The mobile phase composition was similar to that reported by Dua et al. (1996): acetonitrile, methanol, 1 M perchloric acid and deionised water (33:6:1:87). Standard solutions of 0.0125, 0.025. 0.05, 0.1 and 0.25 mg ml^{-1} were used in the construction of calibration plots. Cumulative permeation profiles were constructed from which permeation data were determined, using standard methods.

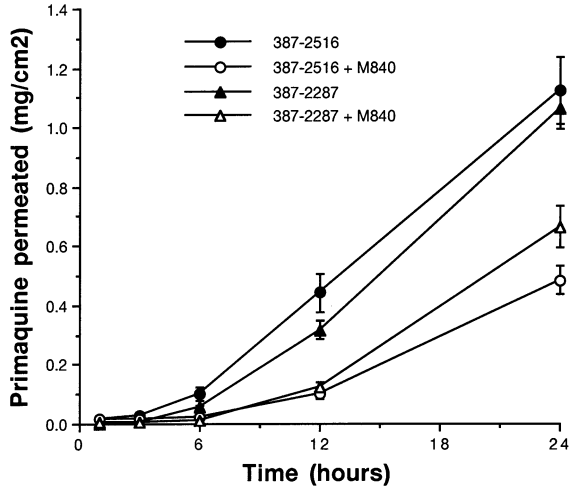

Fig. 1. Cumulative permeation profiles of primaquine across excised human skin from transdermal adhesive/transdermal adhesive + M840 matrices ($n = 12 \pm$ S.E.).

3. Results and discussion

The four permeation profiles are shown in Fig. 1. Good consistency was found within each run indicating the integrity of the skin specimens over the duration of the experiment. Relatively high fluxes were found, the highest being $5.68 \pm 0.30 \times 10^{-2}$ mg cm^{-2} h^{-1}, from adhesive 387-2516 (Fig. 2). This result was almost double that reported by Thassu and Vyas (1993), who found a maximum flux of 3.2×10^{-2} mg cm^{-2} h^{-1} ($n = 3$) from ethylene vinyl acetate co-polymer matrices. Table 2 and Fig. 2 summarise the permeation data. There appeared to be no major difference between the two adhesives in terms of skin permeation, hence rates of release from the matrices. This might have been anticipated in that the loading of primaquine within each patch, hence thermodynamic activity, was the same. However, adhesive 387-2516 has a cross-linked structure that may have provided a more tortuous pathway for drug release than adhesive 387-2287, which lacks cross-linking. However, no evidence for this effect was apparent.

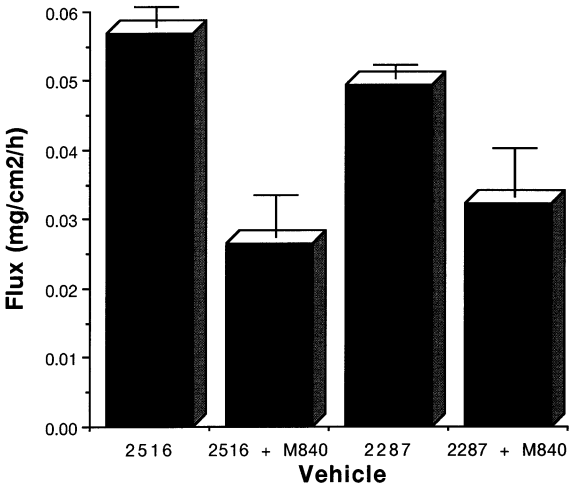

Fig. 2. Histogram showing steady-state flux values of primaquine across excised human skin from transdermal adhesive/transdermal adhesive + M840 matrices ($n = 12 \pm$ S.E.).

From Fig. 1 it can also be seen that the two formulations containing 10% M840 provided an approximate twofold *decrease* in flux relative to the two formulations where M840 was absent. These findings indicate that, in these systems, M840 failed to provide the anticipated enhancing effect (Morris et al., 1998). A possible explanation for these results lies in the difference in thermodynamic activity between the two formulations. Thermodynamic activity is essentially a measure of the 'leaving ability' of a drug from a vehicle and provides the driving force for passive diffusion (Higuchi, 1960). In a saturated solution, the thermodynamic activity is defined as 1, whereas at concentrations less than saturation, the thermodynamic activity is < 1. Assuming no other effects, flux is directly proportional to thermodynamic activity. If the solubility of primaquine in M840 is greater than its solubility in the adhesive, and the concentration is the same in both formulations, it follows that the thermodynamic activity of the two formulations will differ. Therefore, the formulation containing M840 will have a lower thermodynamic activity and thus will provide a lower leaving potential. This would give rise to lower flux (in the absence of other effects). The similarity in solubility parameters of primaquine and M840 suggests that the two species would be inherently miscible, supporting this hypothesis. Morris et al. (1998) postulated that this miscibility effect was responsible for the enhancing properties of M840 and it is likely that this effect was

Table 2
Steady state flux, lag time and permeability coefficient data for the permeation of primaquine across human skin in vitro from transdermal adhesive matrices ($n = 12 \pm$ S.E.)

Experiment	Lag time (h)	Flux (mg cm^{-2} h^{-1})	S.E.	Permeability coefficient (cm h^{-1})
1	4	5.68×10^{-2}	0.003	4.44×10^{-3}
2	12	2.66×10^{-2}	0.006	2.08×10^{-3}
3	5	4.94×10^{-2}	0.002	3.86×10^{-3}
4	6	3.32×10^{-2}	0.007	2.53×10^{-3}

also occurring during these experiments, but at the concentrations used, was overwhelmed due to the stronger effects exerted by the differences in thermodynamic activity. Increasing the level of M840 was not practical as it produced matrices that were excessively fluid and with reduced adhesive properties. The fact that primaquine and M840 are fully miscible *liquids* makes it difficult to fully rationalise the results on grounds of thermodynamic activity.

The pharmacokinetic properties of primaquine have been studied by Mihaly et al. (1985a,b). Based upon the flux achieved from primaquine in the 2516 adhesive formulation, it is possible to estimate the magnitude of a patch that could deliver a therapeutic dose in vivo. Eq. (1) (Benet et al., 1996) describes flux, pharmacokinetic parameters and body weight in relation to the dimensions of a patch required to deliver a therapeutic dose:

$$J \cdot A = Cl \cdot C_p \cdot W \quad (1)$$

where J is the flux (57 μg cm^{-2} h^{-1}), A is the area of application, C_p is the plasma concentration (0.03 μg ml^{-1}; Thassu and Vyas, 1993) and W is the weight of the subject (average male, 70 kg). Cl (clearance rate) was calculated using Eq. (2):

$$t_{1/2} = (\ln 2V)/Cl \quad (2)$$

where $t_{1/2}$ is the half life (7 h) and V is the volume of distribution at steady-state (3.5 l kg^{-1}). Based on the findings in this work, a patch with an area of ≈ 13 cm^2 formulated in the same manner as the prototype (area 0.8 cm^2) would be expected to deliver a therapeutically useful dose of primaquine in vivo.

To summarise, this study has shown that a relatively simple transdermal adhesive patch formulation incorporating primaquine can deliver a dose that could provide new treatment and prophylaxis regimens for *P. vivax* and *P. ovale* malaria. It is also clear that the penetration enhancer M840 is of no benefit in this particular formulation as it effectively retards release, therefore reducing permeation rate.

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