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Interpretation and prediction of plasma levels of primaquine following transdermal delivery in Swiss mice

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Abstract

A therapeutic transdermal system based on ethyl cellulose polymer and matrix diffusion-controlled release of an antimalarial, primaquine (PQ), was investigated with respect to the in vitro percutaneous penetration and in vivo skin absorption profile of the drug. In order to correlate in vitro and in vivo data, pharmacokinetic modelling was performed. Thus, the diffusion of the drug through the polymeric device and its pharmacokinetics parameters were both considered. Franz-type diffusion cells were used for in vitro determinations. In vivo experiments were performed with Swiss mice. Drug plasma profiles following transdermal treatment showed constant primaquine plasma levels, indicating controlled and systemic delivery of the drug over a period of 40 h. © 1997 Elsevier Science B.V.

Keywords: Primaquine; Percutaneous absorption; Transdermal drug delivery system; In vivo; Pharmacokinetic modelling

1. Introduction

Primaquine (PQ) is an 8-aminoquinoline widely employed for the treatment of malaria (radical cure). This compound acts on the hipnozoites of *Plasmodium vivax* and *P. ovale* which may persist in the liver after eradication with chloroquine. PQ also acts as a causal prophylactic on the primary exo-erythrocytic stage and it shows gametocytocidal activity (Nodiff et al., 1991; Bhat et al., 1984).

However, the prophylactic and therapeutical applications of PQ are greatly limited because of its side-effects, marked gastrointestinal disturbances and the development of methaemoglobinaemia and haemolytic anaemia, which are dose-dependent (Clyde, 1981; Winstanley and

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Breckenridge, 1987). Thus, it would be interesting to obtain a therapeutic device able to provide a long-term action without peaks of drug in the plasma.

In order to improve PQ treatment, we propose an ethyl cellulose-based transdermal therapeutic system (TTS) as a viable dosage form for PQ administration. The formulation of this device has already been described (Mayorga et al., 1996).

Considerable attention has been focused on transdermal drug delivery in order to obtain a sustained systemic effect (McDaid and Deasy, 1996; Ogiso et al., 1994; Guy and Hadgraft, 1985). There are many advantages to using this route of administration: (a) avoidance of first-pass metabolism; (b) improvement of biocompatibility and/or reduction of bioavailability problems caused by variability of absorption, in contrast with the gastrointestinal route; (c) reduction of side-effects by elimination of plasmatic peaks and (d) patient convenience and improved patient compliance.

In vivo experimentation and its analysis in terms of pharmacokinetics represents a crucial step in the development of a therapeutic device. However, this stage is time consuming, expensive and may pose ethical problems.

In view of this, some attention has been given to model development in transdermal administration. This approach combines in vivo pharmacokinetic data with absorption rates determined by an in vitro procedure and allow improved interpretation of pharmacological or toxicological data (Bunge et al., 1995; Auton et al., 1993; Chandrasekaran et al., 1978).

Thus, kinetic modelling of transdermal drug delivery may be a valid approach to predict skin penetration and so to examine and optimize a formulation strategy (Shah, 1996; Guy and Hadgraft, 1985, 1986), which is competitive in terms of low cost and high feasibility.

The aim of this work was to investigate the bioavailability of PQ following a single transdermally delivered dose. After this evaluation, we compared the data observed in vivo with the profile predicted by a pharmacokinetic model. This approach was based on the physicochemical and pharmacokinetic properties of the drug and on in vitro release and percutaneous absorption studies, adopting a three compartment pharmacokinetic model.

In order to estimate the basic pharmacokinetic parameters, the plasma concentration profiles after both intravenous (i.v.) and subcutaneous (s.c.) administration of the drug were also evaluated.

2. Materials and methods

2.1. Materials

Primaquine diphosphate was purchased from Sigma Chimie (France) and primaquine free base was obtained in our laboratory by extraction with organic solvent. The TTS formulation components used were a mixture of propylene glycol dicaprylate/caprate as a vehicle (Miglyol® 840) provided by Hüls (Germany), an antioxidant, (±) α-tocopherol, purchased from Sigma Chimie (France), ethyl cellulose polymer (Dow Chemical Company, Netherlands) with diethyl phthalate (Prolabo, France) as plasticizer and a pressure-sensitive adhesive acrylic resin, Durotak® 280 2287 (National Starch and Chemical, France). Ketoprofen was used as internal standard for the HPLC assay. All other chemicals and solvents used were of reagent grade or HPLC quality. Male Swiss mice, weighing 24–26 g (Iffa Credo, France), were used in these experiments.

2.2. Methods

2.2.1. Preparation of TTS formulation

The transdermal system formulation was obtained by the casting method. A mixed isopropanol/acetone solution of the matrix components was cast in PTFE (Teflon®) moulds and dried at 25°C for up to 5 days. The drug vehicle Miglyol® 840 was added at 20% of the dry polymer and the antioxidant (±) α-tocopherol was added at 0.04% of the drug content. Afterwards, the adhesive layer was applied as an ethanol/acetone solution of the acrylic adhesive (Durotak®), loaded with primaquine (5 mg/cm^2) and dried at 25°C for 5 days. The ratio of plasticizer to ethyl cellulose was 20% of diethyl phtha-

late, based on dry polymer. The total amount of primaquine base in the TTS formulation obtained was 15 mg/cm^2. This protocol of TTS preparation as well as the extraction procedure for obtention of primaquine free base have been described in a previous study (Mayorga et al., 1996).

2.2.2. Drug release kinetics studies

Drug release studies were conducted in 2-compartment Franz-type diffusion cells at 37°C. The patch was placed between the donor and receptor compartment, which contained 8.0 ml of a phosphate-buffered solution (pH 7.4) of Tween® 80 (5% w/v). A mixture of the antibiotics penicillin and streptomycin was added to the receptor solution. The diffusion surface was 2.54 cm^2. At suitable time intervals, the whole receptor phase was taken for drug assay. The release of drug from the primaquine patch was tested for 26 h and determined in four sets of diffusion cells. Drug assay was performed by high performance liquid chromatography (Waters 501). The analytical system was equipped with an automatic sampler injector (Waters 712 WISP), a variable wavelength UV detector (Waters 484) and a reversed phase column (C_{18}, 3.9 × 300 mm, particle size, 4 μm). The mobile phase was acetonitrile:methanol: phosphate buffer (3.7 g/l K_2HPO_4, 3.5 g/l KH_2PO_4), 30:35:35. A ion pairing agent, *N*,*N*-dimethyloctylamine, was used (0.5 ml/l). The UV detection was at 356 nm, with a flow rate of 0.8 ml/min.

2.2.3. In vitro percutaneous penetration kinetics

The same Franz-type diffusion cell as described above was used to investigate in vitro skin penetration kinetics at 37°C. The skin samples of 2.54 cm^2 were obtained from the dorsal and abdominal regions of 24–26 g male Swiss mice (Iffa Credo, France). The skin was always freshly obtained and used full thickness with the adherent fat and other debris carefully removed, and the hair cut and shaved. The skin fragments placed on the cell were equilibrated with the receptor phase for 12 h before beginning the experiment. At this moment, the receptor phase was renewed and the TTS samples (2.0 cm^2) were deposited. At regular intervals up to 26 h, the whole receptor phase was taken for drug assay, as described above. In this study, six experiments were performed.

2.2.4. In vivo experiments—intravenous (i.v.) and subcutaneous (s.c.) administration

Primaquine diphosphate, 10 mg/kg (5.7 mg/kg free base), was administered into the tail vein of male Swiss mice. Nine groups of four animals were used to obtain the complete profile and each one corresponds to a single point on the drug plasma concentration profile. Blood samples were collected by cardiac puncture using heparinized syringes at 1, 3, 5, 10 and 30 min and at 1, 1.5, 2.5 and 3 h after administration of primaquine diphosphate.

For subcutaneous pharmacokinetic determination, a similar procedure was used. In this case, primaquine diphosphate at a dose of 50 mg/kg (28.5 mg/kg free base) was administered into the dorsal region of male Swiss mice. Blood samples were collected by cardiac puncture from eleven groups of four mice at 15 and 30 min and 1, 2, 4, 6.5, 8, 10, 12, 15 and 24 h after subcutaneous administration, each group corresponding to a different point of the complete time-course.

The blood samples were centrifuged at 3000 rpm for 10 min to separate the plasma, which was kept in aluminum foil-covered Eppendorf tubes at −20°C until analysis.

2.2.5. In vivo experiments—transdermal administration

Male Swiss mice, weighing 24–26 g (Iffa Credo, France), were used in this study. After the back hair had been cut and shaved, taking care to prevent damage to the surface of the skin, the primaquine patch was stuck on the back skin of each mouse for up to 96 h. The complete time-course was obtained with thirteen groups of four mice, where each one group corresponded to a single point on the drug plasma concentration profile. The blood samples were collected by cardiac puncture at 3, 5, 7, 9, 10, 24, 32, 48, 58, 72, 77, 82 and 96 h after patch application. The plasma was separated by centrifugation (3000 rpm for 10 min) and kept in aluminum foil-covered Eppendorf tubes at −20°C until analysis.

2.2.6. Plasma drug assay

For the determination of drug concentrations in plasma samples, 0.2 ml of acetonitrile containing 4 μg/ml of ketoprofen as internal standard was added to 0.2 ml of plasma. After vortexing for 1 min, the mixture was centrifuged at 10 000 rpm for 10 min. The supernatant was used for HPLC analysis. The analytical system was the same as that used for the samples of in vitro studies, except for the column, which was a C_8, 4 × 125 mm, particle size, 5 μm. The mobile phase of the HPLC system was composed of 7.0 mM monochloroacetic acid, 0.5 mM 1-decanosulfonic acid—acetonitrile—methanol (56:24:20, v/v) (Dean et al., 1994). UV detection was carried out at 254 nm, with a flow rate of 1.5 ml/min.

2.2.7. Pharmacokinetic modelling

To establish a theoretical profile of plasma primaquine concentration after transdermal administration, we developed a three compartment model. A schematic representation of the model is presented in Fig. 1.

The different compartments represent the transdermal system, the skin and the plasma. The rate constants which apply to mass transfer between the different compartments are indicated by k_1, k'_1, k_2 and k_3.

3. Results and discussion

3.1. Drug release kinetics

To describe drug delivery from the device, the first order release rate constant, related to the diffusional properties of the drug through the polymeric matrix, was determined.

Fig. 1. Schematic representation of the three-compartment model and the respective rate constants implied in mass transfer between the different compartments, indicated by k_1, k'_1, k_2 and k_3.

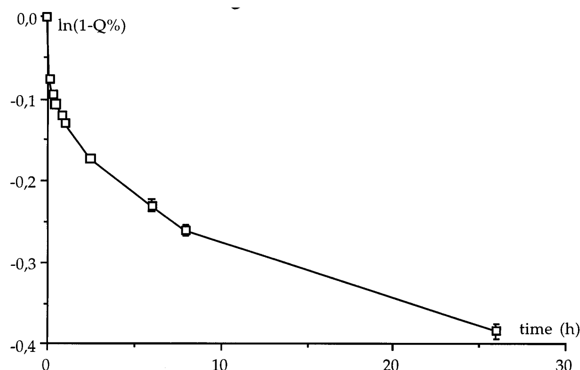

Fig. 2. In vitro drug release kinetics from TTS formulation (15.0 mg of PQ/cm^2); $n = 4$.

In the development of transdermal delivery systems, determination of the drug release profile is required for in vitro characterization of the device. Moreover, Guy and Hadgraft (1985) have shown the effect of the rate at which the device delivers drug on the steady state-plasma concentrations. So, this correlation makes it possible to achieve suitable plasma levels by choosing an appropriate rate of drug release from the device.

The first order release rate constant determined for the TTS in question was 8.31×10^{-3}/h. This value was calculated by logarithmic linearization of the drug release profile (Fig. 2). It was also observed that the device had released 30% of the total drug after 24 h.

3.2. In vitro percutaneous penetration kinetics

The skin penetration profile of TTS formulation showed apparent zero-order kinetics, when the cumulative amount which had permeated was plotted against time (Fig. 3). By application of Fick's law of diffusion, under sink conditions, the percutaneous flux and the lag time, 156.3 ± 30.4 μg/cm^2 per h and 1.12 ± 0.97 h respectively, were determined. In a previous study, percutaneous flux and lag time were determined for this TTS formulation using hairless rat skin. The obtained values were 181.0 ± 25.5 μg/cm^2 per h and 4.32 ± 0.74 h respectively (Mayorga et al., 1996).

Illel et al. (1991) investigated the importance of the transappendageal route in skin penetration.

The authors observed that appendageal diffusion was possible despite the relatively small surface area of the follicles of hairless rat skin, for a large range of substances with varying solubilities. In addition, skin should not always be assumed to be a simple lipoidic barrier and a polar pathway has also been shown to exist, especially for iontophoretic treatment. In a recent paper, Roland et al. (1994) support the theory of 'aqueous pores' in skin penetration. Thus, the difference observed between percutaneous flux values of PQ through hairless rat and normal mouse skin suggests that appendageal diffusion can play a role in skin penetration of PQ from the TTS device, but is probably not the major pathway of diffusion.

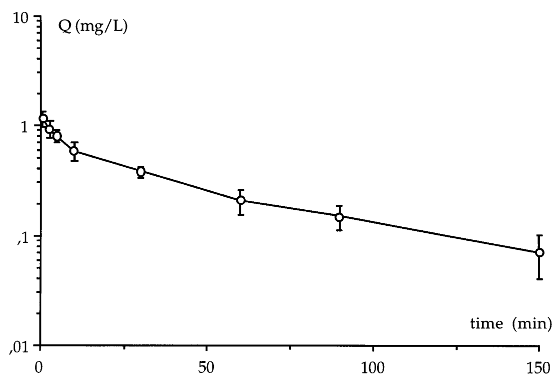

Fig. 4. Drug plasma concentration profile after intravenous administration of PQ (5.7 mg/kg free base); each point corresponds to the average of four animals group.

3.3. In vivo experiments—intravenous (i.v.) and subcutaneous (s.c.) administration

3.3.1. intravenous (i.v.) administration

The drug plasma concentration profile after i.v. administration is shown in Fig. 4. It was observed that the decay in plasma concentrations followed first-order kinetics described by the equation:

$$C_t = A\ \mathrm{e}^{-\alpha t} + B\ \mathrm{e}^{-\beta t} \quad (1)$$

where C_t is the drug concentration at time t, and A, α, B and β are the biexponential equation constants. The half-life of the $\alpha (t_{\alpha 1/2})$ and β $(t_{\beta 1/2})$ phases were calculated as $t_{\beta 1/2} = 0.693/\beta$ and $t_{\alpha 1/2} = 0.693/\alpha$, respectively. The most important parameter in this evaluation is the elimination rate constant, β, which is determined by the slope of the terminal portion of the fitted curve.

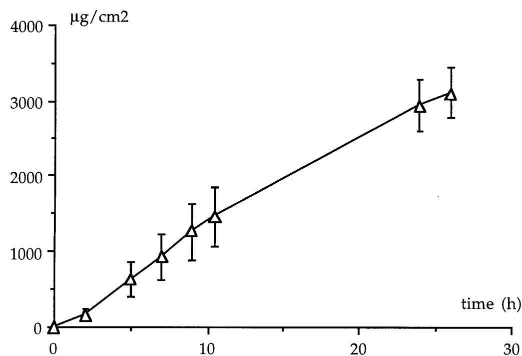

Fig. 3. In vitro percutaneous penetration kinetics of PQ through mouse skin after transdermal application (15.0 mg of PQ/cm^2); $n = 6$.

The total clearance ($\mathrm{Cl_T}$) was determined by the following equation:

$$\mathrm{Cl_T} = \frac{D_0}{\mathrm{AUC}_{0\to\infty}} \quad (2)$$

where D_0 is the applied dose and $\mathrm{AUC}_{0\to\infty}$ is calculated as:

$$\mathrm{AUC}_{0\to\infty} = \mathrm{AUC}_{0\to t_i} + \mathrm{AUC}_{t_i\to\infty} \quad (3)$$

where t_i is the last point of curve which has the respective concentration $C_{t_i} > 0$, $\mathrm{AUC}_{0\to t_i}$ is determined by the trapezoidal method and $\mathrm{AUC}_{t_i\to\infty}$ is calculated by theoretical extrapolation as:

$$\mathrm{AUC}_{t_i\to\infty} = \frac{C_{t_i}}{\beta} \quad (4)$$

where C_{t_i} is the drug concentration at time t_i.

Distribution volume (V_d) was also calculated according to the following equation:

$$V_\mathrm{d} = \frac{D_0}{\mathrm{AUC}\cdot\beta} \quad (5)$$

We used this expression to determine V_d because it is independent of the kinetic model. The pharmacokinetics parameters determined in this way are given in Table 1.

The results show that primaquine is distributed and removed from the circulation very rapidly, as previously described (Pirson et al., 1982), with an apparent elimination half-time of 0.948 h.

Table 1
Pharmacokinetic parameters determined following intravenous, subcutaneous and transdermal administration of primaquine

Parameter	Administration route		
	i.v.	s.c.	TTS
Cl_t (l/h)	0.19	0.165	—
Dose (mg)	0.142	0.712	4.17
AUC (mg/l per h)	0.749	4.31	21.95
Co (mg)	1.2054	—	—
β (h^{-1})	0.73092	0.0789[a]	—
$t_{\alpha 1/2}$ (h)	0.069	—	—
$t_{\beta 1/2}$ (h)	0.948	8.78	–
V_d (l)	0.290	—	—

Cl_t, total clearance; dose, amount administered; AUC, area under the curve; Co, concentration at time O h (extrapolated from the curve); β, rate constant of elimination step; $t_{\alpha 1/2}$, half-life of α phase; $t_{\beta 1/2}$, half-life of β phase; V_d, distribution volume.
[a] Apparent elimination rate constant.

3.3.2. In vivo experiments—subcutaneous (s.c.) administration

The drug plasma concentration profile after s.c. administration is shown in Fig. 5. To interpret the elimination phase after subcutaneous administration we employed the same pharmacokinetic model as used for intravenous data analysis. Thus, the pharmacokinetics parameters, $AUC_{0\to\infty}$ and the apparent elimination rate constant (β), were determined as described above (Table 1).

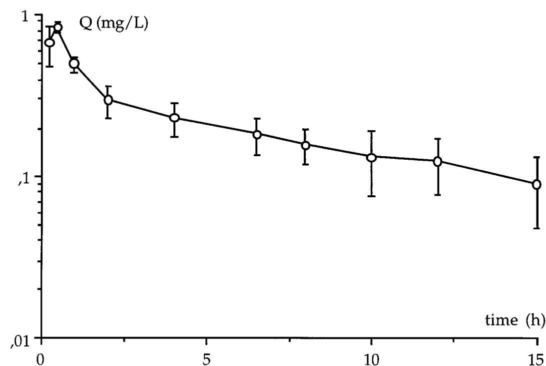

Fig. 5. Drug plasma concentration profile after subcutaneous administration of PQ (28.5 mg/kg free base); each point corresponds to the average of four animals group.

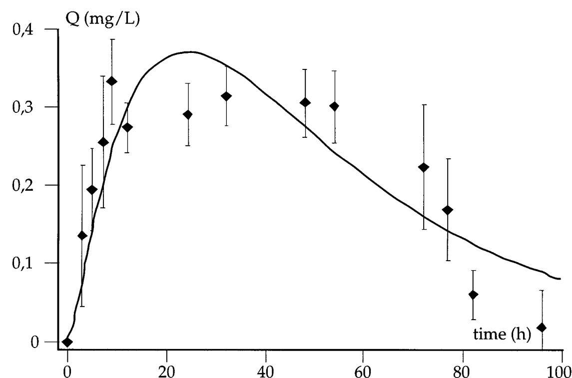

Fig. 6. Drug plasma concentration profile after transdermal administration of 1.0 cm^2 TTS (15.0 mg of PQ/cm^2): experimental data (◆) and theoretical profile from pharmacokinetic modelling (——); each point corresponds to the average of four animals group.

From comparison with i.v. pharmacokinetics data, the absolute bioavailability value was determined as 1.15. This value may be a result of an extrapolation error in $AUC_{0\to t_i}$ which represents 26% of $AUC_{0\to\infty}$. Moreover, it was observed that the apparent elimination rate constant (β) determined after s.c. administration was approximately 10-fold lower than this one determined after i.v. administration. Based on this result, we can assume the presence of a rate-limiting factor, probably as a result of drug retention at the administration site.

3.4. In vivo experiments—transdermal administration

The drug plasma concentration profile following transdermal administration is showed in Fig. 6. Maximal concentration (332.9 ± 56.2 ng/ml) was attained after 9 h of TTS administration. Thereafter, a maintained drug plasma concentration of about 300 ng/ml was observed between 12 and 54 h. From this concentration and the value of the clearance we were able to calculate the in vivo steady-state flux: 223.4 μg/h. This value is higher than the percutaneous flux determined from in vitro percutaneous kinetics which was 156.3 ± 30.4 $\mu g/cm^2$ per h. It is possible that in the in vitro experiments particular skin condi-

tions, such as a high extent of hydration, caused a reduction in the percutaneous flux of the lipophilic drug. This observation is in accordance with the results described above concerning the appendageal or polar routes of primaquine diffusion through the skin, the latter of which is probably of lesser importance. Furthermore, as observed by Diez-Sales et al. (1993), it is possible that in the in vitro experiments the dermal layer acts as an additional barrier, mainly for weakly hydrophilic compounds, in comparison with their in vivo absorption process where the drug may be absorbed at the capillary level after leaving the living epidermis and does not have cross the dermis in order to reach the bloodstream.

The mean area under the curve (0–96 h) was 21.95 mg/l per h. Moreover, based on pharmacokinetics parameters obtained from intravenous data the total amount delivered by 1.0 cm^2 TTS was 4.17 mg over a 96-h period.

3.5. Pharmacokinetic modelling

In the present model, the linear differential equations describing the mass transfer process between the different compartments can be written as follows:

$$\frac{dM_1}{dt} = -k_1M_1 + k'_1M_2 \tag{6}$$

$$\frac{dM_2}{dt} = k_1M_1 - k'_1M_2 - k_2M_2 \tag{7}$$

$$\frac{dM_3}{dt} = k_2M_2 - k_3M_3 \tag{8}$$

Mass transfer equations were solved by using the method of Laplace transformation, leading to the following equation:

$$C_p = \frac{M_0}{V_d} k_1k_2\left[\frac{e^{-\mu t}}{(\mu-\sigma)(\mu-k_3)} + \frac{e^{-\sigma t}}{(\sigma-k_3)(\sigma-\mu)} + \frac{e^{-k_3t}}{(k_3-\mu)(k_3-\sigma)}\right] \tag{9}$$

where μ and σ constants are defined from the pharmacokinetic parameters as follows:

$$\mu + \sigma = k_1 + k'_1 + k_2 \qquad \mu\sigma = k_1k_2$$

and in which C_p is the drug plasma concentration; M_0 is the amount of drug in transdermal system (TTS) at time to; V_d is the distribution volume determined from i.v. pharmacokinetic data; k_1 is the first order rate constant for release from transdermal system (TTS), determined from drug release kinetic study; k_2 is the rate constant of the absorption step; k_3 is the rate constant of drug elimination from plasma, determined from i.v. pharmacokinetic data.

The s.c. pharmacokinetic data revealed a rate-limiting phenomenon, probably as a result of drug retention at the administration site. So, we assumed that the apparent elimination rate constant determined after s.c. administration represented, in our model, the absorption rate constant (k_2) for drug transport from skin to the plasma.

Using Eq. (9) it was possible to estimate plasma levels of PQ after transdermal administration. First-order kinetics were assumed, and the time course of PQ behaviour was considered in terms of three rate constants, k_1, k_2 and k_3; k'_1 being a rate constant for drug transport from the skin to the TTS, representing the partitioning between these two compartments. The unknown parameters μ and σ were estimated to provide the best fit of the data (MCF 1.2 software). The rate constants were defined from the experimental data and assumed to be fixed. The theoretical profile based upon our model is compared with the experimental data in Fig. 6 and the parameters, which served to calculate the theoretical curve, are given in Table 2. In this first approximation, we observed a good correlation between theoretical prediction and experimental data (sum of square errors = 0.045; correlation coefficient = 0.75).

As discussed above, the three constants, k_1, k_2 and k_3 were determined experimentally. The first, which describes the input rate from the device, k_1, was determined as been 8.31×10^{-3}/h. This constant depends on the performance of the device. So, in practice, it is the only parameter that can be adjusted by modifying the formulation in order to obtain an appropriate drug plasma concentration. The other constants, k_2 and k_3, are a function of pharmacokinetic properties of the drug and were determined from s.c. and i.v. pharmacokinetic data, respectively. We observed that k_3

(0.731/h) was higher that k_2 (0.079/h) by a factor of about 10. This observation led to the hypothesis of a reservoir effect of the drug resulting from a rate limiting absorption process. We supposed that real rate constant of elimination following s.c. administration would be masked by the rate constant of drug desorption from the administration site. Estimation of k_2 to obtain the best fit of the data gives a value of 0.077/h, which was very similar to that determined from s.c. pharmacokinetic data (0.079/h). Thus, we identified this apparent rate constant of drug elimination with the absorption rate constant in our model. The last parameter, k'_1, is included in the model for completeness and was determined as 1.4×10^{-4}. It reflects the competition for the drug between the patch and the skin surface. Ideally, if the system is well designed, the partition will favor the skin and k'_1 will be negligible.

Overall, we can conclude that the pharmacokinetic model presented in this study allowed us to predict plasma levels of PQ following transdermal application and represented a simple and useful approach to improve formulation strategies for this type of system. By using this simulation, it is also possible to determine the optimum input kinetics to obtain a specific plasma level. The approach requires knowledge of in vitro release properties of the device as well as predetermined drug pharmacokinetic data. Moreover, it is possible to view this model as both a tool for screening potential transdermal delivery candidates and for general use for the interpretation of transdermal absorption data, but this requires further studies of compounds with different physical and pharmacokinetic properties. Finally, the goal of this work was to show that the studied device provides controlled steady plasma levels of PQ and, as a result, it possesses advantages such as avoidance of first-pass metabolism, improvement of patient compliance as well as reduction of bioavailability problems in comparison with other administration routes.

Table 2
Parameters used in the pharmacokinetic model to predict the theoretical profile of PQ plasma levels after transdermal administration

Parameter	Value
M_0	15.0 mg
V_d	0.290 l
k_1	8.31×10^{-3} h
k_2	0.0789 h
k_3	0.73092 h
μ	6.89×10^{-2}
σ	2.63×10^{-2}

M_0, drug amount in transdermal system (TTS) at time t_0; V_d, distribution volume; k_1, order rate constant of release from transdermal system (TTS); k_2, rate constant of absorption step; k_3, rate constant of drug elimination from plasma; $\mu + \sigma$, $k_1 + k'_1 + k_2$; $\mu\sigma$, $k_1 k_2$.

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References

Auton, T.R., Ramsey, J.D., Woollen, B.H., 1993. Modelling dermal pharmacokinetics using in vitro data. Part I. Fluazifop-butyl in the rat. Hum. Exp. Toxicol. 12, 199–206.

Bhat, B.K., Seth, M., Bhaduri, A.P., 1984. Recents developments in 8-aminoquinoline antimalarials. Prog. Drug Res. 28, 197–231.

Bunge, A.L., Cleek, R.L., Wecchia, B.E., 1995. A new method for estimating dermal absorption from chemical exposure. 3. Compared with steady-state methods for prediction and data analysis. Pharm. Res. 12, 972–982.

Chandrasekaran, S.K., Bayne, W., Shaw, J.E., 1978. Pharmacokinetics of drug permeation through human skin. J. Pharm. Sci. 67, 1370–1374.

Clyde, D.F., 1981. Clinical problems associated with the use of primaquine as a tissue schizontocidal and gametocytocidal drug. Bull. Who 59, 391–395.

Dean, R.A., Ochieng, W., Black, J., Queener, S.F., Bartlett, M.S., Dumaual, N.G., 1994. Simultaneous determination of primaquine and carboxyprimaquine in plasma using high-performance liquid chromatography with electrochemical detection. J. Chromatogr. B 655, 89–96.

Diez-Sales, O., Sayas, E.P., Villodre, A.M., Dominguez, M.H., 1993. The prediction of percutaneous absorption: I. Influence of the dermis on in vitro permeation models. Int. J. Pharm. 100, 1–7.

Guy, R.H., Hadgraft, J., 1985. The prediction of plasma levels of drugs following transdermal application. J. Control. Release 1, 177–182.

Guy, R.H., Hadgraft, J., 1986. Interpretation and prediction of the kinetics of transdermal drug delivery: oestradiol, hyoscine and timolol. Int. J. Pharm. 32, 159–163.

Illel, B., Schaefer, H., Wepierre, J., Doucet, O., 1991. Follicles play an important role in percutaneous absorption. J. Pharm. Sci. 80, 424–427.

Mayorga, P., Puisieux, F., Couarraze, G., 1996. Formulation study of a transdermal delivery system of primaquine. Int. J. Pharm. 132, 71–79.

McDaid, D.M., Deasy, P.B., 1996. Formulation development of a transdermal drug delivery system for amlodipine base. Int. J. Pharm. 133, 71–83.

Nodiff, E.A., Chatterjee, S. and Musallam, H.A., Antimalarial activity of the 8-aminoquinolines. In Ellis, G.P. and West, G.P. (Eds), Progress in Medicine Chemistry, Vol. 28, Elsevier, Amsterdam, 1991, pp. 1–40.

Ogiso, T., Iwaki, M., Jing-Ping, Z., Tanino, T., Noguchi, A., 1994. Percutaneous absorption of betahistine through rat skin and pharmacokinetic analysis of the plasma concentration. Int. J. Pharm. 102, 141–149.

Pirson, P., Steiger, R., Trouet, A., 1982. The disposition of free and liposomally encapsulated antimalarial primaquine in mice. Biochem. Pharmacol. 31, 3501–3507.

Roland, A., Rohr, U., Kreuter, J., 1994. Transdermal delivery of the tetrapeptide histeal (Melanotropin (6–9)) and amino acids: their contribution to the elucidation of the existence of an ‘Aqueous Pore’ pathway. Int. J. Pharm. 107, 23–28.

Shah, J.C., 1996. Application of kinetic model to in vitro percutaneous permeation of drugs. Int. J. Pharm. 133, 179–189.

Winstanley, P.A., Breckenridge, A.M., 1987. Currently important antimalarial drugs. Ann. Trop. Med. Parasitol. 81, 619–627.

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