Preliminary evaluation of primaquine activity on rodent malaria model after transdermal administration

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Summary:
The aim of this preliminary study was to investigate the potential use of the transdermal route for primaquine administration in the treatment of malaria. Thus, the activity of this drug on asexual blood forms of two rodent malaria parasites (P. v. petteri and P. y. nigeriensis) was evaluated following a single TTS patch application. Sustained plasma concentration values were observed for about 60 hours. The results obtained from a prepotency test showed that primaquine was more active towards P. v. petteri than P. y. nigeriensis. This preliminary study showed that the transdermal route for primaquine administration may be a promising strategy for improving the treatment of malaria in both causal prophylactic and prevention of relapses infection.

Key words: primaquine, antimalarial activity, rodent malaria, percutaneous absorption, transdermal drug delivery system.

INTRODUCTION

The goal of this work was the evaluation of the transdermal route of administration of a very active anti-malarial drug whose use is limited by its toxicological effects. Therefore, a Transdermal Therapeutic System (TTS) containing primaquine (PQ) was investigated.

PQ is almost the only drug active against both blood and hepatic (acute and chronic, i.e. hypnozoites) stages. It is also able to prevent relapses of P. vivax infection. At present, PQ does not seem to be subject to induced drug resistance in the same way as other blood schizontocidal drugs (Nodiff et al., 1991). Despite its activity, the use of PQ as a curative drug as well as a prophylactic agent is limited by its side effects, mainly at higher dosages to destroy asexual blood parasites during malaria attack. The most important of these side effects are the development of methaemoglobinaemia, a haemolytic anaemia, especially in people with G6PD deficiency precluding the use of PQ in this group, and gastrointestinal disturbances (Clyde, 1981). In addition, recent papers report effectiveness of PQ as a prophylactic drug against falciparum and vivax malaria (Baird, 1995; Fryauff, 1995; Weiss, 1995).

In order to evaluate the activity of this drug administered by the transdermal route, two rodent malaria models were used. Plasmodium yoelii nigeriensis and Plasmodium vinckei petteri were chosen because of their differences in chloroquine sensitivity. The results of activity on the asexual blood stage of the parasite are discussed with respect to the drug plasma profile. The preliminary results described below showed that the transdermal route for primaquine administration is promising, demonstrating high activity towards different rodent malaria strains associated with a steady drug plasma level.
MATERIALS AND METHODS

MATERIALS

Primquine diphosphate was purchased from Sigma Chimie (France) and primquine 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 (+) ß-tocopherol purchased from Sigma Chimie (France), ethyl cellulose polymer (Dow Chemical Company, Netherlands) with diethyl phthalate (Prolabo, France) as plasticizers and a pressure-sensitive adhesive acrylic resin, Durotak® 280 2287 (National Starch & Chemical, France). Ketoprofen was used as internal standard for 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. Two strains of rodent malaria parasites were chosen for this study, Plasmodium yoelii nigeriensis and Plasmodium vinckei petteri (strain 106 hW).

METHODS

Application of TTS device

TTS formulations were prepared as described by Mayorga et al. (1996). After the dorsal hair had been cut and shaved, taking care to avoid damage to the surface of the skin, the 1 cm² patch containing PQ (15 mg) was stuck on the back of each mouse. This represented the start point of experiment (time 0 h).

Determination of drug plasma concentration profile

A preliminary evaluation of primaquine plasma levels following transdermal administration was carried out. The complete time-course study was obtained with four groups of four mice, where each one group corresponds with a single point on the drug plasma concentration profile. Blood samples were collected from the intraorbital sinus vein at 12, 24, 48 and 72 hours after the 1 cm² patch application (15 mg of PQ). The plasma was separated by centrifugation (3,000 rpm, 10 min) and was kept in aluminium foil-covered Eppendorf tubes at - 20 °C until analysis.

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 one minute, the mixture was centrifuged at 10,000 rpm for 10 minutes. The supernatant was used for drug assay by high performance liquid chromatography. The analytical system (Waters 501) was equipped with an automatic sampler injector (Waters 712 WISP), a variable wavelength UV detector (Waters 484) and a reversed phase column (C8, 4 x 125 mm, particle size, 5 µm). The mobile phase of the HPLC system was composed of 7 mm monochloroacetic acid, 0.5 mm 1-decanosulfonic acid-acetonitrile-methanol (56:24:20, v/v) (Dean et al., 1994). With a flow rate 1.5 ml/min, peaks were detected by UV absorbance at 254 nm.

Infection of mice with Plasmodium parasites

Four groups of three animals receiving patches were infected by intraperitoneal inoculation of 10⁶ of either Plasmodium yoelii nigeriensis or Plasmodium vinckei petteri — infected mouse erythrocytes at different intervals after patch application. The first group received the parasite at the same time of patch (T0) and the three others at T24, T48 or T72 hours after patch application. Each group was compared with a control group which did not receive a TTS device for evaluation of the activity of primaquine after transdermal application. Afterwards, the parasitaemia was evaluated over one month by a prepatency test (Warhurst et al., 1968), i.e., number of days before parasitaemia reached 1 %. In order to evaluate a TTS containing a lower load of PQ (5 mg/cm²), a similar experiment was performed. In this case, the prepatency test was carried out after infection of mice at the same time as either 0.5 or 1 cm² patch application.

RESULTS

DETERMINATION OF DRUG PLASMA CONCENTRATION PROFILE

Plasma levels of PQ following single TTS patch application were determined in healthy mice 12, 24, 48 and 72 hours after the 1 cm² patch application (loaded at 15 mg/cm²). The effective dose of PQ was 4.17 mg (approximately 30 % of patch content). The plasma concentration profile is shown in Figure 1.

ACTIVITY OF HIGH DOSE TTS (15 MG/CM²) ON THE BLOOD STAGES OF RODENT MALARIA Plasmodium vinckei petteri

In this experiment, patch application took place at the time 0 hour and parasites were inoculated at 0, 24, 48 and 72 hours. No mice became infected during the 30-day follow-up. In control mice the parasitaemia reached 1 % after 24 hours.

Plasmodium yoelii nigeriensis

The same protocol as that described above was used in this experiment. In this case, only the group that received parasite inoculation at 72 hours after device
application developed the disease, with a long pre-patent period (21 days). The corresponding control group showed the same parasitaemia after only 24 hours.

ACTIVITY OF LOWER-LOAD TTS (5 mg/cm²) ON THE BLOOD STAGES OF RODENT MALARIA

In this experiment intraperitoneal inoculation of 10⁶ Plasmodium yoelii nigeriensis — infected mouse erythrocytes and patch application were done simultaneously, i.e. at 0 hour. For comparison of parasitaemia, a control group was also inoculated with the parasites. The control group reached a parasitaemia of 1% within 24 hours. Mice groups treated with a 0.5 and 1 cm² patch reached the same parasitaemia in 48 hours and 6 days, respectively.

DISCUSSION

From the plasma concentration data it was observed that the TTS device was able to deliver primaquine through the skin, maintaining a constant plasma concentration for about 60 hours. During this period, the sustained PQ concentration was around 300 ng/ml. This value is much higher than the therapeutic plasma level at the man (30 ng/ml) and probably too high in relation to toxic concentration. Moreover, this result shows that the administration route investigated seemed to be able to deliver PQ in a way which would allow prolonged activity. However, further optimization of TTS patch to achieve lower plasma levels will be necessary. In addition, it would be interesting to study the effect of parasitemia on the bioavailability of PQ after transdermal administration to obtain a more complete information about this route of administration in the rodent malaria model. In this preliminary study we compared the activity of primaquine delivered from a TTS device on two different rodent malaria strains. P. vinckei petteri is a very synchronous strain (Montalvo-Alvarez et al., 1988) and very sensitive to chloroquine (Cambie et al., 1991) producing very few latent merozoites. In contrast, P. yoelii nigeriensis is highly chloroquine-resistant (Peters and Robinson, 1987). It is an asynchronous strain and produces many latent merozoites which are chloroquine resistant.

Our results show that PQ is, like chloroquine, more effective on P. v. petteri than P. y. nigeriensis. However, its activity towards P. y. nigeriensis is relatively high. It is well assumed that side effects reduction of a drug can be obtained by decrease of plasma drug concentration. Thus, after verification that high plasma levels of PQ could be achieved after device application, we were interested in the evaluation of a lower-load TTS patch (5 mg/cm²) after inoculation of 10⁶ parasites of Plasmodium yoelii nigeriensis. The sensitivity of plasma drug assay did not permit the determination of the plasma drug profile following application of this lower-load device. However, from physico-chemical data obtained from in-vitro experiments (study under investigation) we observed that a dose reduction from 15 mg/cm² to 5 mg/cm² produces a high reduction of percutaneous flux of PQ (ten fold aproximately). Thus, we can expect a lower plasma concentration of PQ after lower-load patch administration. In addition, the prepatent period observed with a 1 cm² lower-load patch was also long, indicating that therapeutic levels of PQ had been obtained in the plasma.

In conclusion, it seems that this administration route is efficient for the elimination of intraerythrocytic stages but is not able to eliminate latent merozoites. Moreover, we have shown the potential use of the transdermal route for primaquine delivery in the treatment of asynchronous and chemioresistant rodent malaria strains by using a prolonged action controlled delivery device. Finally, we consider that further studies will be necessary to determine the activity of PQ patch on intrahepatic stages to evaluate the potential of this system for prophylaxis against malaria.

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REFERENCES


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