

## Anti-Relapse Efficacy of Transdermal Primaquine Against *Plasmodium Cynomolgi* B in Rhesus Monkeys

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**Abstract:** Transdermal administration of primaquine has been found to produce radical cure against sporozoite induced *Plasmodium cynomolgi* B infection in rhesus monkeys. Formulation containing 7mg/kg primaquine (base) was curative in 12 out of 13 rhesus monkeys as no relapse occurred during the observation period of 100 days.

**Key words:** *Plasmodium cynomolgi*, Anti-relapse (antimalarial) efficacy, Primaquine, Transdermal drug delivery.

### INTRODUCTION

Primaquine is the only 8-aminoquinoline drug in clinical use for treatment of relapses of *Plasmodium vivax* infections. The drug is usually administered for 7-14 consecutive days to achieve radical cure and is known to produce adverse side-effect including gastrointestinal distress, nausea and methaemoglobinemia with cyanosis (Clyde, 1981). Transdermal drug delivery as an alternative mean of drug administration has gained, in recent years, high patient acceptance in many clinical conditions and the advantages of delivering drugs across the skin for systemic circulation are well-documented (Price *et al.*, 1981; Laufer *et al.*, 1983; Karim, 1985; Shaw, 1983). This mode of drug administration can eliminate some of the side-effects associated with gastrointestinal absorption and also avoid deactivation of the drug by first-pass metabolism.

Recent studies on the use of transdermal route for drug delivery in malaria are limited to the preparations with blood schizontocidal agents like sodium artesunate and artemether (Xuan *et al.*, 1988, 1990), and artemisinic acid (Klayman *et al.*, 1991). Klayman *et al.*, (1991) recorded per cent curative and prophylactic efficacy with transdermal preparations of artemisinic acid against blood stages of *P. berghei* in mice. This is the first report on the successful use of transdermal preparation of tissue schizontocidal drug primaquine to achieve radical cure against sporozoite induced *P. cynomolgi* infection in rhesus monkeys.

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## MATERIALS AND METHODS

*Parasite:*

*Plasmodium cynomolgi bastianelli* strain has been used for the present study. The sporozoite induced infections of *P. cynomolgi* B in rhesus monkeys are being maintained routinely by cyclic transmission through *Anopheles stephensi* (Puri *et al.*, 1989).

*Animals:*

The rhesus monkeys (*Macaca mulatta*) were obtained from approved contractors and quarantined for one month before experimentation. The monkeys were fed on pellets, bread, seasonal fruits and water was provided *ad libitum*. During quarantine, monkeys were tuberculin tested and their blood smears were examined 2-3 times to ensure absence of any natural blood protozoan infections.

*Preparation of primaquine transdermal tapes*

Transdermal tapes were prepared from 1 gm of the matrix filled in molds of 25.6 mm diameter and 3 mm depth. The molds were prepared from polypropylene sheets by hot press technique using a die of appropriate size. All the molds so prepared had constant surface area (5 cm<sup>2</sup>) available for drug release. The matrix was prepared according to method of Jain *et al.* (1992) by dissolving 65.5% of polyvinyl pyrrolidone and 17% of polyethylene glycol in a mixture of 1:1 dichloromethane and ethyl alcohol by aid of slight heat followed by removing the solvent. To the residue, the required amount of primaquine diphosphate and 0.5% urea in 17% propylene glycol were homogenously mixed. Each mold is uniformly filled with 1 gm of this matrix and kept under vacuum for 2-3 hrs to remove entrapped air if any. On the surface of matrix in each mold, 0.1 gm of azone was uniformly spread. The matrix was subsequently allowed to cure for 24 hrs under vacuum at room temperature.

*Assay for anti-relapse activity:*

For efficacy tests, the monkeys were anaesthetized with 5 mg/kg ketamin hydrochloride (i.m.) and their upper arm was carefully shaved to avoid any cuts or abrasions on the skin. The bare skin was cleaned with alcohol before applying the matrix filled molds containing 1.75 mg base/kg to 7.00 mg base/kg primaquine. The molds were kept in contact with monkey's skin by wrapping three-four layers of adhesive tape around the arm. The molds were applied to the infected monkeys when parasitaemia level of approximately 5000/mm<sup>3</sup> was attained after sporozoite induced infections. These tapes were kept in place for 7 days. The anti-relapse activity of primaquine was also established via oral route in 4 monkeys which were administered 1 mg (base)/kg drug for 7 days.

The test protocol for anti relapse activity established at this institute was followed (Dutta and Puri, 1988). Chloroquine at a dose of 5 mg (base)/kg × 7 days was concurrently administered as the companion blood schizontocide to eliminate blood stage parasites. Giemsa stained blood smears from the experimental monkeys were observed to record the occurrence of relapse. If there was no relapse for 100 days after the last chloroquine dose, the monkeys were taken as cured (Dutta and Puri, 1988).

## RESULT AND DISCUSSION

Primaquine administered orally at 1 mg/kg  $\times$  7 days, has been found to produce radical cure against sporozoite induced *P. cynomolgi* B infection in 4/4 rhesus monkeys. This is in agreement with our earlier report (Dutta and Puri, 1988). Transdermal formulation of primaquine was evaluated for radical curative activity at 4 dose levels (1.75-7.00 mg base/kg) (Table-1). The tapes containing primaquine diphosphate equivalent to 1.75 mg base/kg were not curative in any of the four monkeys. The tapes containing drug equivalent to 3.50 mg/kg protected 1 out of 4 experimental monkeys. The tapes containing 5.25 mg base/kg primaquine protected 5 out of 7 monkeys (cure rate 71.43%), while the tapes containing 7 mg base/kg primaquine protected 12 out of 13 monkeys thus giving a cure rate of 92.3%. One of the 13 monkeys which was not protected with the highest dose, showed a delayed relapse on day 36. Four monkeys applied with placebo tapes (without primaquine), relapsed between day 11-19. The log-dose probit analysis of the present data gave the ED<sub>50</sub> and ED<sub>95</sub> values of 4.38 mg/kg and 6.65 mg/kg respectively.

The present study thus establishes that primaquine is active by transdermal route as an anti-relapse drug. Transdermal primaquine formulation is likely to enhance the dose compliance at it would eliminate the need of repeated drug intake and avoid gastric irritation.

Table 1. Anti-relapse activity of primaquine transdermal tape against sporozoite induced *Plasmodium cynomolgi* B infection in rhesus monkeys.

Primaquine*	No. of monkeys treated	Relapse if any		No. of monkeys** protected (%)
		No. of monkeys	Days after last dose of chloroquine	
0.0	4	4	11, 14, 14, 19	Nil (0%)
1.75	4	4	13, 15, 17, 18	Nil (0%)
3.50	4	3	17, 17, 19	1 (25%)
5.25	7	2	18, 22	5 (71.43%)
7.00	13	1	36	12 (92.3%)
Primaquine 1.00mg/kg $\times$ 7 days (oral)	4	—	—	4 (100%)

\* The tape was applied on monkey's fore arm for 7 days and chloroquine 5 mg base/kg  $\times$  7 days was administered orally.

\*\* No relapse observed till 100 days after last chloroquine dose.

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