IJPSR (2018), Volume 9, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 November, 2017; received in revised form, 06 June, 2018; accepted, 12 June, 2018; published 01 August, 2018

IN-VIVO STUDY OF ORODISPERSIBLE TABLET OF PRIMAQUINE

Pankaj Sharma [*1] and Mukul Tailang [2]

Department of Pharmaceutics [1], Shri Ram College of Pharmacy, Morena - 476444, Madhya Pradesh, India. Department of Pharmacognosy [2], S.O.S. Pharmaceutical Science, Jiwaji University Gwalior - 474011, Madhya Pradesh, India.

Keywords:

Orodispersible, HPLC, bioavailability, primaquine

Correspondence to Author:
Mr. Pankaj Sharma

Assistant Professor,
Department of Pharmaceutics,
Shri Ram College of Pharmacy,
Morena - 476444, Madhya Pradesh, India.

E-mail: pankajsharma223@gmail.com

ABSTRACT: The main objective of the study is to evaluate orodispersible tablets of primaquine with different *in-vivo* parameters. Sharma P *et al*., (2015), *in-vitro* study was based on various evaluation parameters of orodispersible tablets of primaquine. From their study results, we have selected formula-6 of orodispersible tablets of primaquine for *in-vivo* study. Taste masking evaluation was performed on five male Swiss Albino rats (200-250 g). The *in-vivo* bioavailability study of drug were performed on six male white New Zealand rabbits (weighing 2-2.5 kg) and rabbits were randomly divided into two groups of equal size using parallel design. Next step of the study was HPLC analysis, in this study two conditions were followed by us. The conditions were- (a) Sample preparation: Protein precipitation method selected for extraction of drug from plasma because it was very simple and cost effective method for primaquine extraction. (b) Chromatographic conditions: HPLC-DAD-UV (Shimadzu Class-VP Japan) was used for evaluation of chromatographic conditions. With the help of UV and HPLC, marketed preparation (Primacip film coated tablet) and primaquine ODTs (test sample formulation) were compared for pharmacokinetic parameters.

INTRODUCTION: Malaria is a common and life-threatening disease in many tropical and subtropical areas [1]. The malaria kills thousands of people in the world and the majority of whom are young children. This death toll exceeds the mortality rate from AIDS (acquired immuno-deficiency syndrome) [2]. Around the world, the malaria situation is serious and getting worse [3]. *Plasmodium vivax* is an important cause of malaria outside Sub-Saharan Africa.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(8).3506-10

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(8).3506-10

The WHO estimates that *P. vivax* comprises 41% of the malaria burden outside of Africa. This translates into 6-11 million cases/year with an estimated 1800-4900 deaths. India, Indonesia and Pakistan account for just over 80% of the global vivax malaria case burden [4]. Relapse frequencies vary by geographical region. South East Asia and Oceania have the highest incidence with relapse rates exceeding 50% [5]. In this context relapse from liver hypnozoites is the main cause of *P. vivax* malaria illness and asymptomatic carriage [6].

The only currently available treatment to eliminate liver hypnozoites and thus prevent future relapses ('radical cure') of vivax or ovale malaria is primaquine, a rapidly eliminated 8-aminoquinoline. Radical curative efficacy depends on the total dose administered [7].

Treatment courses of 14 days are recommended by the WHO but the effectiveness of unsupervised primaquine is often poor [8, 9]. Primaquine use for the treatment malaria as a causal prophylactic on the primary exo-erythrocytic stage and it shows gametocytocidal activity. The primaquine hypno-zoitocidal activity depends on the total dose administered in the body than on the length of the treatment. For the treatment of *P. vivax,* the dosage is 30 mg daily for 14 days, while for *P. ovale*, the dosage is 15 mg daily for 14 days. The need of convenient and acceptable dosage form which provide easy administration, increase patient compliance and increase market size for drug companies and drug uses, coupled with low cost of disease management. The orodispersible tablet has been developed which fulfil the need of drug dosing such as ease of dosing and convenience of dosing in the absence of water or fluid.

Fast dissolving drug formulations have unique properties and advantages like rapid disintegration and dissolution in the oral cavity without the need for water, accurate dosing, rapid onset of action, pleasant taste and improved patient compliance, especially for paediatrics and geriatrics [10]. The primary need of research was, to avoid G.I. disturbances and undesirable side effects by orodispersible tablets of primaquine, taste masking of drug, achieve quick onset of action, to increase patient compliance and increase bioavailability of primaquine [11]. In this article, a conceptual diagram **Fig. 1** of orodispersible tablets shows the disintegration mechanism. An orodispersible tablet may disintegrate by three mechanisms, these mechanisms are wicking, swelling and capillary action. Generally, disintegration mechanism depends upon types of disintegrating agents.

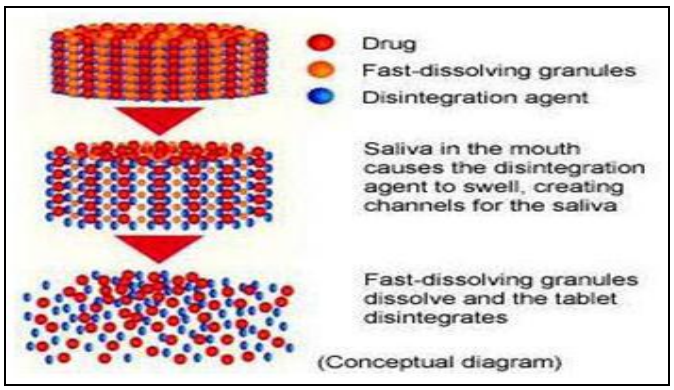

FIG. 1: CONCEPTUAL DIAGRAM OF ORODISPERSIBLE TABLET [12]

MATERIALS AND METHODS:

Materials: Acetonitrile and methanol (HPLC grade) were provided by Shri Ram College of pharmacy, Banmore, Morena. All other chemicals were of analytical grade. DRDE Gwalior provided Swiss albino rats and New Zealand Wistar rabbits for *in-vivo* study. The *in-vivo* study was conducted in accordance with Animal Ethical Guidelines for investigations in laboratory. The Animal Ethics Committee (registration no.: 891/PO/Re/S/05/ CPCSEA) of Shri Ram College of pharmacy, Banmore, Morena M.P., approved animals and the study protocol.

***In-vivo* Study:** Sharma P *et al*., (2015), *in-vitro* study was based on various evaluation parameters of orodispersible tablets of primaquine [11]. From their study results, we have selected formula-6 of orodispersible tablets of primaquine for *in-vivo* study.

***In-vivo* Evaluation of Taste Masking in Rats:** Taste masking evaluation was performed on twenty (4 × 5) male Swiss Albino rats (200 - 250 g). Rats were kept in standard conditions of temperature (22 ± 2ºC), relative humidity (60 ± 5%) and light (12 h of light-dark cycles). Rats were administered standard feed from Shri Ram College of pharmacy animal house (Morena, India). According to the experimental protocol, all twenty (4 × 5) rats were first allowed to drink water, then after permitted to test substances at fixed time intervals and the licking frequency was counted as compared to water.

***In-vivo* Bioavailability Study:** According to study protocol, six male white New Zealand rabbits (weighing 2-2.5 kg) were used in the study and were randomly divided into two groups of equal size using parallel design. The experimental part was performed in accordance with ethical procedures and policies approved by Ethics Committee of Shri Ram College of pharmacy, Banmore, Morena M.P. The work was carried out to compare the pharmacokinetics of primaquine from selected ODTs (F6) containing 15 mg primaquine to a commercially available Primacip tablet (15 mg primaquine, Cipla Ltd.). Food was taken off 10 ± 1 h prior the *in-vivo* study with water *ad libitum*, and no food was allowed 4 h after dosing.

Rabbits of group A were orally administered with primacip tablet, whereas those of group B were administered with our prepared primaquine orodispersible tablet (F6). For rabbits of group B, the orodispersible was placed between the jaws of tweezers and inserted onto the oral cavity and was held by the tweezers until it dissolved. A small incision in the marginal ear vein was made and blood samples (2 ml) were collected into heparinised tubes at the following time points: 0 (pre-dose), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h after administration of the treatment. Plasma was separated from the heparinised whole blood by centrifugation at 4,000 rpm for 15 min. After separation, plasma samples were immediately transferred to Eppendorf tubes and stored at -20 °C until analyses, which were carried out within 3 days of primaquine administration.

Plasma concentration of drug was determined by HPLC analysis and following steps were used for HPLC analysis-

(a) Sample Preparation (Primaquine Extraction from Plasma): Protein precipitation method was very simple and cost effective for primaquine extraction. Protein precipitation conditions were empirically optimized and standardized as follows. Samples of 50 µL of plasma were transferred to 1.5-mL Eppendorf tube into which 50 µL of acetonitrile acidified with 2% acetic acid (w/v) was added. The tubes containing plasma plus acetonitrile were carefully vortexed, avoiding any contact of the mixture with the tube tip, for 30 sec.

Then, 25 µL of an aqueous 12.5% zinc sulfate solution (w/v) was added to each tube, followed by vortexing for 30 sec. The suspension was then allowed to stand for 30 min for full precipitation of the plasma protein. The tubes were then centrifuged at 4,000 rpm for 15 min and the supernatant was analyzed by HPLC-DAD-UV.

(b) Chromatographic Conditions: Analyses were performed by HPLC-DAD-UV using the Shimadzu Class-VP Japan (liquid chromatographer coupled to a Shimadzu UV detector with the diode array SPD M10A VP equipped with a SCL 10A VP controller, DGU14A degasser, 10ADVP LC binary pump, CTO 10ASVP oven, and SIL10AF auto injector). The chromatograms were evaluated using Shimadzu software.

Various combinations of acetonitrile, methanol, and ammonium acetate buffer were used as mobile phases. All solution were filtered through a 0.45-µm pore PVDF filter (Merck-Millipore) before use. Primaquine was analyzed using a HPLC silica-based C18 column (250 mm × 4.6 mm i.d. × 5 µm,) and modified-silica cyanopropyl (250 mm × 4.6 mm i.d. × 5 µm,). The injection volume was 20µL for all analyses. The mobile phase consisted of 50 mM ammonium acetate adjusted to pH 3.5 with glacial acetic acid and acetonitrile (35:65, v/v). The analysis was run at a flow rate of 1.0 mL/min, the detector was set at a wavelength of 259 nm and the injection volume was 20 µL. The calibration curve exhibited an excellent linearity curve over the concentration range of 25 - 1000 ng/mL of primaquin with a correlation coefficient of 0.9999. The pharmacokinetic parameters, namely, maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were obtained directly from the data. The area under the plasma concentration-time curve from zero to the last measurable concentration (AUC_{0-t}) was calculated by the trapezoidal rule. $AUC_{0-\infty}$ was the summation of area under plasma concentration-time curve from zero to time t (AUC_{0-t}) and area under plasma concentration-time curve from time t to infinity ($AUC_{t-\infty}$). $AUC_{t-\infty}$ was calculated by dividing the last measurable plasma concentration with the terminal elimination rate constant (Ke). The value of Ke was calculated using the least-squares regression analysis of the terminal portion of the log plasma concentration vs time curve. The elimination half-life ($t_{1/2}$) was calculated by dividing 0.693 with Ke.

RESULTS AND DISCUSSION:

Taste Masking Evaluation: In this evaluation, we have checked the taste masking effect of ODTs. During this evaluation, the licking frequency of water was considered 100%. A higher licking response up to 86% was found for drug solution of concentration 1 mg/ml. It indicates that the drug solution did not show very bad taste. About 55% licking response was found for drug solutions of concentration 5 mg/ml. When we take 10 mg/ml drug, solution then it exhibited less licking response of about 35%. Licking response variation occurs due to loss of entrapment of drug from β-cyclodextrine and it depends upon concentration of drug-β-cyclodextrine complex.

When taste masked orodispersible tablets permitted to the rats, then it show high licking frequency of up to 74%. This result indicates that the taste masking methods were effective with drug-β-cyclodextrine complex. Licking responses of rats are given in **Table 1**.

TABLE 1: PERCENTAGE OF LICKING FREQUENCY OF RATS

Groups	Conc.	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Average	Standard deviation
1	1 mg/ml	85.42	83.66	89.31	86.56	87.34	86.45	±2.11
2	5mg/ml	53.56	58.91	54.76	54.67	54.98	55.37	±2.05
3	10 mg/ml	31.94	33.46	38.01	33.43	37.85	34.93	±2.80
4	ODTs	72.51	74.01	76.88	79.90	66.21	73.90	±5.14

***In-vivo* Study:** The plasma drug concentration time profiles following oral administration of the commercial product (Primacip tablets) and test formulation (Primaquine ODTs) are depicted in **Fig. 2**.

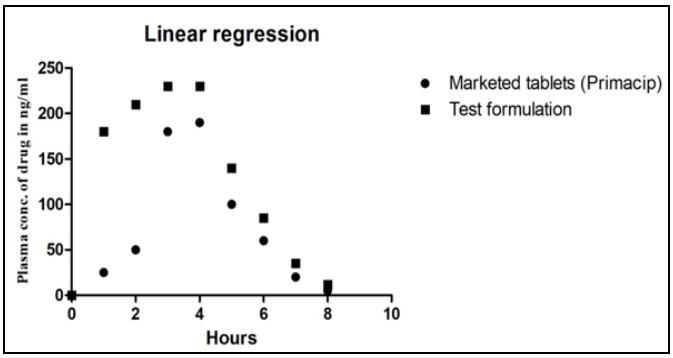

FIG. 2: MEAN PLASMA PRIMAQUINE CONCENTRATION PROFILES AFTER ORAL ADMINISTRATION OF REFERENCE PRODUCT (PRIMACIP 15 mg TABLET®) AND TEST FORMULATION (PRIMAQUINE ODTS ACCORDING TO FORMULA) IN RABBITS

TABLE 2: PLASMA DRUG CONCENTRATION (CMAX) AND MEC ACHIEVEMENT TIME COMPARISON BETWEEN MARKETED PREPARATIONS (PRIMACIP TABLET) AND PRIMAQUINE ODTS (TEST SAMPLE)

Time in hours	Primacip tablets (Plasma drug conc. In ng/ml)	Primaquine ODTs (Plasma drug conc. In ng/ml)
1.0	25	180
2.0	50	210
3.0	180	230
4.0	190	230
5.0	100	140
6.0	60	85
7.0	20	35
8.0	05	12

The data obtained for plasma drug concentration (C_{max}) and MEC achievement time (T_{max}) from UV spectrophotometer Shimadzu. Pharmacokinetic parameters C_{max} 190 ng/ml and 230 ng/ml respectively were achieved for Primacip tablets and Primaquine ODTs. C_{max} differences in both formulation due to changes in bioavailability. Bioavailability was found to be higher for primaquine ODTs comparison to Primacip tablets. Bioavailability of both formulation was different because delay in Primacip tablet release in stomach due to film coating of tablets. Primaquine ODTs achieve MEC level of drug 180 ng/ml within one hour because it release drug within a minute while marketed tablet take time to release drug. C_{max} was decreasing after four hours of administration of both tablets, because $t_{1/2}$ of primaquine was 3 - 6 h.

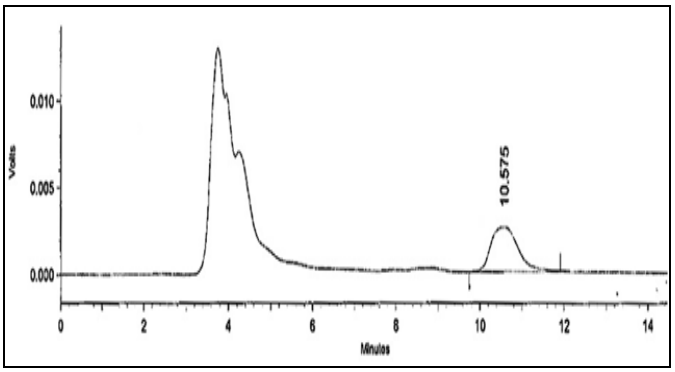

FIG. 3: HPLC CHROMATOGRAM OF RABBIT PLASMA SPIKED WITH PRIMAQUINE

The HPLC chromatogram of rabbit plasma spiked with Primaquine as shown in **Fig. 3**. The mean plasma concentration–time profiles of primaquine following administration of single dose of ODTs (15 mg) to three rabbits (G1) and a single oral dose of the reference marketed tablet (15 mg, Primacip) to three rabbits (G2). The pharmacokinetic data are characterised in **Table 2**. After administration of ODTs, primaquine was quickly absorbed and reached a C_{max} of 180 ng/ml at a T_{max} of 10 min. After the oral administration of primacip tablet, primaquine reached a C_{max} of 200 ng/ml at a T_{max} of 30 min.

CONCLUSION: The formulation of orodispersible tablet was made by using primaquine and β-cyclodextrine complex. Formula F6 were prepared by direct compression to select the disintegrant, from the results. It can be concluded that the tablets containing crospovidone (F6) exhibit quick

disintegration time and followed by tablets containing sodium starch glycolate. Based on *in-vitro* results the F6 formula was selected for *in-vivo* study. The *in-vivo* study showed that the T_{max} of ODTs was significantly shorter than that of the reference with an enhancement of relative bioavailability. Based on data interpretation, it could be concluded that ODTs are a promising delivery system for primaquine rapid absorption of the drug through the oral mucosa. ODTs of primaquine can be considered a pharmaceutical alternative for conventional tablets. For this study, clinical trials should be next level for development ODTs.

ACKNOWLEDGEMENT: Authors are thankful to Shri Ram College of Pharmacy for providing laboratory facilities to conduct research work and thankful to DRDE Gwalior to provide animals to conduct *in-vivo* study.

CONFLICT OF INTEREST: Authors declare no conflict of interest.

REFERENCES:

1. World malaria report 2014 by World Health Organization. Available from: http://www.who.int/malaria.pdf. Accessed May18, 2016.
2. Bloland PB: Drug resistance in malaria. Available from: http://www.who.int/csr/resources/publications/drugresist/malaria.pdf. Accessed July 16, 2014.
3. Promoting rational use of drugs and correct case management in basic health services published by UNICEF's program division in cooperation with the World Health organization, 2000. Available from: http://www.unicef.org./presciber/eng-p18.pdf. Accessed May 28, 2016.
4. Howes RE *et al*.: Global Epidemiology of Plasmodium vivax. Am J Trop Med Hyg. The American Society of Tropical Medicine and Hygiene 2016; 95(6): 15-34.
5. Betuela I *et al*.: Relapses contribute significantly to the risk of *Plasmodium vivax* infection and disease in Papua New Guinean Children 1-5 Years of Age. J Infect Dis. 2012; 206(11): 1771-1780.
6. White NJ: Determinants of relapse periodicity in *Plasmodium vivax* malaria. Malar J. BioMed Central 2011; 10(1): 297.
7. John GK *et al*.: Primaquine radical cure of *Plasmodium vivax*: a critical review of the literature. Malar J. BioMed Central 2012; 11(1): 280.
8. Leslie T *et al*.: Compliance with 14-day primaquine therapy for radical cure of vivax malaria-a randomized placebo-controlled trial comparing unsupervised with supervised treatment. Trans R Soc Trop Med Hyg. 2004; 98(1): 168-273.
9. Douglas NM *et al*.: Unsupervised primaquine for the treatment of *Plasmodium vivax* malaria relapses in southern Papua: A hospital-based cohort study. Garner P, editor. PLOS Med. Public Library of Science 2017; 14: e1002379.
10. Heer DA, Geeta A and Kumar SH: Recent trends of fast dissolving drug delivery system - an overview of formulation technology. Journal of Pharmacophore 2013; 4(1): 1-9.
11. Sharma P *et al*.: To formulate and evaluate orodispersible tablets of primaquine. Indo American J. of Pharm. Research 2015; 5(5): 1625-1632.
12. Yadav G, Kapoor A and Bhargava S: Fast dissolving tablets recent advantages. Int. J. of pharmaceutical sciences and research 2012; 3(3): 728-736.

How to cite this article:
Sharma P and Tailang M: *In-vivo* study of orodispersible tablet of primaquine. Int J Pharm Sci & Res 2018; 9(8): 3506-10. doi: 10.13040/IJPSR.0975-8232.9(8).3506-10.