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Piperaquine phosphate: Reproduction studies

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ABSTRACT

In embryofetal studies in rat and rabbit Piperaquine phosphate (PQP) was not teratogenic at the maximal tolerated dose, even in presence of fetal exposure.

In peri- post-natal study in rat, PQP did not interfere with the course of delivery at the dose of 5 mg/kg/day (treatment Gestation Day(GD)6-Lactation Day(LD)21) as well as up to the dose of 20 mg/kg/day (treatment GD6–17 and LD1–21). PQP at the dose of 80 mg/kg, induced prolonged gestation, dystocic delivery and increase perinatal mortality both with interruption of treatment (GD6 to GD17 and LD1–21) and with continuous dosing (GD19-LD21).

PQP did not interfere with lactation and pup growth and development, in presence of clear exposure during suckling period, irrespective of the dose and treatment schedules.

It was not possible to identify the mechanism leading to the delivery delay. In a comparative study using other antimalarials, only Mefloquine gave similar findings to PQP.

1. Introduction

The treatment of pregnant women who have contracted malaria is an important public health concern in endemic countries where it is estimated that up to 125 million pregnancies occur every year. Having malaria during pregnancy can have serious consequences for both mother and child, significantly increasing the risk of maternal anemia, stillbirth, low birth weight and neonatal death. According to the World Health Organization (WHO), around 10,000 women and 200,000 infants worldwide die every year as a result of malaria during pregnancy.

Primigravidae and secundigravidae women are most susceptible to the maternal and fetal effects of malaria in pregnancy, especially in regions with stable transmission.

Despite the evident and pressing need for more safe and effective antimalarial drugs for use in pregnancy very few drugs have been formally tested for their safety.

The target of Millennium Development Goal 6 is to end malaria deaths by 2015. Maternal and perinatal morbidity and mortality due to malaria may be reduced by implementing preventive measures, early diagnosis of suspected cases, effective antimalarial therapy and treatment of complications.

The fixed dose combination of Dihydroartemisinin (DHA) + Piperaquine phosphate (PQP) was developed by the Guangzhou University of Traditional Medicine in Gaungzhou China. The drug underwent numerous clinical trials in China and South East Asia and was registered for use in China and other countries. Using the basis established by clinical use in China, Vietnam, Cambodia and Thailand, the fixed dose combination of DHA + PQP was developed for registration by a stringent regulatory authority under the partnership of Medicines for Malaria Venture (MMV) in Geneva, Switzerland, and Sigma-tau in Rome, Italy. The development to these standards is needed to assure the international funding agencies of the quality of the drug.

In the course of drug development to the standards set forth in by the ICH guidelines a recommendation is given to explore the possible human developmental or reproductive risks associated with drugs. To achieve this, a series of systematic experiments have been defined and executed.

The partnership MMV and Sigma-tau completed the development and registration of Eurartesim®, a fixed dose PQP (320 mg) + DHA (40 mg) combination. The drug has received European Medicines Agency Authorisation in October 2011 and it has been launched in June 2012. As such it is one of the few antimalarial drugs examined in depth by a stringent regulatory authority.

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Eurartesim® is highly effective against *P. falciparum* malaria in adults and children, has a simple dosing regimen (three administrations over 3 days) and has significantly longer protection against new infections as compared to other antimalarials. Developed to high international standards, Eurartesim® meets WHO clinical treatment recommendations as it combines two active antimalarial ingredients in a single tablet: the highly potent artemisinin-derivative (DHA) with a second antimalarial (PQP) which protects the first one against the emergence of resistance [1,2].

In the course of the development a complete set of Development and Reproduction Toxicity studies have been taken into consideration.

Fertility studies were not performed with PQP or DHA since data produced in 4-week toxicity studies with the DHA + PQP combination did not reveal any significant abnormalities in the reproductive tract in rats or in dogs (EPAR, EMEA/H/C/001199).

The effect of DHA (and other members of artemisinin family) in animal pregnancy is well known with induction of embryolethality and developmental abnormalities [3–5]. Despite this, no evidence of adverse effects on pregnancy in humans have been observed [6–12].

There is scant literature available on the embryofetal developmental toxicity of PQP.

An evaluation of PQP for reproduction was performed. PQP submitted to a classical battery of regulatory tests did not show any genotoxic or clastogenic potential (EPAR, EMEA/H/C/001199). It was demonstrated that Piperaquine (PQ) accumulates in most tissues and organs, including the uterus, the most exposed organ being the adrenals (Table 1). Data on the distribution of PQ in pregnant rats and fetus (Day 12th and 18th of pregnancy) was obtained by autoradioluminography. Radioactivity was detected in the whole uterus and in the fetus where it was mainly concentrated in the liver. This demonstrates placental transfer and consequently fetal exposure after PQP administration to the dams. Moreover, evaluation of excretion in the milk during lactation on Day 4th post-partum indicated a rapid and extensive distribution of total radioactivity in milk (EPAR, EMEA/H/C/001199).

All these data bring the proof that rat embryos/fetuses were exposed in utero to PQ as well as the suckling pups.

Therefore, a full package of embryofetal developmental toxicity studies and pre- and post-natal developmental toxicity studies were conducted according to ICH S5A compliant protocols [13]. In addition, studies were performed to address specific issues that arose from conventional studies.

2. Materials and methods

Sprague-Dawley Crl:CD(SD)BR rats and New Zealand White rabbits were used. Animals were maintained under standard management and animal care conditions and the procedures adopted were in strict compliance with international guidelines for Laboratory Animal Welfare.

Rats and rabbits were maintained in stainless steel or polycarbonate cages in air-conditioned animal house accommodations regulated in accordance with international guidelines for temperature, relative humidity, air exchange rate, and lighting. Fresh drinking water and specific meals or pelletted commercial diet [GLP 4RF25 Mucedola for rats; Altromin CS4 Rieper for rabbits] were supplied ad libitum.

The rat was the species selected to evaluate the effects of PQP on the entire embryofetal development up to and including the postnatal evaluation of offspring and subsequent investigative studies. The rabbit was used, in addition to the rat, in the assessment of the embryotoxic and teratogenic potential of PQP, as it is the conventional nonrodent species in developmental toxicity studies.

In all studies the day of confirmation of spermatozoa in the vaginal smear of rats or the day of copulation for rabbits were designated as Gestation Day (GD) 0.

The dose levels, treatment period and number of animals per group are reported in Tables 2 and 3. Blood concentrations of PQ were determined in most of these studies.

Studies were carried out at a certified GLP facility. The Principles of Good Laboratory Practice are accepted by the Regulatory Authorities of United States of America and Japan on the basis of intergovernmental agreements.

The compound was administered orally by daily gavage, in a single dose, as a suspension prepared in 1%Tween® 80 in 0.5% Methylcellulose 400 cP solution in water for injection. 1%Tween® 80 in 0.5% Methylcellulose 400 cP solution in water for injection was used for control administration.

Doses of the test compound over the studies were expressed in mg/kg/day of PQP.

The intended clinical use of Eurartesim® in patient (adults and children weighing 36–75 kg) is 3 tablets of 320 mg PQP + 40 mg DHA in a single administration, for 3 consecutive days. Therefore, the daily dose of Eurartesim® is about 20 mg/kg/day. This corresponds to 17.7 mg/kg/day of PQP.

The maximal possible dose level of PQP used in pivotal studies was 80 mg/kg/day, corresponding to a Human Equivalent Dose (HED) of about 13 and 26 mg/kg/day in rat and rabbit, respectively [14]. In addition, area under the plasma concentration vs. time curve up to the concentration at 24 h (AUC_{0-24}) in patients (third day of treatment) is very similar to those recorded in rats treated at 80 mg/kg, thus the safety ratio on a daily basis is rather limited (Table 4).

2.1. Embryofetal developmental studies

The studies performed are shown in Table 2. Studies in rat are indicated as Study A (preliminary study – Teratology Subgroup),

Table 1
C_{max} (ng/mL) and $AUC_{(0-168)}$ (ng h/mL) tissue to plasma (T/P) ratio as total radioactivity following a single oral administration of [^{14}C]-Eurartesim® at the dose level of 90 mg/kg ([^{14}C]-PQP 80 mg/kg and DHA 10 mg/kg) to male and non pregnant female albino Sprague–Dawley rats.

Organ/tissue	Male		Female	
	C_{max}, T/P ratio	AUC T/P ratio	C_{max}, T/P ratio	AUC T/P ratio
Prostate/ovaries	110.3	196.7	1003.5	1829.8
Testes/uterus	107.5	240.8	257.8	488.1
Pituitary	602.5	791.9	450.8	751.2
Adrenal glands	1840.3	4622.2	2949.8	4828.0
Bone marrow	408.8	785.2	492.3	791.2
Bone	84.5	199.3	84.5	149.1
Lachrymal glands	675.3	1556.6	654.8	1198.8
Liver	360.3	644.8	363.8	691.2
Lungs	544.8	964.0	564.5	1112.9
Spleen	792.8	2245.2	1538.5	2934.7