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Interspecies comparison of the pharmacokinetics and oral bioavailability of 99-357, a potent synthetic trioxane antimalarial compound☆

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ABSTRACT

The pharmacokinetic data obtained in lower animals is of considerable importance in drug discovery and development. The objective of the present study was to generate *in vitro* and *in vivo* preclinical pharmacokinetic data of 99-357, a synthetic trioxane antimalarial, in rats and rabbits and to scale-up the data in order to apply for further studies. The pharmacokinetic profile of 99-357 was investigated after both intravenous and oral dose in rats and rabbits. Oral studies were carried out at three dose levels 6, 12 and 24 mg/kg in rats while in rabbit only one dose level was selected. Both compartmental and non-compartmental approaches were used to calculate the pharmacokinetic parameters following intravenous and oral doses in both the species. The clearance in rat and rabbit was 45–57% and 60–67% respectively of hepatic blood flow. The plasma protein binding in rats was ~75%. *In vitro* studies showed high RBC partitioning and low to moderate hepatic clearance. Linearity was observed in terms of dose and AUCs suggesting linear pharmacokinetics at the dose levels studied in rats. The oral bioavailability of compound 99-357 in rat and rabbit at 12 mg/kg dose level was comparable and 39% and 41% respectively.

1. Introduction

Malaria is one of the most severe and devastating health problems worldwide. It is a leading cause of death in many developing countries where young children and pregnant women are among most affected groups. In addition, pregnant mothers exposed to malarial infection suffer increased risk of severe anemia (White, 1996; Raynes, 1999; WHO fact sheet, 1998). Malaria kills over a million each year and some 3.2 billion people living in 107 countries/territories are at risk. But malaria is a curable and preventable disease (Winstanley et al., 2002; WHO malaria report, 2005).

Incidence of parasite resistance to the existing front line chemotherapy against malaria has reached alarming stages over the past few years in Africa and Southeast Asia. This has necessitated the quest for novel drugs to combat and control malaria. In this context, World Health Organization (WHO) has recommended artemisinin class of compounds as well as its combination as promising novel antimalarials for further clinical development. Artemisinin and its derivatives have become essential components of antimalarial treatment, used in combination with long acting antimalarial drugs (artemisinin based combination therapy, ACT) to manage drug resistance, recrudescence, and non-compliance. Newer synthetic and semi-synthetic artemisinin like class of compounds are being developed (Roberts, 2001; Greenwood and Mutabingwa, 2002; Ridely, 2002). The rapid onset of artemisinin derivative makes them especially effective against severe malaria (Gupta et al., 2002; Gordi et al., 2002). The artemisinin and artemisinin like compounds are representative of a group of compound, which can be used in the multi-drug resistant *Plasmodium falciparum* malaria especially for cerebral malaria. However, the existing artemisinin class of the drug had to be improved regarding efficacy, neurotoxicity, stability and pharmacokinetic behaviors. Many synthetic antimalarials having peroxides bridge have been prepared but most suffers from low oral activity and toxicity, a defect shared in part by semi-synthetic artemisinins. Therefore, a need exists to identify novel peroxide antimalarial agents with high oral activity, devoid of neurotoxicity and moreover affordable (Sinclair et al., 2009; Li et al., 1998; Genovese and Newman, 2008).

In the quest to develop compounds with improved oral bioavailability, low toxicity and moreover affordable for the treatment of complicated and severe malaria, Central Drug Research Institute (CDRI), Lucknow, India, developed a promising antimalarial compound, 99-357 (Fig. 1) in its drug discovery program (Singh et al., 2002, 2003, 2004a,b, 2005a,b, 2006). 99-357 is a synthetic triox-

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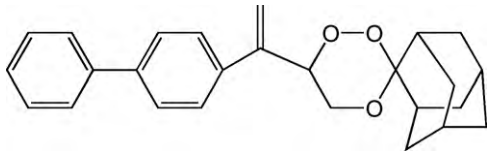

Fig. 1. Structure of 99-357.

ane artemisinin like class of compound. The antimalarial activity of 99-357 is a result of the peroxide bridge in the trioxane nucleus. The aim of the present study was to assess the *in vitro* and *in vivo* preclinical pharmacokinetics of 99-357 in rats and compare with *in vivo* profile in rabbits. The *in vitro* studies were performed to characterize the intestinal permeability, metabolic stability and whole blood uptake of 99-357.

2. Materials and methods

2.1. Chemicals and reagents

Pure reference standards of 99-357 were obtained from the medicinal chemistry division, CDRI, Lucknow, India. Dextran coated charcoal was obtained from Sigma Chemicals, USA. Dulbecco's phosphate buffered saline (DPBS) (Ca^{2+} and Mg^{2+} free) was purchased from Hi Media Laboratories Pvt. Ltd., Mumbai. Orthophosphoric acid was purchased from E-Merck Ltd., Mumbai, India. Dimethyl formamide (DMF), Na_2HPO_4, KH_2PO_4 and NaCl were procured from s.d.-Fine Chemicals, Mumbai. D-Glucose-6-phosphate mono sodium salt (G-6-P), glucose-6-phosphate dehydrogenase type XV from baker's yeast (G-6-PDH) and β-nicotinamide adeninine dinucleotide phosphate sodium salt (β-NADP) were obtained from Sigma Chemicals (St. Louis, MO, USA). Other chemicals and reagents used were of analytical grade or high performance liquid chromatographic grade. Plasma was obtained from drug free male rats (*Sprague–Dawley*) and male *New Zealand* rabbit, which were procured from the Laboratory Animal Services Division, CDRI.

2.2. In vivo experiments

2.2.1. Animals

Male *Sprague–Dawley* rats and male *New Zealand* rabbits were procured and housed at the Laboratory Animal Services Division of the institute. Rats, 7–8 weeks old (225–250 g) and rabbit (2–2.5 kg) were housed in well ventilated cages at room temperature (24 ± 2 °C) and 40–60% relative humidity. Animals were placed on a regular 12 h light–dark lighting cycle for a minimum period of 3 days prior to the experiment. Animals in the oral dose group were fasted for 8–12 h before dosing, but allowed free access to water. Prior to studies, the approval from the Local Animal Ethics Committee (Reg. No. 34/1999/CPCSEA) was sought for maintenance and experimental studies with animals. All experiments, euthanasia and disposal of carcass were executed in accordance with the guidelines laid out by the local ethical committee for animal experimentation.

2.2.2. Formulations

All the formulations used for the study (oral and intravenous) were subjected to quality control (QC) checks to ensure strength, content uniformity of 99-357. Both intravenous and oral formulation were a clear solution of 99-357 in DMF and propylene glycol at a ratio of 40:60 (% v/v). The intravenous formulation was sterilized by filtration before use. For intravenous treatment, single bolus doses were injected into the tail vein (caudal vein) of rats and into marginal ear vein of rabbits (12 mg/kg) (Waynforth, 1980). The volume administered was less than 1 ml/kg. At all times during the study, pain to the animals was minimized by the use of anesthesia.

Oral formulations were administered at three dose levels (6, 12 and 24 mg/kg) to rats and at one dose (12 mg/kg) level to rabbit. The oral formulation was administered using a 20 G gavage needle in rats and using an orogastric catheter in rabbits. The tubing was flushed with drug free vehicle after the dose administration to ensure accurate and complete dose delivery. Food was provided after 1–1.5 h post-dose to rats and rabbit in the oral study group.

2.2.3. Sampling procedures

The composite sparse sampling approach in rats and serial sampling approach in rabbit was used for the present pharmacokinetic studies. Blood samples were collected from three different rats and rabbits for each time point ($N = 3$). Two samples were collected from each rat in the study group, first by cardiac puncture followed by terminal sampling from inferior vena cava. Samples were collected through marginal ear vein in rabbits. The volume of blood drawn per day from a rat was less than 0.5% of its body weight. Samples were collected at 5, 15, 30, 45, 60, 90 min, 2, 3, 4, 5, 6, 8, 10, 12, 24 and 36 h post-intravenous dose in both rats and rabbit, while for oral administration, sampling was done up to 48 h in rats and up to 72 h in rabbits. Plasma was separated by centrifugation at 2000 × *g* for 5 min and stored at −60 °C till analysis. Analyses were performed within 30 days of sample collection.

2.3. Sample analysis

A two-step liquid–liquid extraction method for 99-357 in rats and rabbits plasma (applicable to 200 μl sample) with 5% isopropyl alcohol (IPA) in n-hexane (2× 3.0 ml) was used for the sample cleanup. HPLC-UV and LC–MS/MS procedures developed and validated in our laboratory were used for quantification of 99-357 in rabbit and rat plasma (Singh et al., 2004, 2005, 2008). The LLOQ for the estimation of 99-357 using HPLC-UV was 25 ng/ml and using LC–MS/MS was 1.56 ng/ml. HPLC-UV method was used to for estimation of 99-357 in rabbit plasma and for *in situ* absorption studies. The LC–MS/MS method was used for all other studies.

2.4. Ex vivo studies

2.4.1. Plasma protein binding

The protein binding was estimated using the modified charcoal adsorption method (Khurana et al., 1999). The dextran coated charcoal suspension was prepared by transferring 0.66 g to a 250 ml reagent bottle containing 100 ml of DPBS. The mixture was stirred with a magnetic stirrer at room temperature until the charcoal was suspended. This suspension was prepared at least 18 h before use and stored at 5–10 °C for not longer than 30 days. The stored charcoal mixture was re-suspended before use. Plasma (6.0 ml) was spiked with 30 μl of 100 μg/ml solutions of 99-357 so as to get a final concentration of 500 ng/ml.

The spiked plasma was allowed to equilibrate for 10 min before the start of the study. The charcoal suspension (6.0 ml) was transferred into a 30 ml glass tube, centrifuged at 3000 rpm for 15 min at 25 °C, and the supernatant DPBS was carefully decanted off. Spiked plasma (6.0 ml) was then added on to the charcoal pellet under continuous stirring at 37 ± 2 °C, the temperature was maintained using an oil bath. Serial samples (400 μl) were withdrawn at 0, 5 min, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 4, 6 and 8 h in 0.6 ml polypropylene micro-centrifuge tubes and centrifuged immediately at 11,000 rpm at 37 °C. The supernatant was separated and was immediately transferred into 5 ml glass centrifuge tubes and stored at −60 °C