Study of Efficacy of Methotrexate against *Plasmodium knowlesi* and its Adverse Effects in *Papio Anubis* (Olive Baboon)

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ABSTRACT

New and affordable interventions are required for malaria control. The exploitation of old drugs originally designed against other diseases presents an attractive alternative since it limits the cost of drug discovery. Methotrexate (MTX) is one such drug. This drug is used in high intolerable doses (9–20 g/kg body weight) for cancer treatment and low safe doses (7.5-25 mg/kg body weight) for arthritis. The low doses are beyond MTX concentrations required to kill drug-resistant *P. falciparum* strains *in vitro* with an 50 % inhibitory concentration (IC_{50}) of less than 50 nM. This study reports on thetoxicity and efficacy of MTX in the olive baboon (*Papio anubis*) infected with *Plasmodium knowlesi.* Twelve baboons were randomly allocated to 3 treatment groups of 4 animals each. Group 3 (non-infected animals) were administered with MTX at a doses of 1.0 mg/kg/day for 5 days. Each animal in group 1 and 2 was infected with 1 x 10_6 *P. knowlesi* blood stage parasites. All animals in groups 1 and 2 were administered with MTX at single doses of 1.0 and 0.35 mg/kg/day for 5 days respectively. Due to stringent ethical obligations and high cost of using non-human primates in biomedical research, retrospective data was used for the positive (n = 4, infected and treated with pyrimethamine at 1.0 mg/kg/day for 5 days) and negative (n = 4, infected non-treated) control groups. Pre-infection and pre-treatment baseline data was used for the noninfected, non-treated controls. Baseline samples were collected from animals in the present study once weekly for 3 weeks prior to infection and drug administration. All animals were followed up for 42 days. Clinical chemistry assays, postmortem and histopathological examinations were conducted for all animals. Group 3 animals

remained clinically healthy throughout the experiment. Their clinical chemistry and blood profile parameters fluctuated within the baseline range. Postmortem and histological studies conducted at the end of follow-up period did not show drug-related pathology. In infected animals, there was a significant increase in parasitaemia from the inception to cessation of MTX administration (Group 1: $p = 0.0131$; Group 2: $p = 0.0141$). Infected baboons were euthanized to alleviate suffering.

Outcome of infected animals revealed changes consistent with the negative controls. These findings indicate that MTX, at the dosages used in this study, is safe in the baboon but not efficacious against *P. knowlesi in vivo*.