PHARMACOKINETICS AND PHARMACODYNAMICS OF INTRAVENOUS METHYLENE BLUE IN HEALTHY Rhesus Monkeys (POSTER)

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Purpose: Methylene blue has antimalarial activity in vitro and in vivo in rats and humans. The purpose of this study is to characterize the pharmacokinetics and pharmacodynamics of intravenous methylene blue in healthy rhesus monkeys. Methods: Methylene blue 1% USP solution was administered as a single 30-second intravenous infusion at doses of 2.5 mg/kg, 5.0 mg/kg and 10.0 mg/kg to healthy rhesus monkeys. Serial venous blood samples were collected at 0, 5, 10, 20, 40, and 60 minutes, and 2, 3 and 5 hours post-dose. Plasma samples were analyzed for methylene blue concentration by HPLC and total antimalarial activity (by bioassay). Results: All monkeys maintained normal clinical signs and symptoms except for blue feces and urine in the 10 mg/kg group for up to 4 days after dosing. The bioassay results for 2.5, 5.0 and 10.0 mg/kg demonstrated respective bioassay AUCs of 79.9, 135.1 and 202.7 μM-min. There is a linear relationship between bioassay AUC and dose values with an r² of > 0.9. The HPLC assays of all three doses are pending validation. Conclusions: Methylene blue was well tolerated in healthy non-infected rhesus monkeys, and demonstrated proportional increases in AUC relative to dose. The results of this study will be used to select two different doses of methylene blue to be used in an antimalarial efficacy study of artesunate in combination with methylene blue in a Plasmodium cynomolgi/rhesus monkey model of uncomplicated malaria.


PHARMACOKINETICS/PHARMACODYNAMICS OF INTRAVENOUS ARTELINATE AND ARTESUNATE USING A PLASMODIUM COATNEYI-RHESUS MONKEY MODEL OF SEVERE MALARIA

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Plasmodium falciparum malaria is the cause of 1-1.3 million deaths each year. The mortality is mostly due to severe malaria. P. coatneyi is a sequestering primate malaria and in a splenectomized animal produces a model to assess therapeutic efficacy to severe malaria. Methods: Equimolar dose of parenteral artesunate (8 mg/kg) and artelinate (11.8 mg/kg), a new artemisinin derivative, were intravenously administered to 10 rhesus monkeys when healthy and then again when infected with P. coatneyi. Consequently, each monkey served as its own control of intra-subject variation. The treatment was initiated at parasitemia 3-14 % or > 200,000 parasites/µL when animals were minimally symptomatic. Blood was collected at 0, 5, 20, 40 min, 1, 3, and 6 h post-dose and plasma samples were divided for simultaneous measurement by HPLC and
bioassay. The plasma levels of parent drug and its primary metabolite were measured by HPLC with electrochemical detection (reductive mode). The total antimalarial activity of the drug and all active metabolite(s) in plasma were measured by bioassay against *P. falciparum* (W2 clone). **Results:** The antimalarial activity of artesunate was contributed by both parent drug and its active metabolite, dihydroartemisinin (DHA), until 20 min post-dose, after which DHA remained in the plasma. On the other hand, artelinate was responsible for all the antimalarial activity in plasma, even though 2-hydroxyartellic acid, an active metabolite, was detected by HPLC. Furthermore, no DHA was found in all the plasma from either healthy or infected monkeys receiving AL. The pharmacokinetic/pharmacodynamic (PK/PD) comparison of both I.V. formulations between healthy and malaria infected rhesus were not significantly different. Based on antimalarial activity data from infected monkeys, the bioavailability of the two I.V. formulations were comparable with area under the curve (AUC) values of 400-500 umole.min.L⁻¹ and half-life values of 30 min. However, the first 20-min bioavailability (AUC 0-20) and the highest activity measured at 5 min for AS were 260 umole.min.L⁻¹ and 22 umole.L⁻¹, which were 2-fold and 3-fold greater than that of AL, respectively. Furthermore, the ex vivo potency of AS was 6-fold greater than AL. These values may be important parameters for clinical efficacy and possibly toxicity of these compounds. **In conclusion**, artelinate is effective but utilizes a different metabolic pathway than artesunate. Artesunate is more potent, but both drugs have comparable PK/PD properties.


**PHYSICOCHEMICAL INTERACTIONS AND IN VITRO ANTIMALARIAL ACTIVITY OF ARTESUNATE AND MEFLOQUINE HYDROCHLORIDE**

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The physicochemical properties of artesunate and mefloquine hydrochloride and physical mixtures of artesunate and mefloquine hydrochloride in the ratios of 1: 1 and 2:5 by weight were studied using Fourier transform infrared spectrophotometry (FTm), powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC). FTIR spectra and PXRD patterns showed that artesunate was compatible with mefloquine hydrochloride. The DSC thermogram of the physical mixtures of artesunate and mefloquine hydrochloride showed a disappearance of melting endotherm of mefloquine hydrochloride. Hot stage microscopy (HSM) was further used to interpret the DSC results. HSM analysis indicated that the phenomenon observed in DSC analysis was mainly due to the dissolution of mefloquine hydrochloride in the melted artesunate which took place while performing thermal analysis. These results indicated no physicochemical interaction between artesunate and mefloquine hydrochloride.