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Next generation quinoline methanol (NGQM) lead analog 8SF5MQ (WR 319689) when administered to Aotus monkeys infected with Plasmodium falciparum FVO orally at 6.25 mg/kg cleared and recrudesce but at 2.1 mg/kg had no effect over parasitemia. In contrast, mefloquine at 6.25 mg/kg cleared and cured but at 2.1 mg/kg only suppressed. Additionally, we were able to cryopreserved Aotus blood at high density parasitemias > 50 x 10^3 x µL for both Plasmodium vivax AMRU-1 and P. vivax SAL-1 parasites that will be used at WRAIR for in vitro culture experiments. In another series of experiments, WR621308 at 65 m/kg suppressed parasitemia in Aotus infected with P. falciparum FVO but toxic adverse effects observed in one animal stopped the experiment at a higher dose (130 mg/kg).

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______________ May 25, 2011

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INTRODUCTION

Each year there are 300-500 million new infections and 2-5 million deaths attributable to malaria that occur primarily in countries in the tropics, particularly in sub-Saharan Africa **(4).** During the past 10-20 years the malaria problem has intensified in some parts of the world, because parasites have developed resistance to drugs used for treatment and prevention; the anopheles mosquito, which transmits the parasite to humans, has developed resistance to insecticides, and control efforts have been reduced as resources have diminished in some developing countries **(7)**.

The use of *Aotus lemurinus lemurinus* (Panamanian *Aotus* monkey), kariotypes VIII and IX **(16)** as a model to study malaria drug resistance and vaccine efficacy, have been ongoing at Gorgas Memorial Laboratory since 1976; due in part to the availability of this monkey in Panama **(20)**, and also to the increasing drug resistance exhibited by the highly pathogenic *Plasmodium falciparum* parasites in Asia, Africa, and Latin America, and more recently *Plasmodium vivax* in the Melanesian and Indonesian archipelago **(21)**. Previously, Schmidt **(26,27)** used the Colombian *Aotus* as the experimental host for antimalarial drug studies, but embargoes imposed by South American countries on the exportation of monkeys in the mid 1970's seriously restricted the use of *Aotus* for biomedical research in the United States. In 1976 the project was transferred to Gorgas Memorial Laboratory where Panamanian *Aotus* were available for research. Seven strains of *P. falciparum*, Vietnam Smith, Uganda Palo Alto, Vietnam Oak Knoll (FVO), Indochina I, Camp, Santa Lucia **(5)**, and a C2A mefloquine resistant clone, and three strains of *P. vivax* Chesson (chloroquine sensitive), New Guinea AMRU-1 (chloroquine resistant) and Sal-1, have been adapted to Panamanian Aotus. These strains exhibit diverse susceptibility and/or resistance to standard antimalarial agents. The course of untreated infections in Panamanian *Aotus* has been characterized and compared with that in *Aotus* of Colombia **(25)**. Overall, the virulence of these strains was less in Panamanian than in Colombian owl monkeys, as indicated by lower mortality rates of Panamanian monkeys during the first 30 days of patency. Maximum parasitemias of the Vietnam Smith and Uganda Palo Alto strains were, however; significantly higher during the first 15 days of patency in Panamanian than in Colombian owl monkeys. These quantitative differences in infection parameters between Panamanian and Colombian owl monkeys have not invalidated the use of the former for evaluation of new antimalarial drugs.

Numerous candidate antimalarial drugs of diverse chemical classes have been evaluated against trophozoite-induced infections of one or more *P. falciparum* strains during the course of these contracts. In seeking alternatives to primaquine, two 8-aminoquinolines proved to be active against the blood stages of *P. falciparum* **(2, 18)**. Desferrioxamine, an iron-specific- chelating agent, was shown to suppress parasitemias of the virulent Uganda Palo Alto strain of *P. falciparum* **(23)**. The *in vitro* activity of two halogenated histidine analogs was not confirmed by evaluation against *P. falciparum* infections in owl monkeys **(22)**.

Chloroquine-resistance of *P. falciparum* represents the greatest challenge in developing effective antimalarial drugs. Reversal of chloroquine-resistance in *P. falciparum*, *in vitro*, was achieved by the co-administration of verapamil (a calcium channel blocker) plus chloroquine **(17)**. Other *in vitro* studies have shown that there is a significantly greater efflux of chloroquine from erythrocytes containing falciparum

parasites resistant to chloroquine than from red cells parasitized by chloroquine-sensitive falciparum malaria **(14)**. Calcium channel blockers appear to prevent this active efflux of chloroquine, thus allowing the drug to accumulate to parasiticidal levels.

Based upon the success of *in vitro* reversal of chloroquine-resistance, trials were initiated to determine if resistance could be reversed in *Aotus* infected with the chloroquine-resistant Vietnam Smith strain of *P. falciparum*. Six calcium channel blockers, or similarly acting drugs, were co-administered with chloroquine in diverse regimens. The desideratum of chloroquine-resistance reversal was administration of a single course of treatment, with parasite clearance and infection cure. Suppression of parasitemia was obtained during an initial course of treatment, but parasite clearance and cure occurred in some instances only after re-treatment. Such infection parameters were similar to those in monkeys with self-limited infections and cure could be attributed to acquired immunity.

Limited trials with desipramine, Norpramin, a tricyclic psychotropic drug, demonstrated the feasibility of reversing chloroquine-resistance in vivo **(1)**. Parasite clearance was obtained, but the infection was not cured.

Subsequently, in vivo reversal of chloroquine resistance was obtained with combinations of chloroquine plus chlorpromazine or prochlorperazine. Such reversal was exhibited by rapid suppression and clearance of parasitemia, resulting in infection cure without retreatment **(15)**.

Evaluation of two oil-soluble derivatives of artemisinin, artemether and arteether, demonstrates that both possess similar activity to cure infections of a multi-drug resistant *P. falciparum* strain in *Aotus* **(28)**.

Some strains of *P. vivax* from Melanesia and the Indonesian archipelago have demonstrated resistance to treatment with chloroquine **(19, 24)**. Unlike chloroquine-resistant falciparum malaria, there exists no easy alternative to chloroquine-resistant strains of vivax malaria. Using WR 238605 alone or in combination with chloroquine in Panamanian *Aotus* monkeys, it was demonstrated that WR238605 is an alternative treatment for chloroquine-resistant vivax malaria **(21)**. The compound WR 238605 is a primaquine analog developed by the US Army as a better tolerated, more effective replacement for primaquine. Recent studies done at Gorgas Institute with Artemisin derivative drugs developed by the U.S. Army such as Artelinic acid demonstrated its efficacy against the FVO strain of *P. falciparum* when administered orally to *Aotus l. lemurinus.*

A series of carbamate, carboxamide, succinimide, and alkylamine derivatives of pyrroloquinazolinediamine WR227825 were prepared to search for compounds with an improved therapeutic index. The new acetamides and imide showed potent cell growth inhibition against four clones of *P. falciparum* (D-6, RCS, W-2, and TM91C235), with a 50% inhibitory concentration of approximately 0.01 ng/ml, and were highly active against *P. berghei*, with 100% cure at doses from <0.1 mg/kg of body weight to 220 mg/kg. The carbamates and alkyl derivatives, however, showed weak activity against *P. falciparum* cell growth but were highly efficacious in tests against *P. berghei* by the Thompson test. The best compounds, bis-ethylcarbamate (compound 2a) and tetra-acetamide (3a) derivatives, further demonstrated high potency against the sporozoite *P. yoelii* in mice and *P. falciparum* and vivax in aotus monkeys. Against the AMRU-1 strain of *P. vivax*, which has four dihydrofolate reductase mutations and is highly resistant to

antifolates, tetra-acetamide 3a cured the monkeys at doses of 1 and 3 mg/kg. Compound 2a cured only one out of two monkeys at 3 mg/kg. The new derivatives 2a and 3a not only have retained/improved the antimalarial efficacy of the parent compound WR227825 but also were less toxic to the animals used in the tests **(32)**.

Mefloquine has been one of the more valuable antimalarial drugs but has never reached its full clinical potential due to concerns about its neurologic side effects, its greater expense than that of other antimalarials, and the emergence of resistance. The commercial development of mefloquine superseded that of another quinolinyl methanol, WR030090, which was used as an experimental antimalarial drug by the U.S. Army in the 1970s. We evaluated a series of related 2-phenyl-substituted alkylaminoquinolinyl methanols (AAQMs) for their potential as mefloquine replacement drugs based on a series of appropriate in vitro and in vivo efficacy and toxicology screens and the theoretical cost of goods. Generally, the AAQMs were less neurotoxic and exhibited greater antimalarial potency, and they are potentially cheaper than mefloquine, but they showed poorer metabolic stability and pharmacokinetics and the potential for phototoxicity. These differences in physiochemical and biological properties are attributable to the "opening" of the piperidine ring of the 4-position side chain. Modification of the most promising compound, WR069878, by substitution of an appropriate N functionality at the 4 position, optimization of quinoline ring substituents at the 6 and 7 positions, and deconjugation of quinoline and phenyl ring systems is anticipated to yield a valuable new antimalarial drug **(33).**

The antimalarial activity and pharmacology of a series of phenylthiazolyl-bearing hydroxamate-based histone deacetylase inhibitors (HDACIs) was evaluated. Approximately 50 analogs were evaluated against four drug resistant strains of *P. falciparum* in an *in vitro* growth inhibition assay. The most potent compound, WR301801 (YC-2-88) did not exhibit cures in P. berghei-infected mice at oral doses as high as 640 mg/kg/day for 3 days or in *P. falciparum*-infected Aotus l. lemurinus monkeys at oral doses of 32 mg/kg/day for 3 days, despite high relative bioavailability. Next-generation HDACIs with greater metabolic stability than WR301801 may be useful as antimalarials if combined appropriately with conventional antimalarial drugs **(34).**

Artemisone (single oral dose, 10 mg/kg of body weight) cured non-immune Aotus monkeys of their *P. falciparum* infections when combined with mefloquine (single oral dose, 5 and 10 mg/kg but not 2.5 mg/kg). In combination with amodiaquine (20 mg/kg/day), artemisone (10 mg/kg/day) given orally for 3 days cured all infected monkeys. Three days of treatment with artemisone (30 mg/kg/day) and clindamycin (100 mg/kg/day) was also curative **(35).**

Both the purpose and methods of approach of the present work remains essentially unchanged since 1976, viz. to ascertain the antimalarial activity of drugs against *P. falciparum* and *P. vivax* in *Aotus*. The method of approach may vary on an *ad hoc* basis, such as administering a combination of drugs.

Previous trials of malaria blood stage vaccines have shown that the Panamanian *Aotus\P. falciparum* model to be suitable for this purpose.
(8-10).

Immunogenicity studies of a plasmid DNA vaccines encoding the circumsporozite *P. yoelli* rodent malaria gene (PyCSP) in Panamanian *Aotus* monkeys, demonstrated that the intradermal route of inoculation (ID) induces a higher level of

antibodies than the intramuscular route (IM). Antibody levels induced in this manner reached a peak at week 9 and titers declined to 50% their peak value by week 14. When boosted at week 46 antibody levels increase 4 fold by week 49. This was comparable to antibodies generated with a Multiple Antigen synthetic peptide vaccine (MAP) delivered with an adjuvant **(4).** We have used this immunization scheduled to test single or multi-gene DNA plasmid vaccines in *Aotus* monkeys. Other experiments, tested the ability of recombinant cytokines GM-CSF to enhance the immunogenicity and protective efficacy of the DNA vaccines.

During the course of these experiments, we have also demonstrated that synthetic oligodeoxynucleotides containing CpG motifs enhance immunogenicity of a peptide malaria vaccine when tested in Panamanian Aotus **(11).** Also, different vaccine formulations, routes and methods of administration with a comparable Hepatitis B Plasmid DNA vaccine were explored in Panamanian Aotus in order to elucidate the best route and methods of immunization for a plasmid DNA malaria vaccine **(6).** Further studies with multiple plasmids encoding EBA-175, MSP-1 and AMA-1 did not induced antigenic competition when this vaccines were delivered as a mixture in Panamanian Aotus **(31)**.

Recently, we tested the hypothesis that *a P. falciparum* ligand, EBA-175 region II (RII), can be used as an immunogen in Aotus to induce antibodies that block the binding of RII to erythrocytes and thus inhibit parasite invasion of erythrocytes **(29)**. When this parasite protein was tested in Panamanian *Aotus l. lemurinus* using a plasmid DNA prime-recombinant protein boost approach, no protection was obtained against a *P. falciparum* FVO strain challenge. However. in the same experiment those animals immunized with a recombinant $MSP1_{42}$ protein/peptide vaccine formulation were partially protected (2001 annual report). These results contrast with those obtained previously in *Aotus nacymae* from Peru were partial protection was achieved using the same EBA-175 region II (RII) formulation and approach **(30).**

Sterile immunity was achieved by repeated blood stage infections with *P. falciparum* in Aotus monkeys previously immunized with early generation plasmid DNA vaccines AMA-1 or MSP-1 and non-immunized, but no association was found between immunization status and mean pre-patent period. These animals were protected against and homologous and partially protection against an heterologous challenge **(12)**. Also, evaluated in Aotus monkeys were the characteristics of *P. falciparum*-induced anemia in two different experimental settings were a non-antibody/non-complement-mediated lysis of uninfected erythrocytes seems to be the principal cause of anemia and bone marrow suppression and lysis of infected erythrocytes contributed to the anemia **(13)**.

The purpose of this report is to: Present data on the evaluation of potential antimalarial activity of drugs in the pre-clinical model of *Aotus l. lemurinus* (Panamanian night monkey) experimentally infected with *P. falciparum* or *P. vivax*. The U.S. Army Malaria Program supported these studies.

BODY:

I. Experimental Methods

II. Results

1. Antimalarial efficacy of next generation quinoline methanol (NGQM) lead analog 8SF5MQ (WR 319689) against *P. falciparum* FVO infections in Aotus monkeys.

The objective of this experiment was to determine if the lead NGQM analog 8SF5MQ (WR 319689) can produce single dose cures at 6.25 mg/Kg and 2.1 mg/Kg in the *Aotus/P. falciparum* FVO model of malaria and to directly compare its performance relative to equivalent dose benchmark mefloquine thereby confirming a relative potency of at least 3-fold over mefloquine. Secondary objectives are to generate preliminary pharmacokinetic, safety, and tolerability data in this non-human primate model. A positive result would support the NGQM team decision to invest substantial team ECEresources towards synthesis of an enatiomerically pure WR319689 compound for further more extensive evaluation and possibly further exploration of closely related compounds.

On May 14, 2010 nine male or female *Aotus l. lemurinus* (700-931g) were divided into four of two monkeys each, and one control and infected with 5,000,000 *P. falciparum* parasites IV from a donor monkey. When their parasitemia reached 5,000 parasites/ µL on day 5 PI treatment started. WR 319689 was administered orally once at 6.25 mg/kg to Group 1 and at 2.1 mg/kg to Group 2. This groups were benchmark to Groups 3 and 4 that were treated with mefloquine at the same doses. The control monkey was treated with the rescue treatment of mefloquine 25 mg/kg once when its parasite levels reached 126.54 x 10^3 parasites / µL on day 6 PI. Blood (0.4 ml) was drawn at 0, 4, 24, and 168 hours frozen and shipped to WRAIR for drug concentration determinations. Blood 0.25 mL (EDTA) was collected prior to infection and 8, 12, 15 and 19 days PI for CBC and chemistry profile (ALT, Creatinine and BUN) and each week after infection for three consecutive weeks in all monkeys. Animal weights were recorded at each time point. If primary treatment failed or recrudescence occurred the animals were treated with the rescue regimen of mefloquine 25 mg/kg. The animals were monitored daily for any signs or adverse effects.

As shown in tables 1 and 2, in Group 1, both animals cleared parasitemia on day 6 and 7 post-treatment (PT), but recrudesce on days 14 and 26 PT, being rescue treated with mefloquine on days 16 and 28 PT. In Group 2, WR 319689 had no effect over parasitemia and both animals had to be rescue treated on days 4 and 5 PT. In contrast, in Group 3, both animals cleared their parasitemias on day 5 PT, remaining negative for more than 23 days PT when this report was prepared. In contrast, in Group 4 both treated animals had to be rescue treated on days 8 and 14 PT due to suppression only. In conclusion, next generation quinoline methanol (NGQM) lead analog 8SF5MQ (WR 319689) when administered to Aotus monkeys infected with *P. falciparum* FVO orally at 6.25 mg/kg cleared and recrudesce but had no effect at 2.1 mg/kg. In contrast, mefloquine when administered at 6.25 mg/kg cleared and cured but at 2.1 mg/kg only suppressed.

2. Establishment of *Plasmodium vivax* Donor Aotus Protocol.

The Division of Experimental Therapeutics Walter Reed Army Institute of Research is developing *in vitro Plasmodium vivax* culturing technologies. The ultimate goal is to establish long-term culturing capabilities, which will facilitate: drug discovery/development, vaccine development, resistance monitoring and basic research. *P. vivax* blood stage merozoites invade reticulocytes, which express cell surface Duffy antigen. The lack of an ample supply of well-characterized Duffy-positive human reticulocytes is the primary obstacle to *P. vivax in vitro* culturing.

The Defense Advanced Research Projects Agency (DARPA)'s Blood Pharming program is developing novel technologies to enable *in vitro* production of red blood cells from progenitor cells. The ultimate goal is to provide large quantities of red blood cells of controlled origin for transfusions.

Collaborative work between WRAIR and Cellgene Corp. *via* the Blood Pharming program has established that placenta-derived progenitor cells can be selected for Duffy expression and expansion. At present, red cell populations consisting of 50% enucleated, Duffy positive reticulocytes are routinely produced. Currently, these cells are being assessed for their ability to support *P. falciparum* growth *in vitro* (*P. falciparum* will invade both mature erythrocytes and reticulocytes).

The objective of this proposal is to provide an Aotus *P. vivax* infected blood donor program to establish long-term culturing capabilities of *P. vivax.* This will facilitate *P. vivax*: drug discovery/development, vaccine development, resistance monitoring and basic research.

On August 29, 2010 eight male or female *Aotus l. lemurinus* (700-900 g) were divided into two groups of four monkeys each, and infected with 2,000,000 *P. vivax* AMRU-*1* (Group 1) parasites *or SAL-1* (Group 2*)* parasites IV from two donor monkeys. When their parasitemia peaked on an avg to 50,000 parasites/ µL between days 9-12 PI, blood was collected and the animals rescue treated with mefloquine (WR142490) at 25 mg/kg once. Blood (2.5 ml) was drawn from the femoral vein on heparin tubes at day of peak, centrifuged at 1,200 rpm and separated into two 500 *ul* aliquots of packed red blood cells cryotubes, labeled and cryopreserved in glycerolate and stored in liquid nitrogen until shipment to WRAIR for culture attempts. Additionally, blood 0.25 mL (EDTA) was collected prior to infection and 8, 12, 15 and 19 days PI for CBC and chemistry profile (ALT, Creatinine and BUN) and each week after infection for three consecutive weeks in all monkeys. Weights were recorded at each time point and the animals monitored daily for any signs or adverse effects.

In Group 1, consisting of Aotus MN21022, MN22034, MN23044 and MN25048 which were inoculated with the AMRU-1 strain of *P. vivax*, peak parasitemias were reached on day 9 post-inoculation (PI) in the first three animals and day 12 in the latter. Peak parasitemia fluctuated in the first three animals between 54.4-70.1 x 103 µL and 6.7 x 103 µL in the latter. In Group 2, consisting ofAotus MN23043, MN24061, MN24034 and MN22011 inoculated with the SAL-1 strain of *P. vivax,* peak parasitemias were reached between days 10-11 PI.. Peak parasitemias in this group fluctuated in the first three animals between 57.6-84.9 x 10^3 x µL and 9.0 x 103 x µL in the latter. In conclusion, we were able to cryopreserved high-density parasitemias > 50 x 10^3 x µL of

both *P . vivax* AMRU-1 and SAL-1 passaged in Aotus monkeys for *in vitro* culture attempts.

3. Antimalarial efficacy of next generation quinoline methanol (NGQM) lead analog WR621308 against *P. falciparum* FVO infections in Aotus monkeys.

WR621308 is a new diamine quinoline methanol with promising in vivo efficacy and pharmacokinetic properties. The compound exhibits 100% survival with 80% cure in P. berghei-infected mice at Day 30 after a single dose of 320 mg/kg PO (all data unpublished from recent studies at WRAIR). This is the approximate equivalent of the total clinical dose of mefloquine for treatment (25 mg/kg) after application of an allometric scaling factor of approximately 12 to take into account differences in body surface area. The compound exhibits no evidence of toxicity at this dose. Pharmacokinetic analyses suggest the compound has a half-life of 133 h versus 32 h for mefloquine after intravenous dosing in mice. Bound and unbound brain concentrations are approximately 15 and 5-fold lower than mefloquine after a single dose of 160 mg/kg PO in mice. If WR621308 can be used at the same therapeutic dose as mefloquine this may mean it will induce fewer CNS adverse events. Because WR621308 exhibits cross-susceptibility with mefloquine in vitro, our working assumption is that it is functionally equivalent to mefloquine from a mechanism of action and resistance standpoint. Consequently, it may be necessary to combine the compound with another drug in a treatment setting prior to deployment for IPT studies. It is therefore necessary to document in a preclinical context that WR621308 is effective in the majority of instances at the intended clinical dose against CQ-resistant Pf in a relevant animal. That is the intent of the present study.

The objectives of this study were: a) WR621308 will exhibit a cure rate of at least 60% when administered at a dose of 130 mg/kg PO in Aotus monkeys. This is the equivalent of the intended treatment dose. b) WR621308 will be well tolerated at single doses of 65 and 130 mg/kg PO. When the compound was administered at 65 mg/kg once it didn't show any signs of toxicity in one Aotus monkey.

As shown in tables 3 and 4, on November 19, 2010 four monkeys were inoculated with 4.0 x 10^6 *P. falciparum* FVO parasitized red blood cells from a donor monkey. On day 4 PI WR621308 was administered to the test monkeys at 65 mg/kg once. However, during the experiment one animals died of bronchial-aspiration after exhibiting profuse vomiting that was followed shortly by shock and death. Necropsy revealed a bronchial-aspiration. The other three animals did not show any signs of toxicity and the experiment continued until all three were rescue treated with mefloquine. The test animals only had a transient suppression of parasitemia and were rescue treated on day 12 PI. The control animal was rescue treated on day 7 PI when it reached 709.65 x 103 parasites per ul.

Part II of this protocol at doses of 130 mg/kg was cancelled on January, 2011 do to *a priori* criteria for termination of the study achieved on December, 2010, related to toxicity or adverse events observed in one animal as reported, which warranted cessation of drug treatment. In conclusion WR621308 at 65 m/kg only suppressed parasitemia in Aotus infected with *P. falciparum* FVO. Toxic adverse effects were observed in one animal.

4. Benchmarking of approved antimalarial drugs at prophylaxis doses and the evaluation of treatment efficacy of two lead compounds, WR150008 and WR246976 against *P. falciparum* FVO infections in Aotus monkeys.

Several new scaffolds are being evaluated at WRAIR. The lead compounds, WR246976 and WR150008, depicted in the structure below, represent two of these scaffolds. Both of these compounds cure *P. berghei* malaria in mice when administered as a single dose of 160 mg/kg PO. These compounds have never been screened against Pf in Aotus before. We are proposing to screen both these compounds as a single dose in Aotus, in order to confirm that initial observations in mice translate to a more relevant model. If cures are observed in Aotus and similar exposure levels in mouse PK studies it would suggest that the current screening paradigm is sufficient to find appropriate lead compounds. If this were not the case, it would suggest that the current in vitro/mouse screening paradigm would need to be modified appropriately.

The objectives of this study were: 1) To determine whether patency results after weekly dosing with mefloquine and daily dosing with doxycycline or atovaquone-proguanil after a blood stage inoculation of Pf FVO. 2) Determine whether WR246976 and WR150008 will cure Pf FVO when administered as a single oral dose of 65 mg/kg PO (this is equivalent of mouse curative dose of 160 mg/kg PO).

As per the experimental design the first group of two animals will serve as control animals. They will be inoculated at the same time as the other animals and administered a mefloquine rescue regimen once parasitemia reaches 100,000 parasites/ul. The day of inoculation is Day 0. The second group of animals will be given mefloquine once per week at a dose of 5 mg/kg PO on Days -13, -6, 1, 8, 15 and 22. The third group will be given doxycycline daily at a dose of 10 mg/kg PO from Days 0-6. The dose on Day 6 will be given at least two hours prior to inoculation. The fourth group of animals will be given atovaquone-proguanil daily at a dose of 25/10 mg/kg PO from Days 0-6. The dose on Day 6 will be given at least two hours prior to inoculation. The fifth group of animals will be given WR246976 as a single dose at 65 mg/kg PO once parasitemia reaches 5000/ul. The animals will be re-dosed with the same daily dose for three days if they recrudesce. The sixth group of animals will be given WR150008 as a single dose at 65 mg/kg PO once parasitemia reaches 5000/ul. The animals will be re-dosed with the same daily dose for three days if they recrudesce.

Following infection, all monkeys, to include the donors, will be monitored daily for parasitemia beginning on the day after infection, and, CBC, Chemistry and weight as stated above. This includes a daily prick on the ear marginal vein with a lancet and a phlebotomy of the femoral vein (if femoral vein is inaccessible, the saphenous vein will be used) for 0.25 ml of blood for CBC determination and chemistry profile (ALT, Creatinine and BUN) using a Coulter Counter hematological analyzer and a Reflotron dry chemistry machine. On day 0, 8, 12, 15 and 19 PI when the CBC is done, if an animal has, persistent low grade (<10 parasites) beyond day 15 PI, severe sustained thrombocytopenia (<50,000 platelets/ul) for >5 days, or anemia HCT % less than 50% of baseline a decision will be made by the attending veterinarian to continue or remove the animal from the experiment and treat with 25 mg/kg of mefloquine or/and transfuse with unmatched citrated whole blood (3 mls).

This experiment started on April 29th, 2011 with the inoculation of the donor monkey and pre-treatment of group 2 with mefloquine at a dose of 5 mg/kg PO on Day -13 pre-inoculation. This group then will receive weekly treatment on days -6, 1, 8, 15 and 22. This experiment is in progress at the moment of this report. Results will be included in the next annual report.

KEY RESEARCH ACCOMPLISHMENTS:

1. Next generation quinoline methanol (NGQM) lead analog 8SF5MQ (WR 319689) when administered to Aotus monkeys infected with *P. falciparum* FVO orally at 6.25 mg/kg cleared and recrudesce but at 2.1 mg/kg had no effect over parasitemia. In contrast, mefloquine at 6.25 mg/kg cleared and cured but at 2.1 mg/kg only suppressed.
2. We were able to cryopreserved Aotus blood from high density parasitemias > 50 x 10^3 x µL for both *P . vivax* AMRU-1 and SAL-1 that will be used at WRAIR for *in vitro* culture experiments.
3. WR621308 at 65 m/kg only suppressed parasitemia in Aotus infected with *P. falciparum* FVO . Toxic adverse effects were observed in one animal.

REPORTABLE OUTCOMES:

I. Manuscripts:

1. Obaldia NIII, et al.. Long-term effect of a simple nest-box on the reproductive efficiency and other life traits of an Aotus l. lemurinus monkey colony: An animal model for malaria research. Submitted to the Journal of Medical Primatology, January 22th 2011.

II. Presentations:

1. Pathogenesis of falciparum malaria sequestration in the human *P. falciparum* /Aotus model. Presentation given at the Gorgas Memorial Institute Annual Conference. Hotel Continental, Panama City, Panama. August 6, 2010.

CONCLUSIONS:

1. Next generation quinoline methanol (NGQM) lead analog 8SF5MQ (WR 319689) when administered to Aotus monkeys infected with *P. falciparum* FVO orally at 6.25 mg/kg cleared and recrudesce but at 2.1 mg/kg had no effect over parasitemia. In contrast, mefloquine at 6.25 mg/kg cleared and cured but at 2.1 mg/kg only suppressed.
2. We were able to cryopreserved Aotus blood at high density parasitemias > 50 x 10^3 x µL for both *P . vivax* AMRU-1 and SAL-1 parasites that will be used at WRAIR for *in vitro* culture experiments.
3. WR621308 at 65 m/kg suppressed parasitemia in Aotus infected with *P. falciparum* FVO but toxic adverse effects were observed in one animal and the experiment with a higher dose (130 mg/kg) was canceled.

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