

Publications

Open access | Research: Extended high efficacy of the combination sulphadoxine-pyrimethamine with artesunate in children with uncomplicated falciparum malaria on the Benin coast, West Africa

Alain Nahum et al.

Malaria Journal 2009, 8:37 (3 March 2009)

The paper describes an extended follow-up study of efficacy of SP plus artesunate, which is a response to the concern that the new ACT drug strategy is inefficient when artemisinin is combined with a partner drug in a situation where resistance to the partner drug is prevalent.

Open access | Research: Glatiramer acetate reduces the risk for experimental cerebral malaria: a pilot study

Peter Lackner, Andrea Part, Christoph Burger, Anelia Dietmann, Gregor Broessner, Raimund Helbok, Markus Reindl, Erich Schmutzhard, Ronny Beer

Malaria Journal 2009, 8:36 (27 February 2009)

A study of role of the host immune response in the pathophysiology of murine cerebral malaria, which may lead to the development of new adjunctive treatment strategies.

Open access | Research: AFCo1, a meningococcal B-derived cochleate adjuvant, strongly enhances antibody and T-cell immunity against Plasmodium falciparum merozoite surface protein 4 and 5

Gustavo Bracho, Caridad Zayas, Lina Wang, Ross Coppel, Oliver Perez, Nikolai Petrovsky

Malaria Journal 2009, 8:35 (27 February 2009)

A valid demonstration of new adjuvant activity of the same order as the benchmark, Complete Freund's Adjuvant, without its toxicity.

Open access | Research: Efficacy of chloroquine, amodiaquine and sulphadoxine-pyrimethamine for the treatment of uncomplicated falciparum malaria: revisiting molecular markers in an area of emerging AQ and SP resistance in Mali

Mamadou Tekete et al.

Malaria Journal 2009, 8:34 (26 February 2009)

A paper that shows the importance of molecular markers of drug resistance in guiding any anti-malarial drug policy.

Plasmodium falciparum signal peptide peptidase is a promising drug target against blood stage malaria

Xuerong Li, Huiqing Chen, Noemi Bahamontes-Rosa, Jurgen F.J. Kun, Boubacar Traore, Peter D. Crompton, Athar H. Chishti

Biochemical and Biophysical Research Communications, Volume 380, Issue 3, Pages 454-459

The resistance of malaria parasites to current anti-malarial drugs is an issue of major concern globally. Recently we identified a *Plasmodium falciparum* cell membrane aspartyl protease, which binds to erythrocyte band 3, and is involved in merozoite invasion. Here we report the complete primary structure of *P. falciparum* signal peptide peptidase

(PfSPP), and demonstrate that it is essential for parasite invasion and growth in human erythrocytes.

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Regular Articles: Partial molar volumes of acyl carrier proteins are related to their states of acylation

Sarika Gupta, Rahul Modak, Namita Surolia, Avadhesha Surolia

Biochemical and Biophysical Research Communications, Volume 380, Issue 4, 20 March 2009, Pages 763-768

Acyl carrier protein (ACP), an abundant protein in every cell, plays a central role in a number of metabolic processes requiring acyl group transfer. Conformational flexibility while crucial for its function remains substantially unaddressed. By dual polarization interferometry we establish correlation between the chain length of aliphatic groups covalently linked to *Escherichia coli* and *Plasmodium falciparum* ACP and their respective partial molar volumes in solution which helps to subserve the aforesaid goal.

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Structural Basis for Binding and Selectivity of Antimalarial and Anticancer Ethylenediamine Inhibitors to Protein Farnesyltransferase

Michael A. Hast et al.

Chemistry & Biology, Volume 16, Issue 2, 27 February 2009, Pages 181-192

Protein farnesyltransferase (FTase) catalyzes an essential posttranslational lipid modification of more than 60 proteins involved in intracellular signal transduction networks. FTase inhibitors have emerged as a significant target for development of anticancer therapeutics and, more recently, for the treatment of parasitic diseases caused by protozoan pathogens, including malaria (*Plasmodium falciparum*). We present the X-ray crystallographic structures of complexes of mammalian FTase with five inhibitors based on an ethylenediamine scaffold, two of which exhibit over 1000-fold selective inhibition of *P. falciparum* FTase. These structures reveal the dominant determinants in both the inhibitor and enzyme that control binding and selectivity. Comparison to a homology model constructed for the *P. falciparum* FTase suggests opportunities for further improving selectivity of a new generation of antimalarial inhibitors.

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Fumagillin and Fumarranol Interact with *P. falciparum* Methionine Aminopeptidase 2 and Inhibit Malaria Parasite Growth In Vitro and In Vivo

Xiaochun Chen, Suji Xie, Shridhar Bhat, Nirbhay Kumar, Theresa A. Shapiro, Jun O. Liu

Chemistry & Biology, Volume 16, Issue 2, 27 February 2009, Pages 193-202

The fumagillin family of natural products is known to inhibit angiogenesis through irreversible inhibition of human type 2 methionine aminopeptidase (MetAP2). Recently, fumagillin and TNP-470 were reported to possess antimalarial activity in vitro, and it was hypothesized that this inhibition was mediated by interaction with the putative malarial ortholog of human MetAP2. In this report, we have overexpressed and purified to near-homogeneity PfMetAP2 from bacteria, yeast, and insect cells. Although none of the recombinant forms of PfMetAP2 exhibited enzymatic activity in existing assays, PfMetAP2 proteins expressed in both yeast and insect cells were able to bind to fumagillin in a pull-down assay. The interaction between fumagillin and analogs with PfMetAP2 was further demonstrated using a newly established mammalian three-hybrid assay incorporating a conjugate between dexamethasone and fumagillin. Unlike human (Hs)MetAP2, it was found that PfMetAP2 is bound to fumagillin noncovalently. Importantly, a new analog of fumagillin, fumarranol, was demonstrated to interact with PfMetAP2 and inhibit the growth of both chloroquine-sensitive and drug-resistant *Plasmodium falciparum* strains in vitro. Antiparasite activity of fumagillin and fumarranol was also demonstrated in vivo.

using a mouse malaria model. These findings suggest that PfMetAP2 is a viable target, and fumarranol is a promising lead compound for the development of novel antimalarial agents.

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Invited Review: Primaquine revisited six decades after its discovery

Nuno Vale, Rui Moreira, Paula Gomes

European Journal of Medicinal Chemistry, Volume 44, Issue 3, March 2009, Pages 937-953

Primaquine was firstly synthesized in 1946 in the USA, and is the most representative member of the anti-malarial 8-aminoquinolines. Six decades have passed and primaquine is still the only transmission-blocking anti-malarial clinically available, displaying a marked activity against gametocytes of all species of human malaria, including multi-resistant *Plasmodium falciparum* strains. Primaquine is also effective against all exoerythrocytic forms of the parasite and is used in conjunction with other anti-malarials for the treatment of *vivax* and *ovale* malaria. However, primaquine is often associated with serious adverse effects, in consequence of its toxic metabolites. 5-Hydroxyprimaquine or 6-methoxy-8-aminoquinoline has been considered to be directly responsible for complications such as hemolytic anemia. Primaquine toxicity is aggravated in people deficient of 6-glucose phosphate dehydrogenase or glutathione synthetase. Adverse effects are further amplified by the fact that primaquine must be repeatedly

administered at high doses, due to its limited oral bioavailability. Over the last two decades, Medicinal Chemists have battled against primaquine's disadvantages, while keeping or even improving its unequalled performance as an anti-malarial. The present text revisits primaquine and its properties on the occasion of its 60th anniversary and aims to give a general overview of what has been the path towards the development of effective and safe primaquine-based anti-malarials. Presently, aablaquine and tafenoquine the two most promising primaquine analogues are already in the final stages of clinical trials against *Plasmodium vivax* and *P. falciparum*. Both compounds are a new hope against **malaria** and other primaquine-sensitive illnesses, such as Pneumocystis Pneumonia or the Chagas disease.

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Synthesis and antimalarial activity of pyrazolo and pyrimido benzothiazine dioxide derivatives

Arthur Barazarte, Gricela Lobo, Neira Gamboa, Juan R. Rodrigues, Mario V. Capparelli, Ángel Álvarez-Larena, Simón E. López, Jaime E. Charris

European Journal of Medicinal Chemistry, Volume 44, Issue 3, March 2009, Pages 1303-1310

A series of phenylsubstituted pyrazolo and pyrimido benzothiazine dioxide derivatives were synthesized and investigated for their abilities to inhibit β -hematin formation, hemoglobin hydrolysis and *in vivo* for their antimalarial efficacy in rodent *Plasmodium berghei*. Compounds 3-amino-7-chloro-9-(2'-methylphenyl)-1,9-dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide 2b and 2,4-diamino-8-chloro-10*H*-phenyl-pyrimido-[5,4-*b*]benzothiazine 5,5-dioxide 3a were the most promising as inhibitors of hemoglobin hydrolysis, however, their effect as inhibitors of β -hematin formation was marginal, except for compound 3-amino-7-chloro-9-(3'-chlorophenyl)-1,9dihydro-pyrazolo-[4,3-*b*]benzothiazine 4,4-dioxide 2g. The most active compound to emerge from the *in vitro* and *in vivomurine* studies was 2b, suggesting an antimalarial activity via inhibition of hemoglobin hydrolysis, however, not as efficient as chloroquine.

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Preliminary Communication: Synthesis and antimalarial activity of hydroxyethylpiperazine derivatives

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European Journal of Medicinal Chemistry, Volume 44, Issue 3, March 2009, Pages 1363-1368

The antimalarial activity of hydroxyethylpiperazine derivatives, synthesized from the reaction of (2*S*,3*S*)Boc-phenylalanine epoxide with benzylpiperazines in good yields (76–96%), has been evaluated *in vitro* against the *Plasmodium falciparum* W2 clone (chloroquine resistant). The results show that some compounds have moderate activity against this parasite and none of the active compounds showed cytotoxicity at high concentration (100 µg/ml).

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Regular Articles: Multiplex real-time PCR detection of *P. falciparum*, *P. vivax* and *P. malariae* in human blood samples

Vincent Veron, Stephane Simon, Bernard Carme

Experimental Parasitology, Volume 121, Issue 4, April 2009, Pages 346-351

Two duplex real-time PCR assays were developed to diagnose three human parasites: *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium malariae*. TaqMan duplex real-time PCR was evaluated in 263 blood samples of suspected **malaria** patients by comparing results against those obtained with microscopy and nested PCR. Compared with nested PCR, duplex real-time PCR assays showed 100% sensitivity and specificity. Duplex real-time PCR detected all mixtures of *P. falciparum* and *P. vivax* DNA, except at threshold detection limits for both parasites in which *P. vivax* was not amplified. Threshold detection limits of real-time PCR were 3.1, 0.3 and 0.8 parasites per microlitre of blood for *P. falciparum*, *P. vivax* and *P. malariae*, respectively. Duplex real-time PCR allows the detection of malarial cases, including mixed species infection, it simplifies analysis and reduces cost. Thus, this protocol may prove invaluable for use in the diagnosis of human infection, trial treatments and epidemiologic studies in which high-throughput analyses are often required.

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Regular Articles: *Plasmodium berghei*: Efficacy of 5-fluoroorotate in combination with commonly used antimalarial drugs in a mouse model

Francis W. Muregi, Shigeyuki Kano, Hideto Kino, Akira Ishih

Experimental Parasitology, Volume 121, Issue 4, April 2009, Pages 376-380

Resistance to antimalarial antifolates necessitates a search for new antimetabolites targeting other enzymes of the folate metabolic pathway. In this study, 5-fluoroorotate (FOA), reported to be an inhibitor of thymidylate synthase, was assayed against *Plasmodium berghei* NK 65 in mice, with(out) an oral uridine supplement. FOA (2.5 and 5.0 mg/kg bw.) was tested alone, or in a double and triple combination with a fixed oral dose of 1.25 and 2.5 mg/kg of pyrimethamine (PYR); 1.0 and 2.0 mg/kg of dapsone (DAP); 1.0 and 2.0 mg/kg of artesunate (ART). FOA achieved high suppression which ranged from 95.7% to aparasitaemic, activity that was dose-dependent. At the highest dosages used, FOA–PYR and FOA–DAP–ART combinations were synergistic with 100% cure rate, while FOA–PYR–ART was antagonistic. Drugs in a synergistic combination may exert less resistance selection pressure, thus FOA–PYR and FOA–DAP–ART warrant further evaluation with an ultimate object of possible clinical use against drug-resistant malaria.

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Armigeres subalbatus prophenoloxidase III: Cloning, characterization and potential role in morphogenesis

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Insect Biochemistry and Molecular Biology, Volume 39, Issue 2, February 2009, Pages 96-104

It has long been suggested that phenoloxidases (POs) play key roles in various physiological functions in insects, e.g., cuticular sclerotization, wound healing, egg tanning and melanotic encapsulation of pathogens. Here we report that a mosquito PO, designated *Armigeres subalbatus* prophenoloxidase III (*As-pro-PO III*), is likely involved in the morphogenesis in mosquito. Expression profile analysis found that *As-pro-PO III* mRNA is persistently expressed in adult mosquitoes and is not significantly affected by blood feeding, microfilariae inoculation, or *Escherichia coli* inoculation, but expression levels of *As-pro-PO III* fluctuated in larval and pupal stages. Knockdown of *As-pro-PO III* expression in pupae using double-stranded RNA resulted in high pupal mortality and deformed adults that subsequently died following emergence. Promoter activity analyses by electrophoretic mobility-shift assays and transfection assays suggest that the *As-pro-PO III* gene is positively regulated by a putative Zeste motif, a developmental regulatory element. These results suggest that *As-pro-PO III* is associated with morphogenesis of mosquitoes.

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The Anopheles gambiae adult midgut peritrophic matrix proteome

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Insect Biochemistry and Molecular Biology, Volume 39, Issue 2, Pages 125-134

Malaria is a devastating disease. For transmission to occur, Plasmodium, the causative agent of malaria, must complete a complex developmental cycle in its mosquito vector. Thus, the mosquito is a potential target for disease control. Plasmodium ookinetes, which develop within the mosquito midgut, must first cross the midgut's peritrophic matrix (PM), a thick extracellular sheath that completely surrounds the blood meal. The PM poses a partial, natural barrier against parasite invasion of the midgut and it is speculated that modifications to the PM may lead to a complete barrier to infection. However, such strategies require thorough characterization of the structure of the PM. Here, we describe for the first time, the complete PM proteome of the main malaria vector, *Anopheles gambiae*. Altogether, 209 proteins were identified by mass spectrometry. Among them were nine new chitin-binding peritrophic matrix proteins, expanding the list from three to twelve peritrophins. Lastly, we provide a model for the putative interactions among the proteins identified in this study.

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Original Papers: False-positive results of a rapid K39-based strip test and Chagas disease

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International Journal of Infectious Diseases, Volume 13, Issue 2, March 2009, Pages 182-185

Background

The definitive diagnosis of visceral leishmaniasis (VL) requires invasive procedures with demonstration of amastigotes in tissue or promastigotes in culture. Unfortunately, these approaches require laboratory materials not available in poor countries where the disease is endemic. The correct diagnosis of VL is important, and made more difficult by the fact that several common tropical diseases such as **malaria**, disseminated tuberculosis, and enteric fever share the same clinical presentation. Serological tests have been developed to replace parasitological diagnosis in the field. A commercially available K39-based strip test for VL has been developed for this purpose. The endemic area of leishmaniasis in Brazil overlaps the endemic area of Chagas disease, a disease that can cause false-positive serological test results. The aim of this study was to evaluate the incidence of false-positive exams using a rapid test for VL in patients with Chagas disease.

Methods

A rapid test based on the recombinant K39 antigen of *Leishmania* was used in: (1) 30 patients with confirmed Chagas disease, (2) 30 patients with a serological diagnosis of Chagas disease by ELISA, indirect immunofluorescence, indirect hemagglutination, and chemiluminescence, (3) 30 healthy patients from a non-endemic area as the control group, (4) 30 patients with confirmed VL, and (5) 20 patients with proved cutaneous leishmaniasis.

Results

The sensitivity and specificity of the rapid strip test were 100% when compared with healthy volunteers and those with confirmed Chagas disease. One false-positive result occurred in the group with Chagas disease diagnosed by serological tests (specificity of 96%).

Conclusion

The rapid test based on recombinant K39 is a useful diagnostic assay, and a false-positive result rarely occurs in patients with a serological diagnosis of Chagas disease. (No E-mail only fax: +55 11 30697508)

Short Communication: Toxicity studies of *Tithonia diversifolia* A. Gray (Asteraceae) in rats

T.O. Elufioye, O.I. Alatise, F.A. Fakoya, J.M. Agbedahunsi, P.J. Houghton

Journal of Ethnopharmacology, Volume 122, Issue 2, 18 March 2009, Pages 410-415

Objective

To investigate the toxicity of an ethanolic extract of the aerial parts of *Tithonia diversifolia*, used in Nigeria to treat **malaria**, in rats.

Materials and methods

A 70% ethanol extract was administered orally to adult Wistar rats at various dosages (400–1600 mg/kg) and the animals sacrificed and various organs examined at a range of times from 30 min up to 24 h after administration.

Results

The studies showed a dose- and time-dependent toxic effect, which was reversible on the kidney and liver while there was no noticeable adverse effect on the morphology of the heart, spleen and brain.

Conclusion

A 70% ethanol extract of the aerial parts of *Tithonia diversifolia*, which had previously been shown to reduce parasitemia in mice infected with *Plasmodium*, displayed kidney and liver toxicity at the lowest dose tested. The use of this plant extract against **malaria** therefore raises concerns over its safety.

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Correspondence: Ribonucleotide reductase inhibitors with erythropoietin and iron sulfate against malaria

Amir Ata Saei, Somaieh Ahmadian

Medical Hypotheses, Volume 72, Issue 5, May 2009, Page 611

(No Abstract)

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A multiplexed real-time PCR assay for malaria speciation with improved sensitivity for mixed infections

Sandra E. Shokoples, Momar Ndao, Kinga Kowalewska-Grochowska, and Stephanie K. Yanow

J. Clin. Microbiol. published 25 February 2009, 10.1128/JCM.01858-08

The implementation of real-time PCR for the diagnosis of malaria has been hampered by poor sensitivity for the detection of mixed infections. We have optimized a method that enhances the sensitivity for detecting minor species in mixed infections within a single, multiplex reaction. Our assay uses species-specific forward primers in combination with a conserved reverse primer, and largely overcomes primer competition for the minor species DNA. With a blind panel of clinical samples, we successfully speciated 13/16

mixed infections. This assay was further validated with 91 blood samples, and demonstrated a specificity and sensitivity for single infections of 100% compared with nested PCR as the gold standard. This test has been implemented for routine confirmation of malaria species in Alberta, Canada. In comparison with speciation by microscopy, the real-time PCR test demonstrated greater sensitivity for the speciation of low level infections, mixed infections, and discrimination of "non-*falciparum*" species. Our experience supports a role for real-time PCR in the speciation of malaria parasites in conjunction with microscopy.

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Review: Molecular machinery of signal transduction and cell cycle regulation in Plasmodium

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Molecular and Biochemical Parasitology, Volume 165, Issue 1, Pages 1-7

The regulation of the *Plasmodium* cell cycle is not understood. Although the *Plasmodium falciparum* genome is completely sequenced, about 60% of the predicted proteins share little or no sequence similarity with other eukaryotes. This feature impairs the identification of important proteins participating in the regulation of the cell cycle. There are several open questions that concern cell cycle progression in malaria parasites, including the mechanism by which multiple nuclear divisions is controlled and how the cell cycle is managed in all phases of their complex life cycle. Cell cycle synchrony of the parasite population within the host, as well as the circadian rhythm of proliferation, are striking features of some *Plasmodium* species, the molecular basis of which remains to be elucidated. In this review we discuss the role of indole-related molecules as signals that modulate the cell cycle in *Plasmodium* and other eukaryotes, and we also consider the possible role of kinases in the signal transduction and in the responses it triggers.

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Essential Skills: The successful applicant will have a PhD, or have submitted a PhD thesis and be awaiting award. Extensive experience with eukaryotic cell culture and common cellular and molecular biology techniques is essential. The successful candidate will be able to work without constant supervision as part of a dynamic team and have the ability to develop and lead their own independent research project.

Ideal Skills: Experience with *Plasmodium falciparum* in vitro culture is highly desirable, and experience in scientific projects involving *P. falciparum* blood stage biology, particularly experimental genetic approaches, an added bonus.

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Malaria conference call for abstracts and meeting registration

The Luso-American Development Foundation (FLAD) in Lisbon, Portugal, in collaboration with the US National Institute of Allergy and Infectious Diseases (NIAID) continues in 2009 with the second in a series of three research conferences highlighting infectious

diseases in Africa and the importance of sharing scientific and cultural knowledge through collaboration and the development of collaborative solutions.

The first meeting in Lisbon was in October 2007 with a focus on the treatment, diagnosis, and prevention of tuberculosis in Africa. The 2009 conference on July 8 - July 10, 2009 will focus on malaria research and its application in an African setting.

The call for abstracts is open until March 15, 2009, and includes varied topics such as malaria drugs, diagnostics, vaccines, malaria epidemiology, vector/parasite biology, insecticides and resistance, as well as the role of research networks, PPPs, and development. In addition, you can register to attend until the end of June by sending an email with your name, institution, and area of expertise to the emails below.

You can submit your information via email to Kathleen Collins [**collinska@niaid.nih.gov**](mailto:collinska@niaid.nih.gov) and Rui Vallera [**rui.vallera@flad.pt**](mailto:rui.vallera@flad.pt) which is outlined in detail on the two meeting websites. Please review the FLAD and NIAID meeting websites for complete information on the abstract submission process [**www.fladtropicaldiseases.com**](http://www.fladtropicaldiseases.com)
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We are strongly encouraging researchers working on Malaria in Lusophone Africa or working on issues related to malaria which can be applied to Africa to submit abstracts and participate in the meeting.