409

ANTI-PLASMODIAL PROPERTIES OF SOME SELECTED GHANAIAN MEDICINAL PLANTS; IDENTIFICATION OF NOVEL ACTIVE COMPOUNDS AGAINST *PLASMODIUM FALCIPARUM*

Jeffrey Agyapong[1], Mitsuko Suzuki[2], Gordon A. Awandare[3], Michael Ofori[1], Irene Ayi[1], Eric H. Frempong[4], Daniel A. Boakye[1]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Tokyo Medical and Dental University, Tokyo, Japan, [3]University of Ghana, Accra, Ghana, [4]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Malaria, caused by *Plasmodium* spp has been considered as a major disease of public health importance affecting multitudes of people worldwide, particularly in the tropics and sub-tropics. Due to increasing drug resistant parasites to existing drugs, development of new anti-malarial drugs are eagerly awaited. In Africa, there is extensive use of traditional medicinal plants for treatment of various diseases. Many research studies into medicinal plants have shown their significant potentials for anti-plasmodial properties but a few of the active ingredients have been studied.. The present study aimed at screening selected medicinal plants used in Ghana for activity against *Plasmodium falciparum* to determine their active compounds. A high-throughput 96 wells flow-cytometry screening system was established using the method reported by Smilkstein and others, with modification. 50% Et-OH crude extracts of medicinal plants were prepared and applied to synchronized *P. falciparum* (3D7 strains) culture and FACS analysis was carried out to determine the IC_{50} of the extracts. Although screening is still ongoing, one active crude extract, JJNC008L, was found possessing activities against *P. falciparum* and identified two novel compounds from this extract, compound #1 and #2. These compounds have significantly high anti- *P. falciparum* activities with IC_{50} values of 3.89 µM and 1.52 µM respectively. Novel compounds identified in this study could be candidates to develop new chemotherapy for malaria. Furthermore, our high-throughput FACS screening system could be useful tool for malaria drug assay.

410

PRODUCTION AND CHARACTERIZATION OF A LIBRARY OF FULL-LENGTH *PLASMODIUM VIVAX* MEROZOITE PROTEINS

Jessica Hostetler[1], Sumana Sharma[2], Gavin J. Wright[2], Julian Rayner[2], Rick Fairhurst[1]

[1]National Institutes of Health, Rockville, MD, United States, [2]Wellcome Trust Sanger Institute, Hinxton, United Kingdom

A vaccine targeting the illness-inducing blood stage of parasite development is an essential component of any worldwide malaria eradication campaign, but major gaps in our understanding of *Plasmodium vivax* biology, including the protein-protein interactions that mediate erythrocyte invasion, hinder the search for an effective vaccine. Only a single parasite ligand-host receptor interaction is presently known, that between *P. vivax* Duffy Binding Protein (PvDBP) and Duffy Antigen Receptor for Chemokines (DARC), and strain-specific immune responses to PvDBP make this antigen a challenging vaccine target. We are carrying out a comprehensive study of *P. vivax* proteins that mediate erythrocyte binding and invasion in order to identify additional vaccine candidates. As a first step, we produced a library of 39 full-length recombinant *P. vivax* proteins to test for erythrocyte binding and immunoreactivity. To our knowledge, this represents the largest full-length *P. vivax* antigen set ever assembled. Candidates were selected based on predicted localization to the merozoite surface or invasive secretory organelles, and on homology to *P. falciparum* vaccine candidates. 37/39 *P. vivax* recombinant proteins were expressed in the HEK293E cell system, which has been successfully used for expression of full-length *P. falciparum* invasion ligands such as PfRH5. Known or predicted functions, such as the interaction between merozoite surface proteins Pv12 and Pv41, were confirmed and several novel parasite protein-protein interactions were identified. Pilot immunoreactivity screens using sera from Cambodian patients with *P. vivax* malaria showed that IgG variously recognize the majority of antigens tested. The large-scale initial screenings of this library will be presented through protein expression, protein interaction and seroreactivity data, as well as immuno-epidemiological studies.

411

GENETIC VARIABILITY AND POPULATION STRUCTURE OF KENYAN *PLASMODIUM FALCIPARUM* ISOLATES

Luicer I. Olubayo

United States Army Medical Research Unit-Kenya, Kisumu, Kenya

Malaria parasite genetic variability and population structure varies among areas of differing endemicity and are key factors in malaria control strategies. Unlike previously used antigen-encoding loci under strong selection, *Plasmodium falciparum* microsatellite markers are an attractive target for population structure studies. In Kenya, molecular studies on drug resistant malaria and antigenic molecules have been inadequate in providing accurate genetic profiles of parasite populations in the country. Understanding the genetic structure of malaria parasites is essential to predict how fast phenotypes of interest, such as novel antigenic variants or drug resistant alleles, originate and spread in populations. Twelve polymorphic *P. falciparum* microsatellite loci were genotyped in 250 parasite isolates from five locations in Kenya using capillary electrophoresis. Analysis of the generated fragments was performed to determine proportions of mixed genotype infections, genotype diversity among isolates, multilocus standardized index of association, and inter-population differentiation. The data revealed dramatic differences in parasite population structure in different geographical locations. An 80% multiplicity of infection was detected in parasites circulating in Kenya. Samples from Kisumu, a high malaria endemic region had a high diversity (He _ 0.73) revealing genetic crossing. Results have shown a *P. falciparum* population structure as well as significant linkage disequilibrium in Kenyan parasites. Regional diversity was observed in range of population structures. These results could be related to geographic difference and low flow of parasites between sites. These data reveal a range of population structures within a single pathogen species and suggest intimate links between patterns of epidemiology and genetic structure.

412

GENETIC DIVERSITY OF *PLASMODIUM VIVAX* IN AREAS OF HIGH RISK OF MALARIA IN CÓRDOBA-COLOMBIA

Carlos J. Castro, Virginia Rodriguez, Maria Fernanda Yasnot

Universidad de Córdoba, Monteria, Colombia

The aim of this work was to study the genetic diversity of natural populations of *Plasmodium vivax* in areas of high malaria risk in Córdoba-Colombia. Molecular confirmation of infection by *P. vivax* DNA extraction using Chelex-100 was made, thereafter a nested PCR protocol was used to amplify the gene encoding 18S ribosomal small subunit (ssrARN) *P. vivax* and *P. falciparum*. A sample confirmed as *P. vivax* underwent a PCR -RFLP for Pvmsp - 3α gene, restriction enzymes used were Alu I and Hha I, which generated an electrophoretic profile that identified different haplotypes for Pvmsp - 3α gene.Of the 125 samples analyzed by nested PCR for Pvmsp - 3α gene, 116 successfully amplified. The size of the PCR products allowed to demonstrate the movement of three different genotypes for the Pvmsp - 3α gene: A (1900 bp), type B (1500 bp) and type C (1100 bp), being the most frequent genotype A (88%). 97.4 % (113 /116) of the samples showed simple infections and 2,6% (3/116) polyclonal infections, two by types A and B and one by types A and C. Besides finding a band is reported in the electrophoretic profile of the amplification products Pvmsp -3α gene with an approximate size of 800 bp which does not correspond with the sizes reported to date, which may represent a new allele of Pvmsp-3α gene however, these results should be interpreted cautiously, pending the results of sequencing. Digestion of PCR products obtained for the Pvmsp - 3α gene with enzyme Alu I showed ten different restriction patterns while the with the enzyme Hha I produced nine. The results of