

Tropical Disease Research



▶ Results Portfolio 3

A collection of Final Reports

from projects selected by

independent scientific experts



Malaria

▶ Artemisinin represents an important class of antimalarial drugs. To improve upon the pharmacokinetic profile of artemisinin and related second-generation antimalarial drugs, a series of analogues has been designed that are more bioavailable and less likely to be metabolised to dihydroartemisinin, which has been associated with neurotoxicity in various *in vitro* and *in vivo* assays. Preliminary data from mice infected with *Plasmodium berghei* are extremely encouraging, with at least one compound showing considerably improved efficacy. Further studies are in progress.

Natural products have had a fundamental impact upon the treatment of malaria. Extracts from the herb *Artemisia annua* have been used in China since AD341 to treat febrile illness. In 1971, the active component, quinghaosu (now known as artemisinin) was identified and shown to be effective against malaria, including chloroquine-resistant disease. Nature produces many biologically active compounds, but most have toxicological and/or pharmacokinetic limitations. To produce safer, more effective drugs, these compounds need to be improved, including artemisinin, which has a sub-optimal pharmacokinetic profile with poor bioavailability, resulting in high relapse rates.

Artemisinin research has led to several second-generation drugs (e.g. artemether, arteether, artesunate, and artelinic acid – all derivatives of the lactol, dihydroartemisinin (DHA)). Although improvements on artemisinin, none are ideal and suffer such problems as toxicity and poor stability and oral bioavailability. These compounds are rapidly metabolized and one metabolite, DHA, may be associated with neurological side-effects.

Attempts to synthesise novel derivatives with improved metabolic stability are promising. Substituents with basic amino side-chains have also been incorporated into target molecules to increase uptake into parasites. Such molecules have the bonus of being able to form water-soluble salts, aiding

dissolution following oral administration and facilitating formulation for parenteral administration. Initially, benzyloxy derivatives of DHA were synthesized containing a diamine side-chain to establish if the presence of amino functionality would reduce activity. These compounds (type 1) were more active than artemisinin when tested *in vitro* against *Plasmodium falciparum*, with activity similar to that of artemether. Potency was retained against chloroquine-resistant strains. To increase metabolic stability and prevent conversion to DHA, analogues were prepared in which the C-10 benzyloxydiamine substituents were replaced with amino-substituted alkyl groups. These compounds (type 2) were also potent *in vitro* against *P. falciparum*, one proving to be 2-3 fold more efficacious than artemether in *P. berghei*-infected mice. They are extremely promising as they are unlikely to suffer metabolic conversion to DHA and their structure should facilitate oral absorption. Further evaluation of the compounds is under way. ■

References:

O'Neill, P.M. et al. (2001) *Synthesis, antimalarial activity, biomimetic iron(II) chemistry, and in vivo metabolism of novel, potent C-10-phenoxy derivatives of dihydroartemisinin*. *J. Med. Chem.* 44:58-68

Hindley, S., et al. (2001) *Mechanism-based design of parasite targeted artemisinin derivatives: Synthesis and antimalarial activity of new diamine containing analogues*. *J. Med. Chem.* (submitted)

PROJECT NO. 980176

Synthesis of novel antimalarial peroxides

PRINCIPAL INVESTIGATOR

Dr P. M. O'Neill
Department of Chemistry
University of Liverpool
Liverpool L69 7ZD
UNITED KINGDOM

e-mail:
p.m.oneill01@liverpool.ac.uk

COLLABORATORS

S. Hindley
S.A. Ward
R.C. Storr
N. L. Searle
P. G. Bray
B. K. Park
J. Davies



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdrnews@who.int
www.who.int/tdr

Information source:

Dr M. M. Bendig
e-mail: bendigm@who.int

Malaria

▶ A 2-year study of malaria control began in Henan Province following cuts in government malaria spending in 1993. Cost data were collected from all government levels and on treatment-seeking (diagnosis, treatment) from 12,325 suspected malaria cases in two endemic counties. The cost burden was found to fall mainly on patients, but using government infrastructure. Good stewardship requires continuing government investment, to at least current levels, along with improved case-management. In mainland China, vivax malaria is a significant factor in poverty and economic underdevelopment.

A 2-year prospective study of the costs and performance of Henan Province's malaria control programme focused on two counties with endemic vivax malaria (population=2,093,100). Diagnosis, treatment and other expenses were calculated for a sample of suspected malaria cases (n=12,325), as well as grading the performance of Village Doctors. Two non-personal malaria control activities—vector control and blood testing—were also costed. From the patient perspective, the average cost (direct and indirect) of treatment for suspected malaria was 27.85 yuan (US\$3.38), equivalent to 10 days' income for local peasants. Each case cost the government an average of 5.60 yuan (US\$0.70). For Henan, the average annual government expenditure for malaria control (vector surveillance 12%, active blood surveys 25% and case-management 60%) was 798,322 yuan (US\$99,790). Of 12,325 suspected malaria cases, only 131 (1%) received excellent case-management, 4,414 (36%) were managed in a mediocre manner and 7,780 (63%) were inadequately managed. Case management would improve if Village Doctors began antimalaria treatment more promptly (within 3 days of diagnosis) and continued treatment longer (at least 3 days), both correctable without increasing government investment. Other key aspects of case management, rapid patient access to care and choice of drug, are already operating well.

The cost of malaria control in Henan is largely borne by suspected cases and their families. Government spending decrease will increase this burden and patients may delay treatment, with a concomitant risk of recrudescence transmission and ultimate breakdown of control, as has occurred in South Asia but not in China. Researchers concluded that efforts should be made to improve the efficiency of case-management if malaria eradication is to be achieved within Henan, and that good management requires continued government investment in malaria control to at least the current levels. Only then will this section of the health system perform optimally. Vivax malaria is responsible for over 50% of malaria morbidity outside Africa and has been of great importance historically. It should not be underestimated as a factor contributing to poverty and economic underdevelopment in China. Methods devised for this research have produced evidence useful for development of health policy for malaria, and which could also be applied widely within the Chinese health sector. ■

References:

Liu, X. et al. (1996)
Malaria control and fever management in Henan province, China.
Tropical Medicine and International Health.
1; 112-116

Sleigh, A.C. et al. (1998)
Resurgence of vivax malaria in Henan Province, China
WHO Bulletin. 76; 265-270

PROJECT No. 930413
950109

Cost and performance analysis
for malaria control in Henan
Province, China

PRINCIPAL INVESTIGATOR

Prof. Xi-Li Liu
Henan Institute
of Parasitic Diseases
Zhengzhou
Henan Province
CHINA

e-mail:
liuxili@public2.zz.ha.cn

COLLABORATORS

Dr S. Jackson (AUS)
Dr A. C. Sleigh (AUS)



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdrnews@who.int
www.who.int/tdr

Information source:

Dr J. Sommerfeld
e-mail: sommerfeldj@who.int

Malaria

► A stable transformation of the major mosquito vector of malaria, *Anopheles gambiae*, was achieved using the transposable element 'piggyBac', marked with the Enhanced Green Fluorescent Protein (EGFP) gene. The embryo survival, hatching and EGFP transient expression rates were also greatly increased by improvement of the microinjection technique, through covering the embryos to be injected with an aqueous solution of 25mM NaCl. These results represent an important step toward the genetic transformation of *An. gambiae* mosquitoes so that they are unable to transmit malaria parasites.

Advances in the understanding of vector genetics and vector-parasite/virus relationships have provided novel tools to develop new strategies for the control of disease transmission. Recent advances include the development of genetic and molecular tools for engineering mosquitoes resistant to pathogen transmission, the characterization of relevant promoters and improvement of germ-line transformation methods. The main objective of this project was to devise a method for the germ-line transformation of *Anopheles gambiae*. Systematic investigations of the utility of selected class II transposable elements and GFP were conducted. The protocols were optimized for transforming *An. gambiae* using a piggyBac/EGFP construct. Successful transformation of *An. gambiae* was achieved with:

- the plasmid pK[BIG α], a piggyBac transformation vector containing an insertion of the EGFP gene under the control of the baculovirus hr5-iel promoter and the lacZ coding sequence, placed in a kanamycin-resistant vector and,
 - the pB/hs Δ st, a piggyBac element helper plasmid, with the *Drosophila melanogaster* heat-shock (hsp-70) promoter, driving expression of the piggyBac transposase.
- The vector and the helper were injected at a concentration of 0.25 and 0.1 mg/ml respectively.

Of 1,954 embryos inoculated, 457 larvae hatched, 172 surviving to adulthood. Survivors were outcrossed and 36,000 G1 larvae were screened. Two progeny males expressed EGFP and were used to found separate lines, in which both males and females expressed EGFP. Molecular and genetic analyses suggest that there was a precise insertion of piggyBac.

The microinjection technique was also improved by simply covering the embryos to be injected with an aqueous solution of 25mM NaCl, which minimizes yolk flow from the egg wound site. No chemical treatment was used to prevent chorion hardening. Larval hatch rates varied between 8-45% with this method and EGFP transient expression rates among hatched larvae ranged from 67-78%. These rates, much higher than previous observations, are attributable to this simple change in technique.

These findings, which represent a landmark in vector biology, have important implications for the development of new strategies for malaria control. ■

References:

- Grossman, G.L. et al. (2000). *The piggyBac element is capable of precise excision and transposition in cells and embryos of the mosquito, Anopheles gambiae*. *Insect Biochem. Molec. Biol.* 30:909-914
- Catteruccia, F. et al. (2000). *Stable germline transformation of the malaria mosquito, Anopheles stephensi*. *Nature*. 405:959-962

PROJECT No. 970417

Genetic transformation of
Anopheles gambiae

PRINCIPAL INVESTIGATOR

Dr M. Benedict
Centers for Disease Control and
Prevention
Division of Parasitic Diseases
Atlanta 30341-3724
Georgia
USA
e-mail:mqb0@cdc.gov

COLLABORATORS

G.L. Grossman
C.S. Rafferty
J.R. Clayton
T.K. Stevens
O. Mukabayire



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Yeya T. Touré
e-mail: tourey@who.int

Malaria

▶ A phage display library approach was used to identify a 12-amino acid peptide, termed SM1, that binds to mosquito midgut and salivary gland tissues. SM1 inhibits development of a malaria parasite, *Plasmodium berghei*, by interfering with invasion of these tissues. Transgenic *Anopheles stephensi* that express an SM1 tetramer from a blood-inducible and gut-specific promoter are impaired in their ability to sustain *P. berghei* development and transmission. This represents an important milestone toward the goal of controlling malaria transmission by genetic alteration of mosquito capacity to transmit malaria.

In order to genetically engineer mosquitoes that are resistant to pathogen transmission, it is crucial to understand the molecular basis of vectorial resistance through investigation of vector-parasite/virus interactions (midgut, hemolymph and salivary gland targets). The objective of this research was to characterize receptors on the midgut and salivary gland epithelial surfaces that mediate parasite invasion. Blockage of such receptors may lead to the development of novel strategies for reducing disease transmission.

Phage display libraries were used to identify peptides that bind to midgut and salivary gland tissues. Peptides that bound were assessed for their ability to block parasite invasion of the tissues. The most frequently isolated phage (termed SM1) bound to both midgut and salivary gland epithelia. It blocks sporozoite invasion of the salivary glands and ookinete invasion of the midgut epithelium. These results indicate that the peptide and the parasite recognize the same ligand on the surface of the two epithelia and that the peptide inhibits invasion by competing with the parasite for this ligand.

A synthetic gene (termed AgCP[SM1]4) consisting of four SM1 units joined by 4-amino acid linkers attached to the CP (carboxypeptidase) signal sequence and driven by the gut-specific and blood-inducible CP promoter was constructed. This gene was inserted into a piggyBac vector (pBacAgCP[SM1]4), mixed with

a piggyBac helper and introduced into the *An. stephensi* germ line. Of 394 embryos injected, 63 (16%) larvae hatched, yielding 33 (8.4%) adults. The adults were distributed into 14 families, of which 2 yielded Green Fluorescent Protein (GFP)-positive progeny.

To measure the effect of AgCP[SM1]4 transgene expression on parasite development, control and transgenic mosquitoes were fed on the same *P. berghei*-infected mouse and oocysts were counted. In nine experiments, inhibition of oocyst formation ranged between 68.7-94.9% (average 81.6%). Importantly, vector competence of transgenic mosquitoes was severely impaired. In two out of three experiments, no transmission was detected and in the third, transmission was reduced by more than two-fold.

This is the first demonstration of the feasibility of blocking malaria transmission by a transgenic approach. Results suggest that a similar approach can be used to develop transgenic *An. gambiae* that are impaired in the transmission of parasites that cause human malaria.

References:

Catteruccia, F. et. al. (2000)
Stable germline transformation of the malaria mosquito *Anopheles stephensi*.
Nature. 405: 959-962

Ghosh, A., Ribolla, P. & Jacobs-Lorena, M. (2001)
Targeting *Plasmodium* ligands on mosquito salivary glands and midgut with a phage display library.
Proc. Natl. Acad. Sci. USA. 98:13278-13281

PROJECT No. 980689

Targeting *Anopheles* organs with a phage display peptide library

PRINCIPAL INVESTIGATOR

Dr M. Jacobs-Lorena
Case Western Reserve University
School of Medicine
Department of Genetics
BRB 631, 10900 Euclid Avenue
Cleveland, Ohio 44106-4955
USA

e-mail: mxj3@po.cwru.edu

COLLABORATORS

J. Ito
A. Ghosh
L. A. Moreira
E. A. Wimmer (GERMANY)



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Yeya T. Touré
e-mail: tourey@who.int

Lymphatic filariasis

▶ A meta-analysis was undertaken of published data on age-specific prevalence and intensity of lymphatic filariasis infection and a standardized database has been created of infection data for 213 communities from different endemic regions of the world. The patterns in Africa and Asia showed an increasing prevalence until a certain age and a levelling off thereafter. In Africa the maximum prevalence was reached at around 40 years of age, whereas in Asia it was reached much earlier, at 15-20 years of age. These patterns were independent of endemicity level.

The current version of the simulation model that has been developed for the transmission and control of lymphatic filariasis (LYMFASIM) has been quantified using epidemiological data for urban Pondicherry in India, where the prevalence and intensity of infection peaks at 30 years of age and declines thereafter. The age-specific trend in prevalence and intensity of *Wuchereria bancrofti* microfilariae is a reflection of acquired immunity and of age differences in exposure, and to fit the model to the declining infection levels in older age groups in Pondicherry, strong immune effects had to be assumed. However, there was concern that the age-specific infection pattern observed in urban Pondicherry may not be representative of the situation elsewhere. In order to establish a more comprehensive understanding of the general pattern, a meta-analysis was undertaken of all published data on the prevalence and intensity of lymphatic filariasis infection by age. Published literature on lymphatic filariasis was scrutinized to identify studies which provide information on community prevalence of microfilaraemia (mf). From the identified studies, important information such as study area, population size, vector species, blood-sampling technique, volume of blood examined and the number of subjects found to have microfilaria, was extracted. A standardized database on

mf prevalence and intensity for 213 communities in different endemic regions of the world has been created. In most Asian communities, it was found that the prevalence of microfilariae increased with age, to reach a maximum level around 15-20 years of age, thereafter remaining steady at almost the same level. In Africa, the prevalence increased with age until it reached a peak at around 40 years, after which it also levelled off. Thus, the age of peak mf prevalence was strikingly different between Asia and Africa but, within each region, the pattern was remarkably similar and independent of endemicity level. Detailed statistical analysis of data from Haiti and Orissa, India, has been carried out using log likelihood test and logistic regression models. The analyses yielded consistent results and showed that mf prevalence is constant in adult age classes. ■

PROJECT No. A00830

Meta-analysis in the context of the LYMFASIM project

PRINCIPAL INVESTIGATOR

Dr K.P. Ramaiah
Vector Control Research Centre
Indira Nagar
605006 Pondicherry
INDIA

e-mail: mosquito@sancharnet.in

COLLABORATORS

J.D. Habbema (NETHERLANDS)
W.A. Stolk (NETHERLANDS)



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Hans Remme
e-mail: remmej@who.int

Onchocerciasis

▶ A study undertaken in Uganda investigated a new rapid assessment method for ivermectin treatment coverage at the community level. Results suggest that community treatment coverage can be estimated effectively and swiftly using a method of assessing treatment coverage in schoolchildren, monitored by schoolteachers. A follow-up study has been launched to validate the method, after it has been scaled up to district level and implemented by the district health management team.

Ivermectin treatment for onchocerciasis control must be monitored regularly, especially with regard to coverage. Communities with poor or insufficient coverage need to be identified quickly in order to institute timely and appropriate interventions to improve coverage. Efficient use of existing education infrastructure offers the potential to reduce complexities of logistics and manpower requirements in the monitoring of intervention programmes.

A study was carried out in Uganda to discover whether ivermectin treatment coverage in schoolchildren, as monitored by schoolteachers, could be used as a proxy for treatment coverage among the population in general. There was a significant correlation ($P < 0.01$) between coverage estimates based on the school-children survey and those arising from the standard household survey. However, there was poor correlation between estimates based on the household survey and those obtained from the treatment register that is maintained at the community level. The register-based coverage figures are the ones which the district health services normally use to

report treatment coverage. Poor correlation between household survey and the community register figures could indicate that coverage figures from the register may be unreliable.

The results of the study suggest that ivermectin treatment coverage in schoolchildren monitored by schoolteachers could give a good approximation of the total population coverage. A follow-up study has been started to validate the school-based survey method after scaling up to cover all schools and communities in several health districts and implementation of the method by the district health management team. ■

PROJECT No. A00600

Simple, rapid method for monitoring ivermectin treatment coverage in Uganda

PRINCIPAL INVESTIGATOR

Dr R. Ndyomugenyi
Ministry of Health
P.O. Box 1661
Kampala
UGANDA

e-mail: vcdmoh@imul.com
notf@imul.com



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Hans Remme
e-mail: remmej@who.int

Lymphatic filariasis

▶ A study was undertaken in Tamil Nadu, India to determine the long-term impact of annual drug treatment with single dose DEC, single dose ivermectin, or a combination of these drugs on the transmission of bancroftian filariasis. After six annual rounds of treatment there was a dramatic and similar reduction in transmission in both the DEC and the ivermectin arm of the study. However, complete interruption of transmission was not achieved and it is planned to continue the study for another three years.

▶ The goal of this project, which began in 1993, was to determine the impact of repeated mass treatment with single-dose diethylcarbamazine (DEC) or ivermectin on the transmission of bancroftian filariasis. The study is being carried out in 20 villages in Villupuram district in Tamil Nadu, India, covering a total population of some 34,000. Study villages are well separated from one another and are considered as independent transmission zones. The villages were randomly allocated to blocks of five villages, each block being randomly assigned to one of four treatment groups: single dose ivermectin, single dose DEC, ivermectin+DEC, and placebo (this arm was dropped after 4 rounds when the beneficial impact of treatment had been clearly demonstrated). The first two treatment rounds occurred at 6-monthly intervals, subsequent treatments were given annually. The ivermectin+DEC combination was introduced in 1996.

The initially-planned rounds of mass treatment have been completed and results of the entomological evaluation of their impact (as well as for four rounds of combination therapy) are now

available. After six rounds of mass treatment with DEC and ivermectin, the infection rate of resting mosquitoes declined by 83% and 86%, the infectivity rate by 80% and 82%, and the Transmission Intensity Index by 86% and 59% respectively. The Annual Transmission Potential was at only 5% of pre-intervention level in the DEC block of villages and 20% in the ivermectin block. The results suggest that DEC is as effective as ivermectin in drastically reducing all parameters.

Results after four rounds of treatment with DEC+ivermectin suggest that the combination therapy may be even more effective. Complete interruption of transmission was not achieved, suggesting that more rounds of mass drug administration are required to achieve elimination. Hence, it is planned to continue the study for at least three more treatment rounds, to assess if transmission can indeed be interrupted. ■

Reference:

Das, P. K. et al. (2001)

Placebo-controlled community trial of four cycles of single-dose diethylcarbamazine or ivermectin against *Wuchereria bancrofti* infection and transmission in India.

Trans. Roy. Soc. Trop. Med. & Hyg. 95(3): 336-41

PROJECT No. 920702

Evaluate impact of mass chemotherapy with ivermectin or diethylcarbamazine on transmission of bancroftian filariasis

PRINCIPAL INVESTIGATOR

Dr P.K. Das
Vector Control Research Centre
Indira Nagar
605006 Pondicherry
INDIA

e-mail: mosquito@sancharnet.in



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Hans Remme
e-mail: remmej@who.int

Lymphatic filariasis

▶ LYMFASIM is a simulation model of the transmission and control of lymphatic filariasis that has been developed to predict the long-term impact of intervention strategies based on vector control and chemotherapy. In this study, the current LYMFASIM model was used for a sensitivity analysis to estimate the number of treatment rounds and the treatment coverage that are needed to achieve elimination of bancroftian filariasis through annual mass treatment with either diethylcarbamazine (DEC) or ivermectin.

Following the introduction of national programmes to eliminate lymphatic filariasis, it has become essential to fully understand what is needed to achieve elimination. The recently developed LYMFASIM simulation model was used to estimate the number of treatment rounds and population treatment coverage that are required for elimination of the disease.

Analysis involved several steps: simulation of the impact of mass treatment, varying the number of treatment rounds and population coverage (all other assumptions remaining unchanged); determining for each simulation whether elimination occurred; and saving all simulation results in a database for statistical analysis. Logistic regression was used to smooth simulation results and estimate the coverage needed to achieve elimination with 99% probability for different number of treatments. The process was repeated with different assumptions on choice of drug, frequency of treatment, endemicity level and host's immune responses. Results show that the probability of elimination depends in a non-linear fashion on treatment coverage, number of treatment rounds

and endemicity level. If, for example, an area has a microfilaraemia prevalence of 10%, and 80% of the population is covered by each round of treatment, then 4-5 rounds are predicted to be sufficient to achieve elimination. If treatment coverage is 60%, then nine rounds will be required.

The study also found that annual mass treatment using diethylcarbamazine (DEC) or ivermectin is equally effective in stopping transmission, and that the predicted impact of mass treatment strongly depends on assumptions concerning host immune responses. Annual mass treatment with a hypothetical drug that kills 100% of microfilaria, but does not affect adult worms, would never lead to elimination. If a perfect macrofilaricide (100% killing of adult worms) becomes available for use in mass treatment, elimination could be reached sooner than with single-dose DEC or ivermectin. ■

References:

Plaisier, A. P. et. al. (1998)
The LYMFASIM simulation program for modeling lymphatic filariasis and its control.
Methods Inf. Med. 37(1): 97-108

Plaisier, A. P. et. al. (2000)
Effectiveness of annual ivermectin treatment for Wuchereria bancrofti infection.
Parasitol. Today 16(7): 298-302

PROJECT No. 970817

LYMFASIM simulation model for comparative assessment of chemotherapy strategies in Wuchereria bancrofti endemic areas of India/Tanzania

PRINCIPAL INVESTIGATOR

Prof. J.D.F. Habbema
Erasmus University
Rotterdam
THE NETHERLANDS

e-mail: Habbema@mgz.fgg.eur.nl

COLLABORATORS

P.K. Das (INDIA)
P.E. Simonsen (DENMARK)
D.W. Meyrowitsch (DENMARK)
G. Dreyer (BRAZIL)



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Hans Remme
e-mail: remmej@who.int

Malaria

▶ This work, part of a research capacity strengthening plan, investigated aspects of multidrug therapy for malaria. The efficacy and safety of drug combinations and sequentially administered drugs were examined, studying impact in both drug-sensitive and drug-resistant infections. Several resistance-reversing compounds proved effective against resistant malaria parasites, and combinations and sequentially administered drugs showed positive outcomes. The results offer potential for devising new treatment strategies for malaria and for delaying the emergence of drug resistance.

▶ Optimisation of drug therapy is dependent on a sound understanding of the pharmacological basis of therapeutics with respect to pharmacokinetic and pharmacodynamic properties.

The impact of these factors is accentuated when multiple drugs are administered, either in combination or sequentially.

Inadvertent interactions, resulting in altered potency and toxicity, are potential outcomes of such strategies. Existing knowledge of several antimalarials was applied to design a new combination/sequential therapy for falciparum malaria. The goal was to achieve maximum anti-malarial effect in drug-resistant and drug-sensitive infections.

Carried out in the context of a TDR Career Development Grant, the work was within the overall research capability strengthening plan for the University of Ibadan, Nigeria. The project built on previous observations that certain anti-histaminic drugs can potentiate the effect of chloroquine against chloroquine-resistant isolates of *Plasmodium falciparum* and restore toxicity of the drug against the parasite. Dosing regimens incorporating this principle were established and parasite viability (PVR), disposition

kinetics of parasitemias and parasite clearance time (PCT) used as indices of therapeutic impact.

Combination of chloroquine with chlorpheniramine proved effective in treating children and pregnant women with chloroquine-resistant infections. Sequential dosing of chloroquine, chlorpheniramine and sulphadoxine-pyrimethamine (SP) proved effective in children with SP- or chloroquine-resistant malaria. A combination of artemether and mefloquine proved safe and effective when given in the 3rd trimester of pregnancy.

Knowledge of the efficacy of combination therapy and proof of principle of clinical use of resistance-reversing compounds in chloroquine-resistant malaria holds promise for development of new treatment strategies and for retarding the emergence of drug resistance. ■

References:

Sowunmi, A. et. al. (2000)
Plasmodium falciparum kinetics during treatment with antimalarial drugs in children.
Clinical Drug Invest., 20: 43-51

Sowunmi, A. et. al. (2000)
Comparative efficacy and safety of two regimens of chloroquine plus chlorpheniramine in acute uncomplicated falciparum malaria in children.
Clinical Drug Invest., 20: 317-325

PROJECT No. 940786

Novel approach to sequential combination therapy of falciparum malaria

PRINCIPAL INVESTIGATOR

Prof. A. Sowunmi
Department of Pharmacology & Therapeutics
College of Medicine
University of Ibadan, Ibadan
NIGERIA.

e-mail:
malaria.iba@alpha.linkserve.com



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Steven Wayling
e-mail: waylings@who.int

Schistosomiasis

▶ Prof. Zhou Xiaonong, recipient of TDR Ph.D. training support, re-entry grant and RCS applied field research funding has, over the past decade, developed and used state-of-the-art geographic information system (GIS) models to predict snail habitat distribution and the prevalence of schistosomiasis japonicum in China. This digital technology can predict, and help prevent, outbreaks of schistosomiasis. The system can be used to identify populations most at risk for the disease and improve the cost-effectiveness of interventions and disease control programmes.

Following graduate studies at the Jiangsu Institute of Parasitic Diseases in China, Dr Zhou Xiaonong was awarded a research training grant to undertake doctoral studies on malacology at the University of Copenhagen in Denmark. Upon returning to the Jiangsu Institute, he received a re-entry grant for work on the population genetics, cytogenetics, morphology and distribution of *Oncomelania hupensis*, the intermediate host of *Schistosoma japonicum*. He subsequently received RCS funding to blend his knowledge of snails with geographic information systems (GIS) and remote sensing in the field. The GIS model developed by Zhou (in collaboration with colleagues at the Danish Bilharzia Laboratory, Louisiana State University (USA)) used satellite remote sensing and environmental data. Selected data - temperature, soil, elevation, vegetation, hydrology, population centres, land use - overlaid onto a base map produces a digital map that can be of use in predicting disease outbreaks.

In 1998, when China's Yangtze River burst its banks, causing massive flooding and heavy loss of life, the GIS model showed its value. Using satellite images from the summer flood season and the following spring, in combination with various ecological factors, maps were created to predict snail habitats and disease risk. A field survey was also carried out to assess the real situation. When the sets of data were compared,

90% of the snail habitats predicted by the model were confirmed, including two entirely new ones.

The Jiangsu Institute has since hosted a TDR-funded GIS training course for Chinese researchers, established a GIS laboratory, and extended training projects to include researchers from other countries. This programme was completed with assistance from the Centers for Disease Control and Prevention (USA) and the Environmental Systems Research Institute (ESRI), the largest research and development organization dedicated to GIS.

In related work, Zhou is developing new computer models to assess the potential impact on schistosomiasis transmission of China's Three Gorges Dam project (due for completion by 2009) in an effort to predict the possible spread of schistosomiasis into currently non-endemic areas.

In 2001, Dr Zhou was promoted to become Deputy-Director and Professor at the Institute of Parasitic Diseases in Shanghai, the leading research institute for parasitic diseases in China. ■

References:

Zhou, X.N. et al. (2001)
Application of geographic information systems and remote sensing to schistosomiasis control in China. *Acta Trop.* Apr 27;79(1): 97-106

Malone, J.B. et al. (2001)
A global network for the control of snail-borne disease using satellite surveillance and geographic information systems.
Acta Trop. Apr 27;79(1):7-12

PROJECT No. 970990

Snail distribution and assessment of the risk of schistosomiasis japonicum by remote sensing and geographic information systems in the lower Yangtze River basin, China

PRINCIPAL INVESTIGATOR

Prof. Zhou Xiaonong
Chinese Academy of Preventive Medicine
Institute of Parasitic Diseases
207 Rui Jin Er Lu
Shanghai 200025
CHINA

e-mail: zhouxn@hotmail.com

COLLABORATORS

J. Malone (USA)



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Steven Wayling
e-mail: waylings@who.int

Malaria

► The merozoite surface protein-1 (MSP-1) is a primary vaccine candidate antigen. Study of allelic diversity in *Plasmodium falciparum* revealed extensive variation in distribution of MSP-1 variants in the Amazon Basin, with examples of epidemic expansion of clones carrying MSP-1 haplotypes. Occurrence of variant-specific antibodies and the differential profile of IgG subclass antibody recognition of different regions of MSP-1 were revealed by serological studies using MSP-1-derived recombinant peptides. These results have major implications for malaria vaccine development and the molecular evolution of MSP-1.

► Patterns of allelic variation in the malaria vaccine candidate merozoite surface protein-1 (MSP-1) from field isolates of *Plasmodium falciparum*, collected over a period of 14 years from several sites in the Amazon region of Brazil, were investigated in the most extensive study of MSP-1 allelic diversity outside Africa, the only such study done in a hypoendemic region.

Extensive temporal and spatial variation in the distribution of msp-1 variants was found across the Amazon Basin. Sequence micro-heterogeneity in the 5' region of the msp-1 gene, which codes for the C-terminal vaccine-candidate peptide MSP-119, was characterized in 130 isolates collected in Rondônia, with two previously unknown MSP-119 haplotypes also being identified. Contrasting with previous work in Africa, a strong linkage disequilibrium between polymorphic sites >1 kb apart within the 5-kb msp-1 gene was seen, indicating that effective rates of meiotic recombination at this locus are quite variable. Moreover, two instances of epidemic expansion of clones carrying particular msp-1 haplotypes were characterized. Sequence diversity in the most variable region of msp-1, the repetitive block 2, was also characterised in 59 isolates. Insertions and deletions of repeat units in block 2 (that may result from illegitimate [mitotic] recombination) generated several new msp-1 alleles, but retained significant linkage disequilibrium between polymorphisms located in the

non-repetitive regions flanking the repeat array. Variation patterns in non-repetitive block 4 were consistent with gene conversion events. These results indicate a role for non-reciprocal (non-meiotic) exchanges in creating sequence variation in this antigen in natural parasite populations.

Patterns of naturally-acquired antibody responses to MSP-1 were examined in semi-immune or non-immune Brazilian patients with malaria and clinically immune subjects from Senegal, using recombinant peptides representing conserved (blocks 3 and 17), dimorphic (block 6) and polymorphic (block 2) regions of the antigen. Contrasting patterns of IgG subclass response to different regions of MSP-1 were observed, as well as the presence of variant-specific antibodies to block 2. As a rule, antibodies recognized mostly variable regions of this antigen. These results suggest that insertions and deletions of repeat motifs in block 2 may represent a strategy of immune evasion, with implications for the use of MSP-1-derived peptides in malaria vaccines, since new variants generated by either mitotic or meiotic recombination events within repeat arrays may be favored by immune-mediated selection of mutants. ■

Reference:

Silveira, L. A. et al. (2001)
Sequence diversity and linkage disequilibrium within the merozoite surface protein-1 (msp-1) locus of Plasmodium falciparum: a longitudinal study in Brazil.
J. Eukaryot. Microbiol. 48: 433-439

PROJECT No. 971034

Merozoite surface protein-1 of Plasmodium falciparum: allelic diversity and antibody recognition in the Brazilian Amazon

PRINCIPAL INVESTIGATOR

Dr Marcelo Urbano Ferreira
Department of Parasitology
Institute for Biomedical Sciences
University of São Paulo
São Paulo
BRAZIL

e-mail: muferre@usp.br

COLLABORATORS

L. A. da Silveira
M. L. Dorta
M. M. Póvoa
G. Wunderlich
E. A. S. Kimura
F. Kawamoto (JAPAN)
Prof. K. Tanabe (JAPAN)
F. E. McKenzie (USA)
S. M. Rich (USA)
S. Jongwutiwes (THAILAND)
T. J. C. Anderson (USA)



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Fabio Zicker
e-mail: zickerf@who.int

Schistosomiasis

► Sequencing and PCR-based approaches were used to compare the coding regions of mitochondrial genomes of species of *Schistosoma* (*S. mansoni*, *S. haematobium*, *S. japonicum*, *S. mekongi*, and *S. malayensis*), and other parasitic flukes and tapeworms. The study found little intraspecific variation but clear interspecific differences within the nad4L gene. Hence nad4L proteins may be involved in transcription regulation as well as respiration. The elucidation of novel features of mitochondrial genomes of these parasitic flukes and tapeworms could help identify potential novel drug targets.

Mitochondrial genomes, small and circular, have evolutionary dynamics different from those of nuclear genomes. Computational analysis of mitochondrial genomes have been valuable in understanding population genetics, geographical variation, evolution and phylogeny. Very little is known about the mitochondrial genome of the parasitic flat worms or parasitic Platyhelminthes. More importantly, the essential nature of mitochondria in energy production and worm development provides an ideal target for drugs. Using a PCR-based approach, the coding regions of the mitochondrial genomes of various species of *Schistosoma*: *S. mansoni*, *S. haematobium*, *S. japonicum*, *S. mekongi*, and *S. malayensis*; other trematodes: *Paragonimus westermani* and *Fasciola hepatica*; and various cestodes: *Echinococcus granulosus*, *E. multilocularis*, *E. vogeli*, *E. oligarthrus*, and *Taenia crassiceps* were obtained and compared. Comparison of the mitochondrial genomes among the platyhelminths indicated that the atp8 gene is absent, that all genes are transcribed in the same direction, and that the nad4 and nad4L genes overlap. Within the *S. mansoni* and within the *S. japonicum* geographic populations studied, there was limited intraspecific variation. However, there were clear inter-species differences, an observation that is consistent with the known phylogenetic distance between *S. mansoni* and *S. japonicum*. Furthermore, the

gene complement is the same but the gene order in *S. mansoni* is different from that of *S. japonicum* and all other species studied. Other notable findings in schistosome mitochondrial genomes include the presence of long non-coding regions that are variable in length in different geographic strains. In addition, a leucine zipper motif was present in the nad4L gene. This represents the first time a leucine zipper of mitochondrial origin has been reported. It suggests that in addition to their well-recognized role in oxidative phosphorylation, Nad4L proteins may be involved in other biological processes, such as transcription, and may in fact represent a link between transcription regulation and respiration in mitochondria. This research resulted in the production of 11 scientific papers, as well as providing a unique training opportunity for Dr T.H. Le. These studies, which have already revealed novel features of the schistosome mitochondrial genome, open new avenues for developing novel drug targets. ■

References:

- Le, T.H., Blair, D. and McManus, D.P. (2002) Mitochondrial genomes of parasitic flatworms. *Trends Parasitol.* : 18: 206-213
- Le, T.H. et. al. (2001) Mitochondrial gene content, arrangement and composition compared in African and Asian schistosomes. *Mol. Biochem. Parasitol.* : 117: 61-71
- Le, T.H. et. al. (2001) Mitochondrial DNA sequences of human schistosomes: the current status. *Inter. Jour. Parasitol.* : 30: 283-290

PROJECT No. 980479

Nucleotide sequence of the mitochondrial genomes of *Schistosoma mansoni* and *S. japonicum*

PRINCIPAL INVESTIGATORS

Dr D.T.J. Littlewood
Natural History Museum
Department of Zoology
Cromwell Road,
London SW7 5BD,
UK

Dr D. P. McManus
Molecular Parasitology Unit
Tropical Health Programme
Queensland Institute of
Medical Research
300 Herston Road, Brisbane,
Queensland 4029
AUSTRALIA

COLLABORATORS

T.H. Le (Viet Nam)
D. Blair (AUSTRALIA)
D. Johnston (UK)



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Ayo Oduola
e-mail: oduolaa@who.int

Capacity Strengthening

▶ This very successful long-term capacity building project has provided significant scientific information, coupled with high-quality capacity building, through studies covering three important areas of malaria research, namely malaria pathogenesis, malaria vaccine R&D and field studies on the impact of new tools for use in malaria control in Sri Lanka. The Malaria Research Unit at the University of Colombo has matured, over the years, into an internationally recognised malaria research centre with highly competent research staff and well-established, successful research collaborations worldwide.

Dr Handunnetti received TDR funding for her early research training in 1986. She was later awarded a TDR Re-entry grant to help start an independent malaria project in the laboratory of Prof. K. Mendis. In 1998 she assumed leadership of the Malaria Research Unit, as well as responsibility for a TDR Partnership grant. Subsequently, there has been a major contribution toward building a sustainable research activity in Sri Lanka, ranging from the training of over 24 post-graduate researchers, to consolidation of field research facilities. Dr Handunnetti is continuing her research with support from TDR's Vaccine Discovery Research steering committee.

In the area of malaria pathogenesis, studies on host genetics have shown a link between TNF- α and TNF- β allele polymorphisms and the severity of malaria. Indeed, the presence of TNF- α 2 allele specifically in the background of TNF- β 1/2 state was shown to increase the risk of severe/complicated malaria by 6-fold. When cytokine secretory levels in plasma of the same study cohort were measured, significantly higher levels of IL-6, IL-10 and TNF- α were found in severe/complicated malaria patients as compared to uncomplicated malaria, but not in cerebral malaria patients. Studies characterising agglutinating antibody phenotypes suggested that early production of these antibodies in response to parasites causing uncomplicated ma-

laria may have resulted in uncomplicated infections developing into the more severe disease state.

A second focus of the research programme concerned development of a vaccine for *Plasmodium vivax* malaria, based on the Merozoite Surface Protein-1 (MSP-1), a leading blood-stage candidate antigen. Investigators used the toque monkey-*P.cynomologi* natural malaria model available in Sri Lanka to study several aspects of the immune response to MSP-1 antigens and several formulations with novel adjuvants, in collaboration with Dr S. Longacre at the Institut Pasteur in Paris, France. A natural challenge system was established, whereby monkeys are submitted to 3-10 infectious bites from *P.cynomologi*-infected mosquitoes. Compared to infections induced by trophozoite blood passages, peak parasitemias were significantly lower in the sporozoite-induced infections. This model will be important for future evaluation of candidate antigens and adjuvant formulations. The group is now developing protocols for a natural sporozoite challenge system in toque monkeys for *P. vivax* malaria, which would be a significant advance in the field of malaria vaccine development at the global level. ■

Reference:

Perera, K.L. et. al. (1998)
Baculovirus merozoite surface protein 1 C-terminal recombinant antigens are highly protective in a natural primate model for human Plasmodium vivax malaria.
Infect. Immun. 66(4):1500-1506

PROJECT No. 930807

New methods of malaria control based on molecular, immunological and epidemiological studies on Plasmodium vivax and P. falciparum malaria

PRINCIPAL INVESTIGATORS

Dr S. M. Handunnetti
University of Colombo
Faculty of Medicine
Malaria Research Unit
Colombo
SRI LANKA

e-mail: handuns@slt.lk

COLLABORATORS

S. Longacre (FRANCE)
R. Carter (UK)
C. Pasay (PHILIPPINES)



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Steven Wayling
e-mail: waylings@who.int

Capacity Strengthening

► This malaria project was conducted in two African cities, the institutions involved being the Centre International de Recherches Médicales de Franceville (CIRMF, Gabon) and the National University of Benin and the Centre Régional Pour le Développement et la Santé (CREDESA, Cotonou, Benin). Researchers were involved in establishing new techniques at each laboratory and in transfer of acquired techniques between other international institutions, namely the University of Tubingen (Germany), Institut Pasteur (Paris, France), University of Edinburgh (UK) and the University of Masuku (Franceville, Gabon).

A novel technique for collecting and isolating malarial parasite DNA from blood thick smears was established in Gabon. Using the same study design and methodologies, clinical and parasitological features of malaria were studied in 58 and 46 children (<5 years of age) from Franceville (Gabon) and Cotonou (Benin) respectively. Parasite density was significantly higher in Gabon. A higher degree of *Plasmodium falciparum* merozoite surface protein (MSP-2) polymorphism and complexity of infection (mean number of *P.falciparum* genotypes/child) was observed in isolates from Gabon ($P < 0.05$). Results showed that maternal efforts to prevent malaria in children were important but affected parasitological factors so as to possibly contribute to a delay in developing immunity. In the absence of a vaccine, control programmes should best focus on appropriate measures to prevent child morbidity and mortality, such as education of mothers and local health workers.

To help clarify the relationship of the humoral immune response to polymorphic regions of MSP-1 and MSP-2 and protection, allelic-family specific humoral responses were explored using MSP-1 and MSP-2 synthetic peptides. Such proteins, expressed by *P. falciparum* asexual blood stages, have been identified as target antigens in protective effector mechanisms.

Studies were carried out on children, older than 6 months with either asymptomatic or uncomplicated falciparum malaria, living in an urban area where malaria is endemic and perennial. Similar numbers of responders were found against schizont crude extract (>90%), in both groups. A similar prevalence of responders were observed, in isolates from asymptomatic and uncomplicated malaria cases, whatever the family allelic marker considered. Antibodies reactive to K1/MSP-1 and FC27/MSP-2 types were predominant, corresponding to the main parasite genotypes. Infection complexity was age-dependent, significantly decreasing around age 15, while a simultaneous increase in mean numbers of MSP-1 and MSP-2 variants recognised was observed in the asymptomatic group. Follow-up of uncomplicated malaria patients found a rapid development of immune responses to block2 (semi-conserved) and block3 (polymorphic) MSP-2 molecules within 7 days. No comparable reactivity was found using MSP-1 variant molecules (block2). Comparison of humoral responses to MSP-1 and MSP-2 suggest that different pathways of responsiveness are involved in antibody production to these two antigens. ■

Reference:

Ekala, M.-T. et al. (2002)
Plasmodium falciparum merozoite surface protein1 (PfMSP1): Genotyping and humoral responses to allele-specific variants.
Acta Tropica : 81 (1): 33-46

PROJECT No. 971211

Relation between Plasmodium falciparum strains parasite factors and outcome of severe clinical malaria in Gabon and Benin children

PRINCIPAL INVESTIGATORS

Dr Francine Ntoumi
Medical Research Unit
Albert Schweitzer Hospital
Lambaréné
GABON

e-mail: ffntoumi@hotmail.com

COLLABORATORS

Prof. P. Kremsner (GERMANY)
S. Issifou (BENIN)
O. Mercerau-Puijalou (FRANCE)



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Fabio Zicker
e-mail: zickerf@who.int

Capacity Strengthening

► Specific inhibitors have been obtained, through structure-based drug design, to be used as lead compounds for the development of new drugs against Chagas disease. A specialised unit for screening libraries of extracts and pure natural products for potential drug leads as enzyme inhibitors has been established. Several substances with significant inhibitory activity against the target enzymes GAPDH and APRT have been identified. The research group has become a regional reference centre in structural biology and a cadre of expert staff specialising in various aspects of drug design has been established.

The structure of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from *Trypanosoma cruzi*, which plays a central role in glycolysis, essential for parasite survival, was determined by X-ray crystallography. This elucidated the enzyme's catalytic mechanism (subsequently confirmed by structural and biochemical characterization of two site-directed mutants). A series of inhibitors were synthesized and assayed against the enzyme, showing activities (IC₅₀) in the range of 50-200 µM. Natural products were also extensively screened for inhibitors, producing promising compounds with IC₅₀s in the range of 20-100 µM. One inhibitor, chalepin (from *Pilocarpus spicatus*) was co-crystallized with the enzyme, its structure elucidating the mode of binding of the inhibitor, leading to the synthesis of a series of modifications of the natural product which were implemented through classical organic chemistry as well as combinatorial parallel synthesis in solid phase. Some of these new TcGAPDH inhibitors have shown activities approaching the sub-micromolar range. Two complexes of enzyme plus substrate analogues were co-crystallized and show promise of higher potency and specificity.

These results catalyzed the expansion of structure-based drug design which now encompasses over 20 different protein targets covering several tropical diseases, with another 30 protein

targets associated with other human health, agricultural and environmental applications. The crystal structure of the enzymes adenine phosphorybosyl transferase (APRT) and hypoxanthine-guanine phosphorybosyl transferase (from *Leishmania tarentola*); phosphoenol-pyruvate carboxykinase and Fe-superoxide dismutase (*T. cruzi*); Cu,Zn-superoxide dismutase (SOD) and purine nucleoside phosphorilase (*Schistosoma mansoni*); and Fe-superoxide dismutase (*Plasmodium falciparum*) were all determined.

A unit dedicated to screening libraries of extracts and pure natural products for new drug leads has been established. Several compounds with significant inhibitory activity against target enzymes (GAPDH and APRT) have been found. Within the research group, competencies have been built for combinatorial parallel synthesis in solid phase. Two libraries of derivatives of promising natural-product leads were synthesized, one of coumarins and another of flavonoid compounds. Staff trained in this specialized area, included 15 MSc and 17 PhD students. The research group is now consolidated as a reference center in structural biology in Latin America and recently became a state-supported Center for Structural Molecular Biotechnology. ■

Reference:

Pavão, F. et. al. (2002)
Structure of *Trypanosoma cruzi* glyceraldehyde-3-phosphate dehydrogenase complexed with chalepin, a natural product inhibitor, at 1.95 Å resolution. *FEBS Newsletter*; 520: 13-17

PROJECT No. 940854

Structure-based drug design against Chagas: *Trypanosoma cruzi* glyceraldehyde-3-phosphate dehydrogenase as inhibitor target design

PRINCIPAL INVESTIGATORS

Dr G. Oliva, Dr R.C. Garratt and Dr O.H. Thiemann
University of São Paulo
São Carlos
BRAZIL

e-mail: oliva@if.sc.usp.br

COLLABORATORS

P.C. Vieira
A.G. Correia
M.T. Pupo
P. Michels (BELGIUM)



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Fabio Zicker
e-mail: zickerf@who.int

Malaria

▶ The number of effective drugs of the antifolate class is falling due to rapid emergence of resistance. Malarial parasites develop resistance to antifolates through mutations in the target enzyme, malarial dihydrofolate dehydrogenase (DHFR). Based on molecular models of wild-type (sensitive) and mutant (drug-resistant) DHFR enzymes bound to known drugs, novel antifolate inhibitors have been designed and found to be effective against drug-resistant parasites. These will serve as lead compounds for designing even more potent compounds which avoid the resistance effects enabled by mutations in DHFR.

The resistance of *Plasmodium falciparum* to antifolates is an important problem in antimalarial chemotherapy. Resistance develops through mutations in the enzyme, malarial DHFR, which is part of the bifunctional enzyme dihydrofolate reductase-thymidylate synthase (DHFR-TS). Since malarial parasites must maintain a functional DHFR, the number of mutations tolerated in the enzyme are thought to be limited. Investigators sought, using molecular modelling, to understand how inhibitors of DHFR bind to the enzyme, how mutant DHFRs escape inhibition and, using this information, to design and/or select for novel antifolate inhibitors effective against known drug-resistant strains of *P. falciparum*.

Molecular modelling of the structure of *P. falciparum* DHFR was done by comparing the amino acid sequence with those of DHFRs from other species where the structures are known. Models of wild-type and mutant DHFRs, and their interaction with various inhibitors, were analyzed with the aim of designing new inhibitors which would be effective against antifolate-resistant *P. falciparum*. The models clearly showed that the binding of one inhibitor, cycloguanil, would be affected by a mutation of alanine to valine at amino acid position 16, as found in some of the drug-resistant DHFRs. The bulky side-chain of valine at position 16 interferes sterically with one

of the 2,2-dimethyl groups of cycloguanil. This is a major cause of cycloguanil resistance. New compounds were designed to avoid this steric clash and shown to be highly effective inhibitors of parasites harboring the mutant enzyme. A similar analysis of multiple mutations at positions 51, 59, 108 and 164 enabled the design of new compounds against pyrimethamine-resistant parasites. Analogues of known inhibitors such as cycloguanil, pyrimethamine, and trimethoprim were designed and synthesized, and then analyzed for their ability to inhibit wild-type and mutant DHFR. Both wild-type (sensitive) and mutant (drug-resistant) DHFR were produced as recombinant proteins using synthetic genes. Several compounds were shown to be effective inhibitors of the mutant DHFR enzymes, fulfilling predictions of the models. Many of the novel compounds showed good activity against resistant parasites carrying mutant DHFRs. As well as designing new inhibitors based on molecular modelling, a method for selecting high-affinity inhibitors from combinatorial libraries was developed and validated. Further work on the synthesis and selection of inhibitors of malarial DHFR is being supported by the Medicines for Malaria Venture (www.mmv.org). ■

Reference:

Yuthavong, Y. (2002)
Basis for antifolate action and resistance in malaria.
Microb. Inf. 4:175-182

PROJECT No. 990221

Development of new antifolate antimalarials based on binding/selection of combinatorial libraries by recombinant malarial dihydrofolate reductase (DHFRs)

PRINCIPAL INVESTIGATORS

Prof. Y. Yuthavong
National Science and Technology
Development Agency
73/1 Rama VI Road
Rajdhevee, Bangkok 10400
THAILAND

e-mail: yongyuth@nstda.or.th

COLLABORATORS

S. Kamchonwongpaisan
B. Tarnchompoo



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Mary M. Bendig
e-mail: bendigm@who.int

Capacity Strengthening

▶ A genome sequencing unit has been founded at the Instituto de Investigaciones Biotecnológicas, located in the National University of General San Martín, Buenos Aires, using TDR funds. A skilled and specialised group of staff have been developed in situ, and the unit has established extensive national and international collaborations. Results produced by the unit include gene discovery through EST sequencing in *Trypanosoma cruzi* and a random sequencing approach for analysis of the *T. cruzi* genome. Work is under way to identify novel genes in *T. cruzi* and other trypanosomatids.

In 1997, the basic set-up of the new Genome Sequencing Unit included an automated, 36-lanes ABI Prism 377 slab sequencer that allowed about 18,000 bases (60 sequences of good quality) to be produced daily. Today, the capacity has been increased significantly, to over 200 sequences per day, mostly due to a sequencer upgrade that duplicated the number of lanes per run, and to improvements in the organization and management of the unit. However, the most important improvement was the incorporation of a small bioinformatics unit in 1999-2000. Bioinformatics within the laboratory provides support in basic aspects of laboratory information management. More importantly, it facilitated expansion of the capacity for sequence analysis, both through automation and through the incorporation of new and emerging technologies in this field.

Since 1997, the unit has participated in the *Trypanosoma cruzi* Genome Project through the Expressed Sequence Tag (EST) and the Genome Sequence Survey (GSS) work. Through these activities about 2,000 epimastigote ESTs and over 12,000 GSSs, totalling more than 4.3 Mb, were generated.

Current work is focusing on i) addressing the lack of stage-specific sequence information for *T. cruzi* and ii) applying new strategies to identify novel genes in *T. cruzi* and other trypanosomatids.

In this context, EST sequencing from subtracted libraries is being explored, and from evolutionarily-related *Trypanosoma* species.

Another of the goals for setting up a sequencing unit, was to gain knowledge and experience in new technologies (automated sequencing) and areas of research (genomics). As a result, skills have been enhanced and individuals trained (technicians, graduate and post-graduate students) in specialist areas. Collaborations with several groups, within the institute, nationally and with international institutions have been developed, allowing other research teams to benefit from the installed capacity. Notable projects carried out or in development include: shotgun sequencing of *T. cruzi* cosmids containing genes of interest; random genomic sequencing (GSSs) in pathogenic bacteria (*Brucella abortus*, 1,900 sequences; *Campylobacter fetus*, 13,000 sequences), and gene discovery (ESTs) in other *Trypanosoma* species (*Trypanosoma carassii*, 2,000 sequences).

Additional information can be found online at: <http://genoma.unsam.edu.ar>.

Reference:

Agüero, F. et al. (2000)
A random sequencing approach for the analysis of the *Trypanosoma cruzi* genome: general structure, large gene and repetitive DNA families and gene discovery.
Genome Research, 10 (12): 1996-2005

PROJECT No. 960682

Establishment of a genome sequencing unit at the Institute for Research in Biotechnology, Buenos Aires.

PRINCIPAL INVESTIGATORS

Dr D. O. Sánchez
Universidad Nacional de
General San Martín
Instituto de Investigaciones
Biotecnológicas
San Martín
ARGENTINA

e-mail: dsanchez@iib.unsam.edu.ar

COLLABORATORS

A.C.C. Frasch
F. Agüero
R. Verdún
V. Ruiz Moreno
I. Cuevas
V. Tekiel
N. Di Paolo



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Fabio Zicker
e-mail: zickerf@who.int

Leishmaniasis

► *Leishmania donovani* is the major causative agent of visceral leishmaniasis. Two formulations of pentavalent antimony are currently available for clinical use but clinical resistance to these compounds has been increasingly reported from many disease-endemic countries. In this project, investigators helped elucidate the mechanisms of resistance to antimony. The work paves the way for the development of new and improved drugs to treat what has hitherto been a relatively overlooked disease.

Visceral leishmaniasis (VL) causes significant morbidity and mortality worldwide. Treatment of choice for VL is pentavalent antimony (SbV), but its mode of action is unknown. This fact, especially in the context of the recent emergence of widespread clinical resistance of *L. donovani* to SbV, is indicative of the urgent need to delineate the mechanisms of SbV susceptibility and resistance, and to develop better tools for assessing SbV susceptibility in *Leishmania*.

Leishmania parasites have a life cycle which includes an extracellular flagellated promastigote stage (in vector sandflies) and an obligatory intracellular, non-motile amastigote stage (in humans). A method for culturing *L. donovani* amastigotes has been established, allowing for direct evaluation of biological and biochemical processes in the amastigote. This culture system is a good model for studying drug resistance in *Leishmania*.

Investigators first determined that novel stage-specific intracellular reduction of SbV occurs in *L. donovani* and that this reduction activity correlates with parasite susceptibility to antimony. Essentially, *L. donovani* amastigotes (but not promastigotes) reduce SbV to SbIII, thus making amastigotes much more susceptible to SbV than promastigotes. A mutant of *L. donovani* (Ld1S.20), that is resistant to pentavalent antimony, was isolated and characterized. It lacks SbV reducing activity. In order to clone

the gene that codes for the mutation in Ld1S.20, a genomic DNA library of *L. donovani* was screened to discover genes that would complement the SbV-resistant phenotype of Ld1S.20 amastigotes. A 7-kilobasepair DNA fragment was identified that is able to restore the SbV reduction activity in the mutant to near wild-type levels. This DNA fragment contains four open reading frames coding for four putative proteins that are novel and do not match any known proteins. Work is continuing to identify the gene/protein that reverses the SbV resistance in the mutant.

Amastigotes metabolize SbV intracellularly, either by reduction reactions or by interaction with intracellular proteins. To elucidate the intracellular fate of SbV, chemically-induced, SbV-resistant mutant amastigotes were isolated. These will be characterized and used in gene cloning experiments in the near future.

Although this work does not propose new drugs, it has already yielded very useful information about how existing drugs work. This information should help in designing effective strategies for the discovery of new anti-leishmanial drugs and generally help combat the threat of increasing drug resistance. ■

Reference:

Zilberstein, D. & Ephros, M. (2002) *Clinical and laboratory aspects of Leishmania chemotherapy in the era of drug resistance. (In World Class Parasites (Vol. 4): Leishmania; Ed. Black, S.J. & Seed, J.R.; Kluwer Academic Press, London: 115-136)*

PROJECT No. 980111

An in vitro system to determine susceptibility and assessing the mechanism(s) of resistance of Leishmania donovani to anti-leishmanial compounds

PRINCIPAL INVESTIGATORS

Dr. D. Zilberstein
Technion, Israel Institute of
Technology
Department of Biology
Technion City
Haifa 32000
ISRAEL

e-mail: dzilbers@tx.technion.ac.il

COLLABORATORS

N. Ulrich (GERMANY)



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Mary M. Bendig
e-mail: bendigm@who.int

Malaria

▶ Trained volunteers can use prepackaged drugs to effectively treat African children with fever within 24 hours of onset of illness. Community-based agents can provide effective near-home treatment. Prepacks were widely acceptable and greatly improved health-seeking behaviour of mothers and carers. Over 90% of those using antimalarial prepacks complied with treatment. Home visits and follow-up, rather than availability, led to effective use and compliance. A good IEC programme resulted in improved care. The findings are causing national health services to review their care delivery systems and products.

Most children in rural Africa have no contact with clinical services that can offer appropriate diagnosis and drugs, and there is little prospect of substantial change in the near future. The majority of childhood febrile illnesses continue to be treated at home and 50-70% of children that die never come in contact with modern health services. In Ghana, Burkina Faso, Nigeria and Uganda, multi-site studies developed and piloted a set of interventions for early, appropriate home management of fevers in pre-school children, aimed at providing at least 60% of children with appropriate treatment within 24 hours of onset of symptoms. Interventions in the sites (each of about 10,000 population) consisted of prepackaging of chloroquine and cotrimoxazole (separately in unit doses), establishment of a network of Community-Based Agents (CBAs) who were trained to treat children with the drugs, and an Information, Education and Communication (IEC) programme focusing on the whole community. Other activities included supply management to ensure CBAs always had ample drugs, supervision for quality assurance and monitoring and evaluation. In Ghana, chloroquine was used to treat children with uncomplicated malaria (fever but no other complications), in line with national treatment guidelines. Prepacks were produced by a local pharmaceutical company in two forms; a white pack with an image of a

crawling infant (for ages 6-11 months) containing 75mg base of chloroquine, to be given once per day for 3 days, and one (yellow, with the image of a walking child) for children up to 6 years, containing the standard 150mg base of chloroquine, to be given once daily for 3 days. The treatment regimen conformed with national guidelines, except for the 75mg tablet which was produced in Ghana for the first time. Infants received either syrups or pieces of the standard tablet. Children who, as well as fever, had symptoms suggestive of acute respiratory infections received cotrimoxazole in addition to chloroquine (national standard in Ghana). Cotrimoxazole was produced as a pink prepack of 200/40mg-paediatric formulation to be given twice daily for 5 days. To restrict antibiotic use to children at risk of pneumonia, CBAs were trained to count breathing rate and treat infants whose breathing rate was 40 or more per minute and older children breathing faster than 50 breaths per minute (as recommended by Ghana IMCI). Ghana's Ministry of Health is using these results to decide the key elements of their scale-up programme for malaria home management, and is rethinking the use of syrups and loose tablets in care delivery systems. ■

PROJECT No. 980285

Early appropriate home management of fevers in children aged 6 months to 6 years in Ghana

PRINCIPAL INVESTIGATOR

Dr E.N.L. Browne
Ghana Home Management
of Fevers Team
Dept. of Community Health
School of Medical Sciences
Kwame Nkrumah University of
Science and Technology
Kumasi
GHANA

e-mail: ebrowne@ghana.com
enlbrowne@yahoo.com

COLLABORATORS

Ministry of Health
Ghana Health Service
UNICEF (Ghana)
BASICS-Ghana



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Dr Jane Kengeya-Kayondo
e-mail: kengeyakayondo@who.int

Health Sector Reform

▶ The 1990s witnessed a wave of reforms in health systems in many countries aimed at improving health status and client satisfaction, technical and allocative efficiency, and equity of access to care. Major elements of these reforms included reorganization of government health agencies, user charges for public services, establishment of health insurance systems and service contracting. Research undertaken to analyse policy changes and monitor and evaluate impact not only provided valuable insights for the future, but also helped improve the base of individuals with skills and expertise in this area of work.

The last decade saw health systems reforms introduced in several countries aimed at improving overall health and client satisfaction, technical and allocative efficiency, and to provide more equitable access. The reforms included the reorganization of national health agencies, user charges for publicly provided services, health insurance schemes and service contracting. Unsuccessful reforms can have long-lasting and profound effects on society, but fundamental changes in health systems are advocated and undertaken based on less evidence than it requires to license a new antibiotic. National and international forces behind reform often have little interest in policy research, and rigorously measuring the impact of complex reforms is not easy. Many developing country researchers lack the resources and experience to conduct such research. TDR, using a contribution from the Norwegian government, supported several such researchers to take up the challenge. A study on pharmaceutical cost containment policy for hospitals in Shanghai, China found that a combination of a limited hospital formulary and a pharmaceutical revenue cap reduced drug costs from 67% of all hospital expenditures in 1992 to 51% in 1996, without negative effect on equity and quality of care. A hospital financing reform in Indonesia produced unexpected results. The reform, intended to generate additional revenue to be used for

providing services for all patients, turned out to generate income for physicians and to drain resources away from care for indigents.

A study of the 'big bang' approach to overhauling the health care financing system for the poor in Colombia, identified implementation problems and positive and negative impacts of the reform, which was accompanied by a substantial increase in health sector resources. Studies of formal attempts for decentralization and community participation in Colombia and the Philippines respectively provided evidence that legislation cannot create true community participation in societies with limited history of democratic participation in health care systems. Research on attitudes among key players towards proposal to establish a system of hospital accreditation in Mumbai, India found real interest and willingness among those involved to participate in creation of such a system. A prospective view of policy formulation on the relationship between the public and private health care sectors in Uganda, found the distinction blurred and little correspondence between official policy and the reality, with the conclusion that achieving consensus on future policy is an elusive goal. ■

Reference:

Learning from experience: Research on health sector reform in the developing world
Eds.: Blas, E., & Hearst, N. (2001)
Health Policy & Planning: 16(S2) 87pp.

**PROJECT No. 960702,
970791, 960732, 980390,
960699, 960703, 970612**

*Learning from experience:
Research on health sector
reform in the developing world*

PRINCIPAL INVESTIGATORS

S. Hu, W. Chen, X. Cheng, K. Chen,
H. Shou & L. Wang: CHINA

A. Suwadono, A. Gani, S. Purwani,
E. Blas & R. Brugha: INDONESIA

B. Plaza, A. Beatriz Barona &
N. Hearst: COLOMBIA

M. Mosquera, Y. Zapata, K. Lee,
C. Arango & A. Varela: COLOMBIA

L. Ramiro, F.A. Castillo, T. Tan-Torres,
C. E. Torres, J. G. Tayag, R. G. Talampas
& L. Hawken: THE PHILIPPINES

S. Nandraj, A. Khot, S. Menon &
R. Brugha: INDIA

H. Birungi, F. Mugisha, X. Nsabagasani,
S. Oluonzi & A. Jeppson: UGANDA



**UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases**

Contact:

Tel: (+41) 22-791-3725
Fax: (+41) 22-791-4854
e-mail: tdr@who.int
www.who.int/tdr

Information source:

Erik Blas
e-mail: blase@who.int

Portfolio 1

► This collection of Final Reports illustrates significant scientific progress arising from TDR-supported research projects during 1998/99. It is the first volume in a regular series. The reports contained in Portfolio 1 are as follows:

disease	project
1 Chagas disease	No. 960295
2 Quality of Care	No. 961008 and 960754
3 Malaria	No. 960448
4 Malaria	No. 950625
5 Malaria	No. 960431 and 960387
6 Malaria	No. 950559
7 Filariasis	No. 950549, 950550 and 950551
8 African Trypanosomiasis	No. 960720, 960721 and 960722
9 Health Sector Reform	No. 960698
10 Schistosomiasis	No. 940897
11 Malaria	No. 930817
12 Schistosomiasis	No. 920479
13 Malaria	No. 950195
14 Leprosy	No. 960147
15 Malaria	No. 950476
16 Chagas disease	No. 950279
17 African Trypanosomiasis	No. 960557 and 970468
18 Malaria	No. 940434 and 960603
19 Malaria	No. 960617
20 African Trypanosomiasis	No. 980785

To obtain copies of Portfolio 1, Portfolio 2, Portfolio 3, or individual reports, please visit the TDR website www.who.int/tdr or contact the TDR Communication Centre (for contact details see back cover).



Portfolio 2

► This collection of Final Reports illustrates significant scientific progress arising from TDR-supported research projects during 2000/01. It is the second volume in a regular series. The reports contained in Portfolio 2 are as follows:

	disease	project
21	Chagas disease	No. 970194
22	Training	No. 910331, 950885 and 990630
23	Onchocerciasis	No. 970524
24	Chagas disease	No. 930772 and 970152
25	Lymphatic filariasis	No. 970746
26	Malaria	No. 930812
27	Schistosomiasis + Filariasis	No. 930815
28	Filariose lymphatique	No. 970696
29	Malaria	No. 971060
30	Malaria	No. 970071 and 980370
31	Schistosomiasis	No. 981108
32	Chagas disease	No. 970502 and 980653
33	Malaria	No. 980241
34	Malaria	No. 980254
35	Technology transfer	No. 950277, 960174 and 971230

To obtain copies of Portfolio 1, Portfolio 2, Portfolio 3, or individual reports, please visit the TDR website www.who.int/tdr or contact the TDR Communication Centre (for contact details see back cover).



TDR/GEN/02.1

This document is not a formal publication of the World Health Organization (WHO), and all rights are reserved by the Organization. The document may, however, be freely reviewed, abstracted, reproduced or translated, in part or in whole, but not for sale or for use in conjunction with commercial purposes.

The views expressed in documents by named authors, are solely the responsibility of those authors.

© TDR 2002

▶ The Special Programme for Research and Training in Tropical Diseases (TDR) is an independent global programme of scientific collaboration. Established in 1975 and co-sponsored by the United Nations Development Programme (UNDP), the World Bank and the World Health Organization (WHO), it aims to help combat 10 major tropical diseases: African trypanosomiasis, Chagas disease, Dengue, Leishmaniasis, Leprosy, Lymphatic filariasis, Malaria, Onchocerciasis, Schistosomiasis and Tuberculosis.

Objectives

- i) Research & Development: to develop safe, acceptable and affordable methods of prevention, diagnosis, treatment and control of target diseases.
- ii) Training & Strengthening: to strengthen the capability of developing disease-endemic countries to undertake the research required to realize new disease control technologies.

TDR operates as a knowledge management organization. It does not work alone, but selects, guides, funds, encourages and develops research done by others. In this way, TDR acts as a 'global facilitator' of both research and training, creating and working within a series of networks. Great emphasis is placed on forming partnerships with a variety of organizations around the world, including research institutions, industry and non-governmental organizations.



UNDP/World Bank/WHO
Special Programme for
Research and Training in
Tropical Diseases

Contact:
WHO/TDR
Avenue Appia 20
CH-1211 Geneva 27
Switzerland
Tel: (+41-22) 791-3725
Fax: (+41-22) 791-4854
E-mail: tdr@who.int