LECTURE SESSIONS

RECENT ADVANCES IN MALARIA RESEARCH: PARASITE BIOLOGY, CHEMOTHERAPY AND HOST/PARASITE RELATIONSHIPS

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Summary.—Malaria is still the most important parasitic disease in the tropics. In 1982, the most recent year for which reliable figures are available, nearly one-twelfth of the world's population were living in areas where malaria is still endemic and where no specific antimalarial measures are being applied. A further 2217 million, 46% of the world's population, were living in areas where malaria is still endemic but where control measures have somewhat reduced its level of endemicity. This epidemiological picture has not significantly changed over the past decade. In areas other than tropical Africa the incidence of the disease is currently estimated to be around 20 million cases per year and in Africa south of the Sahara some 200 people are believed to be chronically infected.

The tools and methods available for malaria control have not changed significantly over the last three decades and many have steadily declined in efficacy. Resistance of anopheline vectors to insecticides, exophilic behaviour of the vector and socioeconomic factors are major constraints to effective malaria control operations. Resistance of P. falciparum to drugs, however, has probably become the most important technical threat to effective control of the disease. By the end of 1984, chloroquine-resistant P. falciparum was present in !4 countries in eastern Asia and Africa and resistance to the second-line drug, a sulfonamide/pyrimethamine combination, is already widespread in South East Asia and South America and recent reports indicate an increasing loss of treatment efficacy in other areas including East Africa. A global programme on the susceptibility of P. falciparum to these and other antimalarial drugs has been initiated by WHO to monitor the emergence and spread of these drug resistant parasites.

During the last decade there has been a renaissance in malaria research in an attempt to provide the new tools required for malarial control. Mefloquine, the first new antimalarial drug to be tested in 30 years, and its combination with sulfadoxine and pyrimethamine have been registered in certain countries for limited use. Other drugs with novel structures and modes of action have also been discovered and may have potential in the future for treatment and suppression of malaria infections. Of these qing-hao-su and its derivatives may have particular relevance for the treatment of cerebral and other complicated forms of the disease.

Primaquine is still the only drug available for the radical treatment of vivax malaria. In spite of its being in use for a long time, little is known of its mode of action, its metabolism or the basis of its toxicity. However, the recent development of sensitive assay techniques have lead to the identification of several putative metabolites whose efficacy and toxicity are now being assessed in newly developed in vitro systems.

Recent advances in malarial parasite biology and immunology allied with the
application of monoclonal antibodies and DNA hybridization techniques have led to the identification of parasite specific antigens in several parasite stages. The development of sporozoite, asexual blood stage and transmission blocking vaccines, therefore, appears feasible. Advances in these fields are also leading to the development of a new generation of diagnostic tests.


Gli strumenti ed i metodi disponibili per il controllo della malaria non sono cambiati in modo significativo nelle ultime tre decadi, e diversi di essi hanno subito un costante declino nell'efficacia. La resistenza dei vettori anofelini agli insetticidi ed il comportamento esofilico del vettore rappresentano, insieme a fattori socio-economici, i principali limiti alle misure di controllo della malaria. La resistenza ai farmaci da parte del P. falciparum tuttavia è venuta a rappresentare probabilmente il principale ostacolo tecnico che minaccia la possibilità di controllo della malattia. Verso la fine del 1984, P. falciparum resistente alla cloroquina era presente in 14 paesi in Asia orientale ed in Africa, e la resistenza al farmaco di seconda linea (una combinazione di sulfanomide e pirimetamina) era pure già largamente diffusa nel Sud Est asiatico ed in Sud America, mentre rapporti recenti indicano una progressiva perdita di efficacia del trattamento in altre aree, comprendenti l'Africa orientale. Un programma globale sulla suscettibilità del P. falciparum a questi e altri farmaci antimalarici è stato iniziato dall'OMS per seguire l'insorgenza e la diffusione dei parassiti resistenti ai farmaci.

Durante l'ultima decade, vi è stata una ripresa nella ricerca sulla malaria, nel tentativo di ottenere nuovi strumenti di controllo. La meflochina, il primo nuovo farmaco antimalarico negli ultimi 30 anni, e le sue combinazioni con sulfadossina e pirimetamina, sono state registrate in alcuni paesi per un uso limitato. Altri farmaci con struttura e meccanismo d'azione completamente nuovi sono stati scoperti e potrebbero rivelarsi utili in futuro per il trattamento e la soppressione di infezioni malariche. Tra questi il qing-hao-su ed i suoi derivati potrebbero avere particolare importanza nel trattamento di forme cerebrali e di altre forme complicate della malattia.

La primachina è ancora l'unico farmaco disponibile per il trattamento radicale della malaria da P. vivax. Nonostante essa sia in uso da lungo tempo, poco si sa del suo meccanismo d'azione, del metabolismo e della base della sua tossicità. Tuttavia il recente sviluppo di tecniche di saggio più sensibili ha portato alla identificazione di diversi metaboliti (o ritenuti tali), la cui efficacia e tossicità sono oggetto di studio in sistemi in vitro sviluppati recentemente.

Progressi recenti nella biologia ed immunologia del parassita uniti all'applicazione di anticorpi monoclonali e a tecniche di ibridazione del DNA hanno portato alla identificazione di antigeni specifici del parassita in diversi stadi del ciclo vitale. Appare quindi possibile lo sviluppo di vaccini per gli stadi di sporozoite e per gli stadi del sangue e di vaccini che bloccino la trasmissione. Progressi in questo campo stanno anche portando allo sviluppo di una nuova generazione di saggi diagnostici.
Fig. 1. - Epidemiological assessment of status of malaria, 1982.
Introduction

Interest in malariology has ebbed and flowed ever since the turn of the century when it was first demonstrated that the disease was caused by protozoan parasites transmitted by mosquitoes. This interest was stimulated in the early years by European scientists and other groups working in the tropics; but, later, strategic considerations played a major role leading to large investments in malaria research made by certain countries during the war years. In fact, immediately after the Second World War, two events occurred which determined and dominated the fight against malaria for the next twenty years. The first was the success of the residual insecticides DDT and BHC in dramatically curbing the transmission of malaria in many countries, including some in which the disease had been highly endemic. The second was the availability in rapid succession of a series of new synthetic drugs which gave the prospect of successfully treating and preventing malaria infections. Both events were the result of intensive research during the war years.

In 1955, the World Health Organization declared its policy of global eradication of malaria and the results over the next decade or more were excellent in Europe, in the Americas, some parts of Asia, USSR and Australia but not so elsewhere. Malaria eradication was not attempted in Africa south of the Sahara. The policy of eradication therefore, had certain successes, a fact which is sometimes forgotten today. However, it also had certain other immediate and important consequences. The concept engendered the belief that the methods for controlling and eradicating the disease were available and as a consequence support for basic malaria research, and even for research on insecticides and drugs, dwindled away at a dangerous rate throughout the world. Worse still, the prospect of total eradication of the disease induced many administrators to consider the training of scientists and doctors in malariology and the maintenance of essential services as no longer necessary. It took the deterioration in the malaria situation in the late 1960's and 1970's to halt this trend and to cause WHO to modify its policy of eradication to a more flexible one of control with the ultimate objective of eradication of the disease.

The malaria situation and drug resistance

The current distribution of malaria in the world shows practically no major changes from that in the mid 1970's with the exception of its disappearance from certain parts of North Africa (Fig. 1). Excluding Africa south of the Sahara, 7.8 million cases were reported to WHO in 1981 compared with 8.0 million in 1980 and 7.0 million in 1979. The provisional figures for 1982, the most recent year for which reliable figures are available, were 6.5 million cases. Caution, however, should be exercised in interpreting these figures since, as a result of many countries having been forced to scale down their control activities, case detection and identification of malaria have declined over the past 15 years and the reporting of accurate information has also been affected. The true incidence of the disease in areas other than tropical Africa is currently estimated to be around 20 million cases a year. A further 200 million people are believed to be chronically infected in Africa south of the Sahara and of these about one third suffer from acute manifestations of disease in the course of the year. Most of these infections (estimated to be 85%) are caused by Plasmodium falciparum. This species also accounts for an important share of the mortality in infants and young children, according to an often quoted estimate of one million infants and young children in Africa alone.

Clearly, the world malaria situation does not give cause for complacency. At best, it reflects a holding operation with little or no change in areas which are most affected by the disease. Mounting problems in the form of insecticide resistance, exophily of vectors, population movements and social, economic and
Fig. 2. - Areas where chloroquine-resistant *P. falciparum* has been reported.
political constraints all favour the existence and spread of the disease. In addition, the rapidly spreading drug-resistant strains of P. falciparum in wide areas of the world now threaten to deprive mankind of the means of curbing the effects of malaria in areas where the disease is a major cause of death and suffering. By the end of 1984, chloroquine-resistant P. falciparum was present in 14 countries in eastern Asia and Africa (Fig. 2). Resistance of P. falciparum to the second-line drug, a sulfonamide/pyrimethamine combination, is already widespread in South-East Asia and South America and recent reports indicate an increasing loss of treatment efficacy in other areas including East Africa (1). In fact, the spread of multi-resistant strains of P. falciparum has become so serious a challenge to malaria control that in certain parts of the world effective chemotherapy now relies on the last line of available antimalarial drugs such as quinine plus tetracycline.

The occurrence and spread of drug-resistant malaria parasites are being monitored by WHO, as part of a global programme on the susceptibility of P. falciparum to antimalarial drugs, using both in vivo and in vitro techniques. In vitro micro-tests based on the methodology of Rieckmann and his co-workers (2) are now available and WHO has developed kits for measuring the parasite's susceptibility to chloroquine, amodiaquine, quinine, and mefloquine. These methods provide quantitative concentration response data and are suitable not only for measuring the parasite's response to a drug but also for longitudinal observations on drug sensitivity (Fig. 3) as well as projections of in vivo response in nonimmunes.

This micro test, although it has been used to determine baseline data and subsequent changes in drug susceptibility of P. falciparum to chloroquine, mefloquine, amodiaquine and quinine, is not capable of measuring the susceptibility of isolates to the sulfadoxine/pyrimethamine combination or other similar combinations of antifolate drugs due to the high levels of para-aminobenzoic-acid (PABA) and folic acid in the medium. However, studies carried out under WHO auspices in Thailand, Haiti and Brazil using culture media with reduced concentrations of PABA and folic acid indicate that this medium is suitable for measuring the susceptibility of P. falciparum to pyrimethamine, sulfonamides, their combinations and surprisingly also to 4-aminoquinolines, quinine and mefloquine.

The development of drug resistance in areas in which previously susceptible parasites existed has so far always been associated with the type of drug used, the degree and duration of drug pressure and the degree of host/parasite contact. Resistance is also genetically determined and, according to Walliker (3), follows Mendelian types of inheritance. Resistance to pyrimethamine and chloroquine, once established, persists also in the absence of drug pressure and it has been shown in rodent malaria models that highly chloroquine-resistant parasites have a selective biological advantage over sensitive ones. This situation is totally contrary to that commonly found in other organisms where resistance is usually accompanied by concomitant biological disadvantage. Chloroquine-resistant P. falciparum parasites also appear to have a biological advantage which may explain their rapid dissemination in nature once foci have been established or selected (4,5). Laboratory studies also show that resistance to dihydrofolate reductase inhibitors, such as pyrimethamine, appears to be genetically independent from resistance to chloroquine, and resistant parasites do not seem to have a biological advantage (3). There is some evidence to suggest that one mechanism by which such resistance arises is gene amplification as observed in methotrexate resistance in Leishmania but this requires confirmation.

Recent advances in malaria research

Clearly the present malaria situation requires the improvement and wider
Fig. 3. - Chloroquine sensitivity of *P. falciparum* in vitro (WHO standard test). EC\textsubscript{99.0} is the concentration which kills 99.0% of the sensitive parasites and is equivalent to 1.0x10\textsuperscript{-6} mol per litre of blood. Regression lines to the left of the vertical threshold line indicate sensitive isolates whereas those to the right indicate resistant isolates.