Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *86*, Suppl. II, 181-184, 1991

ANTIMALARIAL CHEMOTHERAPY WITH NATURAL PRODUCTS AND CHEMICALLY DEFINED MOLECULES

LUZIA HELENA CARVALHO & ANTONIANA U. KRETTLI

Departamento de Parasitologia, Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais (UFMG) and Centro de Pesquisas René Rachou – FIOCRUZ, Caixa Postal 1743, 30190 Belo Horizonte, MG, Brasil

In the present work we have described the in vivo *antimalarial activity of six different plants. Two of them* (Vernonia brasiliana *and* Eupatorium squalidum) *were tested in a randomic approach among 273 crude extracts from plants; four* (Acanthospermum australe, Esenbeckia febrifuga, Lisianthus speciosus *and* Tachia guianensis*) were selected after screening 22 crude extracts from different medicinal plants used in Brazil against fever and/or malaria. We also studied chemically defined molecules and some of them showed antimalarial activity* in vitro. *Some aspects of recent research with natural products aiming to produce drugs are discussed.*

Key words: malaria – natural products – chemotherapy

Malaria is still the most important parasitic disease in the tropics. Despite of considerable efforts to control this disease the number of cases has been increasing with 5.3 million cases of disease reported by WHO in 1984. An accurate global evaluation of the frequency of clinical malaria cannot be made because of underdelection or underreporting, but a more realistic estimate is about 500 million cases a year (Struchler, 1989). The incidence of malaria in Brazil has also rised lately. In 1987 509,000 cases were reported more that 96% of them from the Amazon region (WHO, 1988). This rapid rise is directly related to the influx of non-immune settlers attracted by opportunities for farming and mining (Marques, 1987).

According to WHO drug resistance is probably the most important factor affecting malaria control at the present time. Since its development in the forties chloroquine has been successfully used by millions of human beings for prevention or treatment of all malaria species (Canfield, 1980). However, *P. falciparum* strains are now frequently resistant to this drug. Chloroquine-resistance (CR), originally reported in South-East Asia and South America, is now found in East Asia and Africa (Peters, 1985) and in Brazil it seems widespread in the endemic area (Reyes, 1981; Ferraroni et al., 1981). Treatment of CR malaria has been performed with alternative drugs or drug combinations, rather expensive and sometimes toxic. Furthermore, these combinations are not always based on pharmacokinetic principles, due to the inadequate knowledge of the metabolism and mechanism of action of most antimalarial drugs. Although these drugs are only one arm of the fight to reduce the quantum of malaria in the world, they are essential reducing morbidity and mortality (Peters, 1985). The need to develop new, safe and effective antimalarial drugs is urgent, considering also that the malarial vaccines had limited protective activity in human trials (Herrington et al., 1987; Patarroyo et al., 1988).

Some antimalarials originated directly from natural products – quinine, an alkaloid from the bark of *Cinchona* (Rubiaceae) has been used for centuries in the treatment of malaria, others have been chemically produced using natural products as a template – chloroquine, amodiaquine, mefloquine and, primaquine. The best example of drug development based on tradicional medicine is *Qinghaosu*, emerged from the Chinese experience, a sesquiterpene-lactone with potent antimalarial activity against multiple-resistant *Plasmodium falciparum*. It was rescued from a milenary tradicional use of the plant *Artemisia annua* L. (Gen & Lin, 1986; Phillipson, 1986). The Chinese scientists contributed with a novel antimalarial drug and their results encouraged the investigation of folk medicine (Klayman, 1985) including ours (Brandão et al., 1985).

The potentiality of the Brazilian flora in offering compounds of biological interest is enormous. There are here a rich folklore and exuberant vegetation in the endemic region. Therefore, it is also expected that search of tradicional plants in Brazil may lead to development of new drugs a task herein partly undertaken.

PLANT SELECTION AND ANTIMALARIAL TESTS

We have used two approaches for plant selection: (1) plants randomly collected, mostly received from our chemist collaborators at the University of São Paulo (NPPN-RP/SP) and formerly from the University of Rio de Janeiro (CPPN/RJ), their extracts being obtained with organic solvents (hexane, chloroform, ethanol); (2) plants popularly used as antimalarials or against fever and, selected after a survey among natives and migrants in the Amazon Region (Brandão et al., in preparation), their extracts being obtained with water because the plants are used popularly as teas.

All crude extracts have been screened *in vivo*, in mice infected with blood forms of *P. berghei*, using the suppressive test (Peters, 1965). The dried extracts were reconstituted in water plus 0.2% Tween 80. Doses of 100, 500 and 1000 mg/kg of extracts were administered to the infected mice orally for 4 consecutive days. Blood films were taken on the 5th day, Giemsa stained and examined microscopically. Drug activity was determinated by percent reduction of parasitemia in treated groups as compared to untreated controls.

Extracts showing antimalarial activity were tested *in vitro* using *P. falciparum* cultures (Rieckmann, 1978) in differents drug concentrations. After 24 and 48 h incubation the culture medium, with or without drug, was replaced and after 72 h blood smears were taken for evaluation of parasitemia.

In some *in vitro* experiments we have also studied the blood schizontocidal effect of chemically defined molecules obtained from plants (mostly Bignoniaceae family) or by synthesis. These compounds were received from the Department of Chemistry (ICEX/UFMG) and some of them, active *in vitro*, were produced in more quantity for the *in vivo* tests. Our main findings are as follows:

(a) Among 273 crude extracts obtained with organic solvents from plants randomly selected only two (0.7%), hexanic extracts of *Vernonia brasiliana* and *Eupatorium squalidum*, had a significant antimalarial action. Among 22 medicinal plants selected based on the rational approach four (18%) had some activity (Table I). Our results emphasize the importance of studying the ethnomedical use of plants which now is the approach guiding our work. The medicinal plants showing antimalarial activity were: *Acanthospermum australe* (Compositae), *Esenbeckia febrifuga* (Rutaceae), *Lisianthus speciosus* and *Tachia guianensis* (Gentianaceae). Water extracts of these plants inhibited about 40% parasite growth in mice infected with *P. berghei*. One of them, *A. australe*, was also active *in vitro* against *P. falciparum*.

TABLE I

Crude extracts from plants randomly collected or from medicinal plants used by Brazilian populations as antimalarials and tested against experimental malaria infections (*Plasmodium berghei* in mice)

Crude extracts	Total tested	Number of active (%)
Randomly selected plants	273	2 (0.7)
Medicinal plants	22	4 (18.0)
Total	295	6 (18.7)

(b) The number of chemically defined drugs tested and the number of those active are shown in Table II. Only among the naphthoquinones we found activity, some of them inhibiting in 100% *P. falciparum* growth. Four active naphthoquinones *in vitro* were also tested in mice with *P. berghei* and one was active. Synthetic analogues of the active molecules have been also prepared (by Oliveira et al.) in an attempt to improve their antimalarial activity. In preliminary assays some of these analogues were more active then the original compound. This is a promissing result since there are naphthoquinones already used in clinical trials (Gutteridge, 1989). Thus, we hope to be able to study them further as to determine their toxicity choosing the most active ones by titrating the inhibitory activity *in vitro* and *in vivo*.

TABLE II

Chemically defined compounds tested *in vitro* against *Plasmodium falciparum* and number of actives

Class of compounds	No. tested (origin)	No. active
Naphthoquinone	19 (9N/10S)	14
Anthraquinone	3 (N)	0
Benzoquinone	3 (N)	0
Xanthone	7 (N)	0
Others	2 (1S/1SS)	0

N: natural; S: synthetic; SS: semi-synthetic.

Testing the pharmacological activity of plants is a difficult task and clear-cut data leading to dose-response curves are quite rare (Farnsworth, 1984). Such limitations have also emmerged during our work and part of the difficulties seem to result from the low solubility of most extracts studied. Homogeneous solutions for the *in vivo* and *in vitro* tests are difficult to obtain even though we have used tensioactive agents.

The ethnopharmacological approach we used to search new antimalarial compounds among natural products was more promising then the randomic approach corroborating our previous suggestions (Brandão et al., 1985). At least 119 distinct chemical substances derived from plants can be considered as important drugs currently in use in one or more countries. About 74% of them were discovered as a result of studies to isolate the active substances responsible for the use of the original plants in tradicional medicine (Farnsworth et al., 1985).

There are many other reports about the pharmacological action of plant extracts but without the complementary phytochemical studies. There are also hundreds of isolated compounds from plants without further studies on its possible pharmacological action (Elizabetsky, 1987). Any effective and successful program to select pharmacologically active compounds from plants can only be antecipated in which a multidisciplinary group of scientists work harmoniously in a collaborative way and situated in proximity (Farnsworth, 1984).

In our study there has been a multidisciplinary collaboration which include pharmacologists, chemistries, phytochemistries, parasitologists and botanists, all located in various places, even in different states. Furthermore, this research is not always the main line of work, which results in a somewhat fortuite collaboration and sporadic contacts. The creation of an institution entirely dedicated to the research of natural products, similarly to what exists in China, seems a rather ideal but unrealistic solution in our country, at the present time.

Several problems in our country make research difficult, i. e., lack of funds, insuficient number of skilled personal, the financial support rather discontinuous and unpredictable. Furthermore, the production of a new medicine for humans is extremelly expensive. It has been estimated that one drug can cost over 50 million dollars, and take many years of research (Farnsworth, 1984). In addition, the development of new drugs implies a political decision which means, substantial emphasis on basic research, rules as to facilitate importation of reagents and equipments. Without a political decision plus a decisive participation of our industry in such projects it is unlike any new drugs will be developed here.

The potenciality of our forests and of our medicinal plants is enormous but it is not being well explored here. The same is not true as far as foreigner institutions which often collect, study, extract medicinal drugs from Brazilian plants, have patents for them, to finally export them back as medicines (Motidome, 1987).

ACKNOWLEDGMENTS

To Drs David S. Filho and João L. C. Lopes (USP, Ribeirão Preto) and, Maria das Graças L. Brandão (UFMG, Belo Horizonte), for all crude extracts; to Dr Alaide B. Oliveira and her group (ICEX, UFMG), Dr Carlos L. Zani (C. P. René Rachou, FIOCRUZ/MG) and, Dr Tanus J. Nagem (ICEX/UFMG), for the chemically defined molecules.

REFERENCES

BRANDÃO, M. G. L.; BOTELHO, M. G. A. & KRETTLI, A. U., 1985. Quimioterapia experimental antimalária com produtos naturais: I. Uma abordagem mais racional? *Ci. Cult. 37:* 1152-1163.

CANFIELD, C. J., 1980. Antimalarial aminoalcoohol alternatives to mefloquine. *Acta Trop., 37:* 232-237.

ELISABETSKY, E., 1987. Pesquisas em plantas medicinais. *Ci. Cult., 39:* 697-702.

FARNSWORTH, N. R., 1984. The role of medicinal plants in drug development, p. 17-30. In P. Krogsgaard-Larsen; S. B. Christensen & H. Kofod (eds). *Natural products and drug development.* Munksgaard, Copenhagen.

FARNSWORTH, N. R.; AKERELE, O.; BINGEL, A. S.; SOEJARTO, D. D. & GUO, Z., 1985. Medicinal plants in therapy. *Bull. WHO, 63:* 965-981.

FERRARONI, J. J.; SPEER, C. A.; HAYES, J. & SUZUKI, M., 1981. Prevalence of chloroquine-resistant *falciparum* malaria in the Brazilian Amazon. *Am. J. Trop. Med. Hyg., 30:* 526-530.

GEN, X. P. & LIN, F. S., 1986. Traditional anti-parasitic drugs in China. *Parasitol. Today, 2:* 353-355.

GUTTERIDGE, W. E., 1989. Parasite vaccines versus anti-parasite drugs: rivals or running makes? *Parasitol., 98:* 587-597.

HERRINGTON, D. A.; CLYDE, D. F.; LOSONSKY, G.; CORTESIA, M.; MURPHY, J. R.; DAVIS, J.; BAGAR, S.; FELIXT, A. M.; HELMER, E. P.; GILLESSEN, D.; NARDIN, E.; NUSSENZWEIG, R. S.; NUSSENZWEIG, V.; HOLLINGDALE, M. R. & LEVINE, M. M., 1987. Safety and immunogenicity in man of a synthetic peptide malaria vaccine against *Plasmodium falciparum* sporozoites. *Nature, 328:* 257-259.

KLAYMAN, D. L., 1985. *Ginghaosu* (Artemisinin): an antimalarial drug from China. *Science, 228:* 1049-1055.

MARQUES, A. C., 1987. Human migration and spread of malaria in Brazil. *Parasitol. Today, 3:* 166-170.

MOTIDOME, M., 1987. Os dados químicos publicados sobre plantas brasileiras são recuperáveis do ponto de vista de aplicação biológica? *Oréades, 8:* 246-250.

PATARROYO, M. E.; AMADOR, R.; CLAVIJO, P.; MORENO, A.; GUZMAN, F.; ROMERO, P.; TASCON, R.; FRANCO, A.; MURILLO, L. A.; PONTON, G. & TRUJILLO, G., 1988. A synthetic vaccine protects humans against challenge with asexual blood stages of *Plasmodium falciparum* malaria. *Nature, 332:* 158-161.

PETERS, W., 1965. Drug resistance in *Plasmodium berghei* Vincke and Lips, 1948. I. Chloroquine resistance. *Exp. Parasitol., 17:* 80-89.

PETERS, W., 1985. The problem of drug resistance in malaria. *Parasitol., 90:* 705-715.

PHILIPSON, J. D., 1986. The case for "plant medicines". *Parasitol. Today, 2:* 327-328.

REYES, S., 1981. Infecções maláricas por *Plasmodium falciparum* resistente ao tratamento com cloroquina. Situação no Brasil (1960-1981). *Rev. Brasil. Malariol. D. trop., 33:* 109-130.

RIECKMANN, K. H.; SAX, L. J.; CAMPBELL, G. H. & MREMA, J. E., 1978. Drug sensitivity of *Plasmodium falciparum.* An *in vitro* microtechnique. *Lancet, i:* 22-23.

STURCHLER, D., 1989. How much malaria is there world-wide? *Parasitol. Today, 5:* 39.

WORLD HEALTH ORGANIZATION, 1988. *Report on a technical consultation on research in support of malaria control in the Amazon basin.* 27 p. Brasília, (TDR/FIELDNAL/SC/AMAZ/88.3).