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RECENT ACQUISITIONS ON CHEMOTHERAPY AND CHEMOPROPHYLAXIS OF MALARIA

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Summary. - *The most recent acquisitions on chemotherapy and chemoprophylaxis of malaria are reviewed. With regard to chemotherapy, candidate antimalarial compounds have been divided into four groups, according to their stages of development. Mefloquine and the combination of mefloquine with sulfadoxine/pyrimethamine belong to the first group: they have completed clinical trials and have been registered in several countries for routine clinical use. The second group is characterized by chemical compounds which are in an advanced stage of development, including clinical trials. The compounds considered in this group are: a) the 9-phenanthrenemethanols, among which halofantrine is the most promising one; b) the sesquiterpene lactones such as Qinghaosu, artemether, artesunate, artesunic acid and arteether which must be further tested in order to find more effective drug regimens capable of eliminating recrudescences and for the completion of toxicity studies; c) pyronaridine, which appears to be a promising antimalarial, effective also against chloroquine-resistant* P. falciparum, *but still requiring further investigations on resistance and cross-resistance, as well as its pharmacokinetics, tolerability and bioavailability; d) enpiroline, another promising compound, which needs to be further studied in Phase II and Phase III investigations with naturally acquired malaria. The third group is composed of seven chemical classes of compounds that are in an advanced pre-clinical development, namely: the 4-aminoquinolines, such as dabechin, piperaquine, hidroxypiperaquine, tripiperaquine, dichlor-quinazine and the Mannich bases compounds, the 8-aminoquinolines, the 4-quinolinemethanols, the quinolones, the naphtoquinones, the quinazolines and the dihydrotriazines. Among the many antimalarial compounds of interest, which can be considered at the moment as leads for further studies, only the acridandione derivatives such as floxacrine, the antibiotics, antifungal agents or their metabolites, plant substances such as Yingzhaosu A and quassinoids have been mentioned. Malaria chemoprophylaxis, especially in chloroquine-resistant* P. falciparum *areas, has become a real problem. The attempts to secure protection under these circumstances with the utilization of amodiaquine, the combination of sulfadoxine/pyrimethamine (Fansidar), sulfalene/pyrimethamine (Metakelfin), of pyrimethamine/dapsone (Maloprim), with or without chloroquine, had to be abandoned or to be used with caution in view of the severe complications following the weekly administration of these drugs. The combination of chloroquine with proguanil or chlorproguanil, which could be recommended on theoretical bases, did not meet the expectations when tested in the field. Mefloquine can be given for short periods, 6 weeks, to international travelers to areas where chloroquine-resistant* P. falciparum *is prevalent; for longer periods it is not recommended at the moment, pending the results of prolonged longitudinal studies. The combination of tetracyclines, especially doxycycline with chloroquine at weekly intervals, needs to be further studied, though reservations can already be expressed in view of their toxicity.*

Riassunto (Recenti acquisizioni sulla chemioterapia e sulla chemioprofilassi della malaria). - *In questo articolo vengono presentate le più recenti acquisizioni sulla chemioterapia e sulla chemioprofilassi della malaria. Riguardo alla chemioterapia, gli antimalarici sono stati suddivisi in quattro gruppi, a seconda dello stadio di avanzamento degli studi farmacologici e clinici. Nel primo gruppo vengono trattate la meflochina e la sua associazione con la sulfadoxina/pirimetamina, ampiamente studiate dal punto di vista clinico e registrate in numerosi paesi. Il secondo gruppo è rappresentato da composti chimici con avanzato stadio di sviluppo, ampiamente valutato anche sotto il profilo clinico. I composti che sono stati presi in esame in questo gruppo sono: a) i 9-fenantren-metanoli, di cui l'alofantrina è il più promettente; b) i lattoni sesquiterpenici, come il Qinghaosu, l'artemetere, l'artesunato, l'acido artesunico e l'arteetere che dovrannno essere ulteriormente sperimentati per poterne stabilire più efficaci regimi farmacologici in grado di eliminare le recrudescenze e per completare gli studi sulla tossicità; c) la pironaridina, che sembra essere un promettente antimalarico, efficace anche in caso di clorochinoresistenza del* P. falciparum, *ma che necessita ancora ulteriori studi sulla resistenza, resistenza crociata, farmacocinetica, tollerabilità, e biodisponibilità; d) l'empirolina, un altro promet-*

tente composto, la cui attività deve ulteriormente essere studiata. Il terzo gruppo è costituito da sette classi di composti chimici che presentano un avanzato studio preclinico: le 4-amino-chinoline, come il dabechin, la piperachina, l'idrossipiperachina, la tripiperachina, la diclorochinazina e le basi di Mannich, le 8-aminochinoline, i 4-chinolinometanoli, i chinoloni, i naftochinoni, le chinazoline e le diidrotriazine. Dei numerosi promettenti composti antimalarici sono stati menzionati solamente i derivati acridandionici, come il floxacrin, gli antibiotici, gli agenti antimicotici o i loro metaboliti, le sostanze estratte dalle piante come lo Yingzhaosu A ed i quassinoidi. La chemioprofilassi, specialmente nelle zone di clorochinoresistenza del P. falciparum, *è diventata un vero e proprio problema. L'uso della amodiachina, le associazioni sulfadoxina/pirimetamina (Fansidar), sulfalene/pirimetamina (Metakelfin), pirimetamina/dapsone (Maloprim), con o senza clorochina, per poter assicurare una buona protezione nelle attuali circostanze ha dovuto essere abbandonato o prescritto con cautela a causa delle serie complicazioni derivanti dalla somministrazione settimanale di questi farmaci. L'associazione clorochina, proguanil o cloroproguanil, che, su basi teoriche, potrebbe essere consigliata, una volta sperimentata su campo non ha soddisfatto le nostre aspettative. La meflochina può essere somministrata per brevi periodi di tempo (6 settimane) ai viaggiatori che si recano all'estero in quelle zone in cui prevale la clorochinoresistenza del* P. falciparum. *Per periodi più lunghi, per il momento, è preferibile non somministrarla, in attesa di risultati provenienti da altri studi longitudinali. La associazione di tetracicline, particolarmente di doxiciclina e clorochina ad intervalli settimanali, necessita ancora ulteriori studi, sebbene si possano già esprimere delle riserve data la loro tossicità.*

Introduction

With the advent of chloroquine resistance in *Plasmodium falciparum* some 15 years after the extensive use of the drug [1, 2], research organizations and industry were again motivated to invest or engage in the development of new antimalarial drugs. In this respect the major programme of antimalarial drug development was carried out by the Walter Reed Army Institute of Research (WRAIR) in the United States. In the course of this programme more than 300,000 chemical compounds were screened, some of which, approximately 300, reached the stage of preclinical development, a few have been registered or are undergoing clinical trials and several other compounds have qualified for clinical development.

Worthy of mention are also the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, which, through its Scientific Working Group of CHEMAL devotes efforts and funds in research on chemotherapy of malaria, development of new compounds and improvement of malaria treatment, and the Chinese Group of experts who have so much contributed to the discovery and development of new classes of antimalarials. Several pharmaceutical industrial firms have also been induced to resume malaria research.

In this presentation, an attempt has been made to summarize the most recent acquisitions in the chemotherapy and chemoprophylaxis of malaria, resulting from research activities carried out in recent years.

Chemotherapy

Numerous candidate antimalarial compounds are in various stages of development: those which have completed clinical trials and are already available for use, those in an advanced stage of investigation including clinical trials, those in an advanced preclinical development and finally other compounds of interest.

New antimalarials which have completed clinical trials

The chemical class of interest belonging to this group is represented by the quinolinemethanols. During the Second World War the first quinolinemethanol to be developed and tested (in the United States) was SN 10,275 which, despite an encouraging evidence of activity, was not further developed because of its phototoxicity.

This old lead was reexamined always in the United States in the 1960s when new compounds, that might retain the antimalarial activity of SN 10,275 but would not have phototoxic characteristics, were synthesized and tested. One of the more active compounds which was produced during these studies was WR 30,090 which advanced to the stage of clinical trials. It showed great antimalarial activity but also poor bioavailability with consequent erratic absorption and the need for at least 6 days of drug administration.

Among the other quinolinemethanols which were synthesized and tested, the one which was selected for advanced testing was WR 142,490 (racemic erythro-a-(2-piperidyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol which was later named mefloquine [3] (Fig. 1).

Mefloquine. - This 4-aminoquinolinemethanol derivative was subjected to extensive pre-clinical toxicological studies that demonstrated sufficient safety for continued development. New methods for assessing the concentration of the parent active substance and of its metabolite in various body fluids was elucidated in rats [4, 5].

The absorption, distribution in different organs and the elimination of C-labelled mefloquine were determined in mice, rats and Aotus monkeys [6-9].

In pharmacokinetic studies it was demonstrated that the absorption of mefloquine is rapid, 0.36-2.0 h, the plasma concentration is about 1.0 mg/l 2-12 h after administration with a terminal half-life of elimination ranging from 15 to 33 days (mean 21.4) [8, 10].

In the plasma more than 99% of mefloquine is bound to proteins, with a much higher concentration (nearly 70% of that in the plasma) in or on erythrocytes where about half

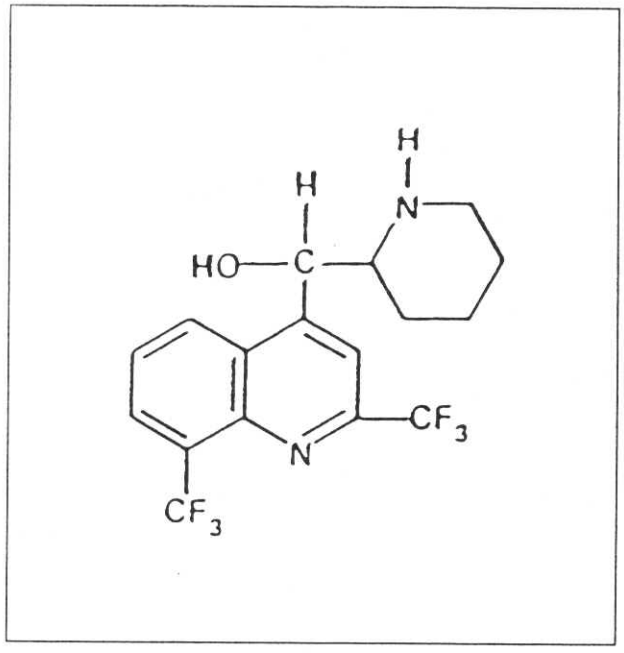

Fig. 1. - Mefloquine (WR 142, 490) α-(2-piperidyl-2,8-bis(trifluoromethyl)-4-quinoline methanol.

of the mefloquine appears to be bound to the cell membrane [5]. Meanwhile, sufficient evidence had been accumulated indicating that mefloquine was a potent, long-acting blood schizontocide active against multi-resistant falciparum malaria and *P. vivax*.

In addition it had the great advantage of being effective in a single dose so avoids the problem of patient compliance.

Clinical trials of mefloquine started in 1972 within the research programme of the WRAIR and continued from 1976 onwards when they were the object of a collaborative effort of CHEMAL, WRAIR and the pharmaceutical company Hoffman-La Roche. Many articles have been published regarding the utilization of mefloquine as monosubstance for the treatment of *P. falciparum* especially multi-resistant infections [11-17].

The conclusion which can be drawn from these studies is that mefloquine is generally well tolerated and safe and has a potent blood schizontocidal activity against parasites resistant to other antimalarials such as chloroquine and pyrimethamine. Cure rates higher than 95% were obtained with single doses of 750 mg or 1000 mg base. The drug has a long but variable half-life in man. This accounts for its single dose therapeutic activity but also suggests that at 750 mg, or 1000 mg or even higher single doses, there are likely occasional treatment failures due to host factors. The side-effects, which are transient and generally mild, include nausea, giddiness, vomiting and diarrhoea. Sinus bradycardia has been noted in some patients, lasting for a few weeks without symptoms and needing no treatment.

No biochemical or haematological abnormalities have been reported. Mefloquine is being tried in pregnant women, in patients with liver dysfunctions and anemia and in children below 2 years of age. Preliminary results indicate that the drug is effective and well tolerated but great numbers of patients must be studied before reaching definite conclusions.

Mefloquine as monosubstance was first registered in Switzerland under the trade name of Lariam (R) for prophylaxis and treatment in adults and children over 2 years of age. Subsequently Lariam (R) has been registered in Australia, Brazil, France, West Germany and New Zeland.

Mefloquine with sulfadoxine and pyrimethamine. - The decrease in susceptibility of *P. falciparum* to mefloquine in continuous culture [18], the clinical evidence of resistance to mefloquine in Thailand, where the drug had only been available in small quantities [19], which was later confirmed in cloning experiments [20], the reports of *in vitro* mefloquine-resistant *P. falciparum* from the Philippines [21, 22] and of one case of falciparum mefloquine-resistant malaria from Tanzania where the drug had never been used and which was enhanced by treatment [23], induced to think that parasites resistant to mefloquine may exist in nature without previous exposure to the drug or may be selected.

To delay the emergence of resistance to mefloquine, studies were initiated by CHEMAL and by industry on the development of mefloquine drug-combinations.

Studies at the London School of Tropical Medicine and Hygiene have shown that the response of *P. berghei* to mefloquine in association with other antimalarials is at least additive (probably even potentiating) and that free-combinations, especially with mixtures of sulfadoxine and pyrimethamine, have a pronounced delaying effect on the development of resistance to the individual component [24]. Observations in several animals models suggested that the combination would be safe and effective in human patients, with the exception of those sensitive to sulfamides.

Dose-finding studies in adult males were carried out in Thailand, Colombia, Burma, Brazil and Zambia.

Good results were obtained in Thailand with a single oral dose of mefloquine (750 mg/base) and a single oral dose of MSP (750 mg mefloquine, plus 3 tablets of Fansidar) were equally effective in the treatment of falciparum malaria [25]. Single oral doses of two or three tablets regimens of the triple combination were also tested in Thailand with a cure rate of 93% with the two tablets and 98% with the three tablets with no statistically significant differences among the two groups [26].

Two drug regimens were tested in Colombia: one group of the patients receiving single dose treatment of a combination of mefloquine 280 mg, sulfadoxine 800 mg and pyrimethamine 40 mg, and another group a combination of mefloquine 420 mg, sulfadoxine 1200 mg and pyrimethamine 60 mg. The cure rates in both groups (28 days study period) was 100% [27].

In Burma, 54 semi-immune adults with falciparum malaria were given single dose of a fixed combination of 750 mefloquine, 1500 mg sulfadoxine and 75 mg pyrimethamine. All the patients were cleared of asexual parasites by days 7, giving a cure rate of 100% [28].

In Brazil, 150 falciparum infections were treated with 1, 2 or 3 tablets of the triple combination. Of those receiving one tablet, 81% were cured and 19% exhibited RI recrudescences. All the patients receiving 2 or 3 tablets were cured [29].

In Zambia, where there seems to be no resistance to sulfadoxine-pyrimethamine, a single tablet or 2 tablets of the triple combination cured all the patients treated [30, 31].

The main side-effects which were observed in the course of these trials were abdominal discomfort, nausea, vomiting, dizziness and diarrhoea, but were mild, transient and required no specific treatment. The results of various haematological, biochemical and urine analysis were not adversely altered by the administration of the drug. Sinus bradycardia and arrhythmia were occasionally revealed by electrocardiograms of some patients in Thailand, but these conditions were transient, symptomless and clinically non significant.

A few cases of severe skin reactions were reported from Thailand where the triple combination had been introduced in 1984/85 by the National Malaria Programme for the treatment of microscopically confirmed cases of multiresistant falciparum infections [32]. At an informal consultation purposely convened by the Malaria Action Programme of WHO in March 1986, further studies were recommended to reexamine the risk/benefit ratio of the use of mefloquine in combination with pyrimethamine/sulfadoxine, particularly related to the effect of such combinations on the rate of emergence of resistance strains and on their efficacy and tolerance in human populations.

The triple combination, under the trade name of Fansimef, has been registered in Thailand, Brazil, Burma, Colombia, Ecuador, France, New Zeland, the Philippines, Surinam and Zimbabwe.

One case of R II and one of R III resistance *in vivo* has been reported from Indonesia out of 36 *P. falciparum* infections treated with Fansimef [33].

It has been argued that the WHO classification was developed to evaluate chloroquine resistance and may not be applicable to slow acting drugs such as mefloquine or sulfadoxine-pyrimethamine. Longer follow-up of the two patients might well have revealed complete clearance shortly after day 7 [34].

Compounds in an advanced stage of development, including clinical strials

Four chemical groups are included in this category: the 9-phenanthrenemethanols, the sesquiterpene lactones, pyronaridine and enpiroline.

The 9-phenanthrenemethanols. - During the Second World War it was demonstrated that several 9-phenanthrenemethanols possess blood schizontocidal activity [35, 36] and two of them (SN 8867 and SN 9160) were successfully tested in man. However, further studies were not pursued as at that time the 4-aminoquinolines were more active and easier to administer.

The exploration of these compounds was resumed by the WRAIR after the advent of chloroquine resistant *P. falciparum*. Among the compounds studied, WR 33,063 was found to be rapidly effective in falciparum and vivax malaria and to be well tolerated in therapeutic doses in clinical studies [37-40], but a poor suppressant [41] and requiring the administration of 1.6 g (in four divided doses) daily for six consecutive days for satisfactory clinical treatment.

In further research, two other compounds, WR 122,455 and WR 171,669, were found to be more active than WR 33,063 in experimental malaria [42-44].

R 171,669 had the advantage over WR 122,455 to be better tolerated in man and to be active with a single day treatment [45] whereas WR 122,455 had to be administrated over several days [46]. For these reasons, WR 171,699 was selected for further clinical studies and has been given the generic name of "halofantrine" (Fig. 2).

Halofantrine. - The median curative dose, CD_{50}, of halofantrine has been assessed in the *P. berghei*/mouse model [47], in the *P. falciparum/Aotus trivirgatus* model [48] and *in vitro* [49]. Toxicological studies were carried out in various animal models without evidencing significant toxicity at the dose level used in human studies [45].

Phase I and II trials have been conducted in volunteers confirming that the drug is a potent blood schizontocide against multiresistant *P. falciparum* [45, 46]. It appears that a problem of bioavailability exists with halofantrine, probably due to poor absorption.

The best results have so far been obtained with the administration of three doses of 500 mg at 6 hourly intervals which cured 28 out of 29 infections [50]. Cross-resistance between mefloquine and halofantrine has been reported in *P. berghei* [47], but in further studies it was found that the sensitivity to mefloquine is independent from that to halofantrine and that cross-resistance is not absolute [50].

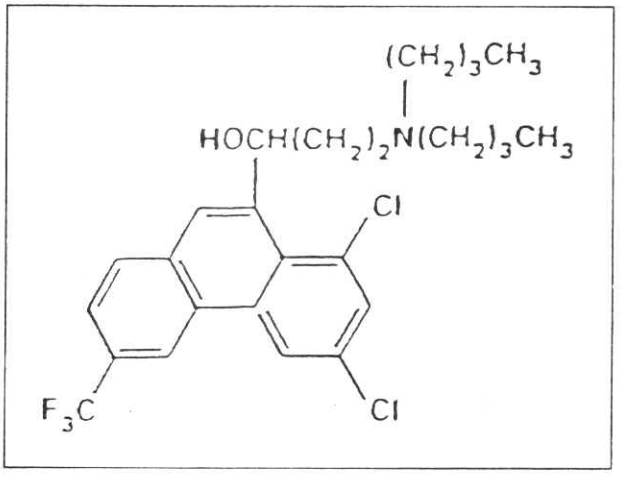

Fig. 2. - Halofantrine (WR 171,699) 1,3-dichloro-α-[2(dibutylamino)-ethyl]-6-(trifluoromethyl)-9-phenanthrenemetanol.

Collaborative studies in human subjects involving CHEMAL, WRAIR and the pharmaceutical company that intends to develop and market the drug are being conducted in Malawi and Kenya, and results may soon be available.

The sesquiterpene lactones. - An indigenous plant, *Artemisia annua* has been used in China as a herbal medicine for more than 2000 years. The active principle from the crude antimalarial fraction, a pure sesquiterpene lactone, was isolated and identified in 1972, when it was named Qinghaosu (or artemisinine or arteannuin) [51]. In the following years, Qinghaosu and its derivates were studied in China for their efficacy in laboratory malaria models, and as regards their pharmacology, pharmacokinetics and toxicology.

Qinghaosu, which is practically insoluble in water, sparingly soluble in oils and which dissolves in ethylalcohol, *artemether*, the methyl ether of Qinghaosu, soluble in lipids such as tea seed oil, and *artesunate*, the sodium succinyl salt of Qinghaosu, rather soluble in water but rather unstable in aqueous solutions, were finally chosen for further preclinical and clinical development (Fig. 3).

Another compound, *artesunic acid* or Qinghaosu succinic acid was found to be not hygroscopic and as effective as the sodium salt. Since 1985, attention has been focused on the development of *arteether*, the beta-ethyl ether derivative of artemisinine, which, being quite stable in storage and highly soluble in oils, may well be suited for the preparation of injectable preparations.

Acute toxicity studies in mice have shown that Qinghaosu, artemether and artesunate have higher LD50 values and chemotherapeutic indices than chloroquine. Subacute toxicity studies in monkeys have indicated that the toxic effect of Qinghaosu may be exerted mainly on the erythroid haemopoietic cells of the bone marrow with liver damages at higher doses.

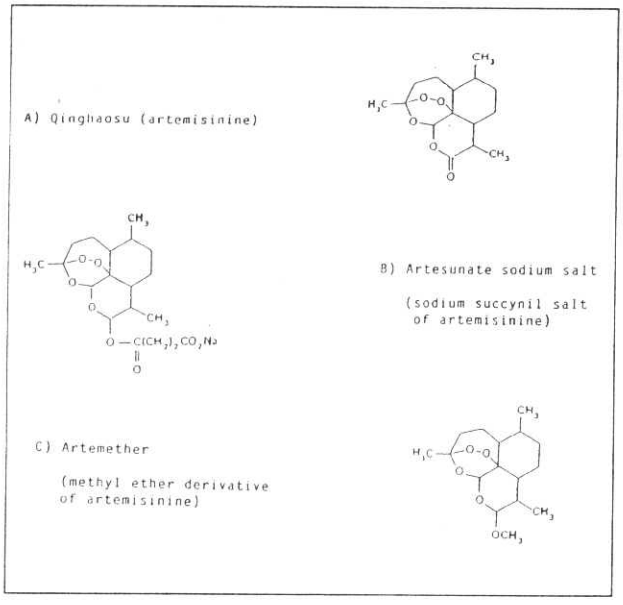

Fig. 3. - Qinghaosu (A) and its derivates (B, C).

In all animal species examined, the three drugs were well tolerated, adverse effects occurring only when large doses were given [52]. Studies, always conducted in China, have shown that artemether is strongly bound to plasma protein and is rapidly transferred to the erythrocytes of human or monkey blood *in vitro* [53]; when a fat emulsion was given intravenously to rabbits, the highest concentration of artemether was found in the brain, indicating that the drug could easily cross the blood-brain barrier, the peak level being attained after only 15 minutes.

Clinical studies with Qinghaosu and its derivates were conducted in Hainan Island, where *P. falciparum* is predominantly chloroquine-resistant, and Henan Province in China since 1973, even before toxicity studies had been undertaken. From 1973 and 1980, 3368 cases of falciparum and vivax malaria were treated with Qinghaosu, artemether and artesunate [54]. Cure rates of over 90% were obtained in all groups and results showed that the schizontocidal action of Qinghaosu and its derivates is significantly higher than that of chloroquine, quinine and mefloquine [55]. 157 cases of cerebral malaria were also treated with the three drugs: 106 with Qinghaosu (9 deaths), 29 with artemether (1 death) and 22 with artesunate (2 deaths). The over all cure rate was 92.4%, well above the average recorded with other forms of treatment. Qinghaosu in water suspension appeared to have the slowest effect, sodium artesunate the fastest [53]. No toxic effects were reported with the three drugs.

Although parasite clearance and fever subsidence are fast following treatment with all formulations of Qinghaosu and its derivatives, the rate of recrudescences are significantly high. This dose, and probably also a time dependent phenomenon, may be eliminated with more effective drug regimens, conducive to an improved radical cure rate. Further clinical drug regimen studies are also needed to evaluate the effect on the haemopoietic system and their phototoxicity.

In an attempt to eliminate or reduce the relatively high recrudescence rates following treatment with Qinghaosu and its derivates, different drug combinations have been used [56-59]. The best results have been obtained with the quadruple combination of mefloquine, sulfadoxine, pyrimethamine and Qinghaosu, but it is too early to draw conclusions from limited clinical studies and perhaps also too early to embark on clinical combination studies in the absence of exhaustive toxicological and pharmacological observations with Qinghaosu and its derivates.

Pyronaridine. - Pyronaridine, which is a 2-methoxy-7-chloro-10-[3,5'-bis(pyrrolidino-1-methyl)-4'-hydroxyanilinol] -benzo (b)-1,5-naphthyridine tetraphosphate (Fig. 4) was synthesized in Shangai, China in 1972 [59].

The compound showed marked antimalarial activity in rodent and simian malaria: when given to mice infected with chloroquine-sensitive and chloroquine-resistant

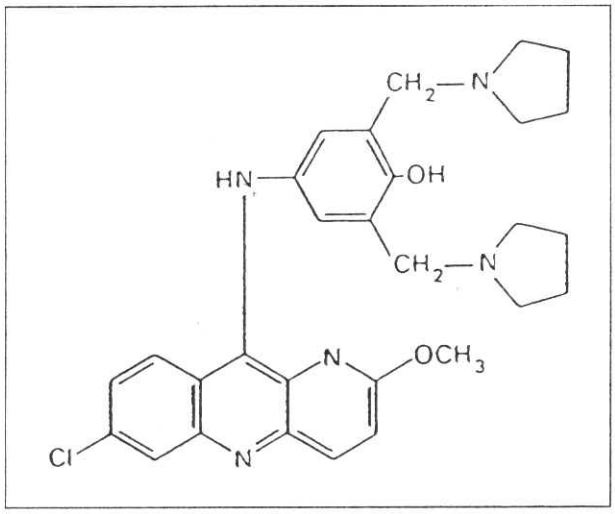

Fig. 4. - Pyronaridine (7351) 2-methoxy-6-chloro-9 [3,5-bis(1-pyrrolidinyl methyl)-4-hydroxy]anilino-1-aza-acridine.

P. berghei, pyronaridine acted as a highly effective blood schizontocide, whereas results in *Macaca mulatta* infected with *P. inui*, *P. cynomolgi*, or *P. knowlesi* were ambiguous probably due to inadequate drug regimens [60, 61].

Teratogenicity tests in rats with high doses yielded no evidence of teratogenic effects although the rate of fetal resorption was increased, but induction of mutations was seen in the *Salmonella typhimurium*/microsome system with strain TA 1537 [62].

Resistance to pyronaridine could be induced through increasing sub-curative doses of the drug in the *P. berghei* model. Without drug pressure the pyronaridine-resistant line reverted to sensitivity within five passages [63].

Pharmacokinetic studies in rabbits indicate peak plasma concentrations 15 min after i.m. injection of pyronaridine. The drug does not appear to have cross-resistance with chloroquine.

More than 1000 patients with vivax or falciparum malaria were treated in the Hainan Island, including patients with cerebral malaria. The drug was given either using oral administration (6 mg base/kg body weight twice on the first day, and once on the second and third day), or by i.v. infusion (total dose 6 mg/kg body weight). 8.8% recrudescence rate was observed among 342 falciparum patients who were followed for 30 days, all recrudescences being found in patients having received oral treatment. Better results were obtained in the parentally treated groups whereas no difference resulted in the parasite clearance time. Clearance of fever and parasitaemia was relatively faster in vivax malaria.

Side effects included nausea, diarrhoea, slight abdominal pains, vomiting, palpitations, headache and skin rash and were less common when the drug was given parentally.

Pyronaridine appears to be a promising antimalarial, effective also against chloroquine-resistant *P. falciparum*.

Further studies are needed on resistance and cross-resistance, as well as on the pharmacokinetics, tolerability and bioavailability of the compound. The causal prophylactic activity in *P. berghei* or *P. yoelii*, superior to that of primaquine [64], merits further investigations.

Enpiroline. - Of the 21 trifluoromethyl substituted a-alkylaminomethyl-2, 6-bis(aryl-4-piridinemethanols which were synthesized in 1972 [65], some of which had shown antimalarial activity in the *P. berghei*/mouse screen [66] and in the *P. falciparum*/*Aotus trivirgatus* system [67], two compounds were finally selected for clinical trials: WR 172,435 and WR 180,409 (enpiroline) (Fig. 5).

Both drugs have shown blood schizontocidal activity against *P. falciparum* and *P. vivax:* they are ineffective against exoerythrocytic forms. They lack phototoxicity and specific organ toxicity and have shown acceptable levels of toxicity in mice, rats and dogs [67]. They also showed a lower subacute toxicity than mefloquine in rats and dogs [47]. Clinical studies on WR 172,435 were discontinued as it caused reversible leukocytosis in healthy male volunteers [68].

Toxicological and pharmacokinetic studies with enpiroline in man showed good tolerability of single doses up to 1 g. The drug seemed to be well absorbed and distributed in the tissues and excreted via the bile in the faeces in the course of 20 days. The mean half-life was estimated to be six days [68].

In a clinical phase II dose finding study, enpiroline was administrated to 22 non-immune adult males with induced infection of the chloroquine, quinine and pyrimethamine-resistant Vietnam Smith *P. falciparum* isolate [68]. Three oral drug regimens were tested: a) three doses of 500 mg every 12 hours; b) 500 mg, 500 mg and 250 mg, or c) 500 mg, 250 mg and 250 mg at 12 hourly intervals: they were all curative. The drug was generally well tolerated: nausea, vomiting and diarrhoea, which are also associated with falciparum malaria, were occasionally encountered.

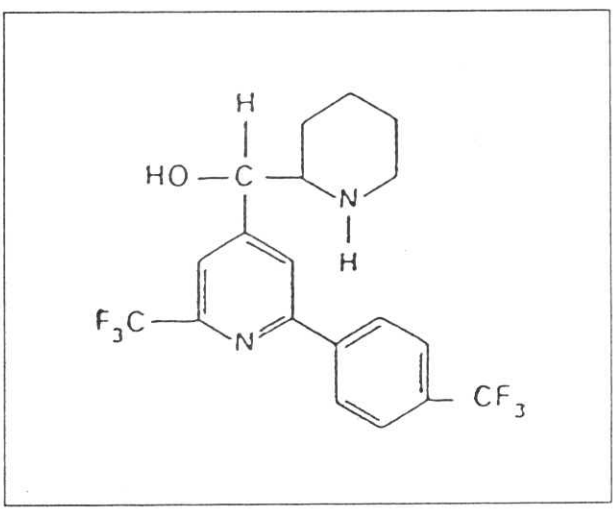

Fig. 5. - Empiroline (WR 180,409) 2-trifluoromethyl-4-[α-hydroxy-α-(2-piperidyl)-methyl]-6(4-trifluoro-methylphenyl)-pyridine.

No signs of emerging resistance to the 4-pyridinemethanols were ever found in the course of the investigations. Enpiroline seems to be a promising antimalarial compound; further phase II and phase III studies in naturally acquired malaria are required to confirm this expectation.

Compounds in an advanced preclinical development

Seven chemical groups are included in this category: the 4-aminoquinolines, the 8-aminoquinolines, the 4-quinolinemethanols, quinolones, naphtoquinones, quinazolines and diydrotriazines, the majority of which were identified and tested by the WRAIR drug development programme.

The 4-aminoquinolines. - Once it became clear that differences in activity exist between chloroquine and amodiaquine and that there is not always cross-resistance between the 4-aminoquinolines, attention was focused again on this class of compounds.

a) *Dabechin.* (benzo (g)-4-(diethylamino-ethylamino) quinoline. The compound was synthesized in the Soviet Union [69].

Its blood schizontocidal activity in the *P. berghei*/mouse model was found to be almost equal to that of chloroquine. The half-life in mice was demonstrated to be longer than that of chloroquine [70], and it was better tolerated. Strong reservations on the future of this compound have been expressed, however, in view of its cross-resistance with chloroquine.

b) *Piperaquine.* A symmetric compound linking (two molecules of 7-chloro-4(1-piperazinyl)-quinidine linked through a propyl group). This compound possesses blood schizontocidal activity similar to that of chloroquine and experimental studies carried out in China with the *P. berghei*/mouse model suggest that is more effective, longer active and less toxic than chloroquine [71]. Field studies carried out in Hainan Island in 1972, showed that piperaquine had higher suppressive and therapeutic activity than chloroquine but actually no comparisons were made with chloroquine [72].

c) *Hidroxypioeraquine.* (1,3-bis/1 (7-chloro-4-quinilyl)-4-piperaziny1)-2-hydroxypropane).

Hydroxypiperaquine, a derivate of piperaquine, was synthesized in China [73].

In acute, sub-acute and chronic toxicity studies carried out in animals, it appeared to be better tolerated than chloroquine. However, some degree of cross-resistance with chloroquine was found in laboratory studies, after field trials in China had indicated that drug was active against chloroquine-resistant *P. falciparum* and *P. vivax* [74].

d) *Tripiperaquine.* (1,4-bis[1-(7-chloro-4-quinoline)-4-ethylpiperazinyl] piperazine).

Another derivative of hydroxypiperaquine, tripiperaquine has been studied in mice and monkeys.

In the *P. berghei*/mouse model the compound was more active than chloroquine and the suppressive blood schizontocidal activity persisted longer against *P. berghei* and *P. cynomolgi*. However, in established infections with *P. cynomolgi* and *P. inui*, the blood schyzontocidal activity was incomplete.

Good results were obtained in clinical trials against *P. falciparum* and *P. vivax* using tablets containing 100 mg tripiperaquine, 25 mg sulfadoxine and 2.5 mg pyrimethamine (six tablets as a first dose, followed by four tablets six to eight hours later) [75].

e) *Dichlorquinazine.* A compound structurally related to tripiperaquine, which was synthesized by Rhône-Poulenc. Dichlorquinazone has shown no cross-resistance to chloroquine *in vitro* [76] and seems to be worth further evaluation.

f) *Mannich bases compounds.* Among this group of compounds, WR 194,965 has proved to be approximately ten times as effective as amodiaquine in the *P. cynomolgi*/rhesus and *P. falciparum/Aothus* models, [77] and good tolerability was demonstrated in preclinical studies. Another Mannich base compound, WR 228,258 was found to be more active than WR 194,965 or amodiaquine in the *P. berghei*/mouse screen [77, 78]. In *in vivo* animal studies and *in vitro* studies with *P. falciparum* isolates, lack of cross-resistance with chloroquine was demonstrated [79, 80]. Considering that WR 228,258 has also a long biological half-life, it may be concluded that this hybrid compound is one of the most interesting and promising leads in malaria chemotherapy.

The 8-aminoquinolines. - Primaquine is the only tissue schizontocidal drug available today for the radical curative treatment of *P. vivax* and *P. ovale*, although it is associated with side-effects which at times limit its use.

Several years ago a modest screening effort to search for alternative drugs was initiated by the U.S. Army Antimalarial Programme. Approximately 4000 compounds of diverse structure were screened in rodent and simian malaria models, 700 of which showed causal prophylactic and tissue schizontocidal activities [81].

Only the 6-and 8-aminoquinolines were capable of curing persistent exorythrocytic infections of *P. cynomolgi* in rhesus monkeys, but the 6-aminoquinolines were less active than primaquine.

Among the 8-aminoquinolines, four phenoxy-primaquine derived compounds were found to possess a radical curative activity five times higher than that of primaquine, namely WR 225,448, WR 233,195, WR 233,078 and WR 242,471.

WR 225,448 has shown a higher blood schizontocidal activity than primaquine in the *P. berghei*/mouse, *P. cynomolgi/Macaca mulatta* and in the *P. vivax/Aotus trivirgatus* models, and little cross-resistance with primaquine against the asexual blood stages of the primaquine-resistant line of *P. berghei* [82]. In toxicity studies in rodents, the acute toxicity of WR 225,448 is less than that of primaquine, but chronic toxicity appears to be higher and the haematotoxicity more marked than with primaquine [77].

Tolerance of the other compounds WR 233,078, WR 233,195 and especially WR 242,471 remains to be assessed. A series of 5,6-bis(alkoxy)-8-aminoquinolines were synthesized in the early eightees [83]. The most active compounds were two (5,6-dimethoxy-4-methyl-8-quinolinyl)-hexanediamines, which though apparently having the same toxicity of primaquine, showed a higher curative effect and suppressive action at relatively low doses.

The 4-quinolinemethanols. - In addition to mefloquine, a drug now available on the market, investigations were carried out with other 4-quinolinemethanols.

Two compounds are of interest: WR 184,806 (a-(tert-butylaminoethyl)-2,8-bis-(trifluoromethyl)-4-quinolinemethanol) [84] and WR 226,253 (a-(2-piperidyl)-2-trifluoromethyl)-4-quinolinemethanol) [85]. The two compounds have been tested in *Aotus trivirgatus* with induced *P. falciparum* and *P. vivax* infections and both proved to be highly effective especially against *P. vivax*. WR 184,806 was one-third as effective and WR 226,253 twice as effective as mefloquine [86]. A full curative dose of WR 184,806 could be administered with a single i.v. (as the phosphate salt) injection.

Quinolones. - Several of the 350 quinolones, which were synthesized by Imperial Chemical Industry Ltd in the course of a research programme aiming at the development of effective coccidiostatic agents, showed also antimalarial activity against *P. berghei* in mice. They are well tolerated compounds and causal prophylactic activity in both *P. berghei* and *P. yoelii* was observed with ICI 56,780 with a single s.c. dose of 30 mg/kg [87].

This causal prophylactic activity may merit further investigations so as the possibility of potentiating their schizontocidal effect through their combination with suitable partners.

Naphtoquinones. - Since the antimalarial activity of naphtoquinones was recognized in the mid 1940s, many quinone derivatives have been synthesized and tested, especially against *P. berghei* in mice [88, 89], which showed a marked effect on tissue forms [90]. One compound, monoctone WR 49,808) was utilized in patients infected with the Malaya (Camp) isolate of *P. falciparum* [91].

Only a slight blood schizontocidal or sporontocidal effects were observed with an oral administration of 0.4-0.5 g for three days, which was attributed to poor absorption from the gastrointestinal tract.

Recently, one compound, BW 58 C (2-(4-t-butylcyclohexyl)-3-hydroxy-1,4-naphtoquinone), was shown to be highly effective against *P. falciparum in vitro* and *P. berghei* and *P. cynomolgi in vivo* [92].

The compound, which has a low mammalian toxicity, was also active against chloroquine, mefloquine, pyrimethamine, sulfonamide and primaquine-resistant lines of *P. berghei*. Investigations are in progress with a more promising derivative.

Quinazolines. - A group of compound which act as DHFR inhibitors. Some of them have been tested in *P. berghei*/mouse model and against *P. cynomolgi* and *P. knowlesi* in *Macaca mulatta* [93, 94] and in *P. falciparum*/*Aotus trivirgatus* [95]. Suppressive schizontocidal activity was observed with most of them, but radical cure was rarely obtained. Investigations carried out in China with one quinazoline in experimental infections with *Aedes albopictus, P. gallinaceum, P. cynomolgi*/*A. stephensi* or *A. balabacensis* s.l. and with *A. balabacensis* s.l. membrane fed with blood from patients with vivax or falciparum malaria, showed a sporontocidal effect [96].

Marked synergism was found with sulfodiazine and two quinazolines (WR 158,122 and WR 159,412) in *Aotus trivirgatus* infected with drug-sensitive and drug-resistant isolates of *P. falciparum* and *P. vivax* [97].

Only one (Arylthio)quinazoline, WR 158,122, has been tested in man: in doses of 1 g daily for 3 days cured one of two volunteers with drug-sensitive falciparum malaria. When the drug was given in combination with sulfodiazine (200 mg/2 g) daily for 3 days to three volunteers with the same drug-sensitive strain, all 3 were cured [98].

Dihydrotriazines. - Another DHFR inhibitors group of compounds synthesized by Beechman Research Laboratories [99]. In the *P. berghei*/mouse model, WR 38,839, showed blood schizontocidal activity but recrudescences were observed [100]. Full causal prophylactic action was exerted by the compound in the *P. berghei*/mouse system of single doses of 10,3 and 1 mg/kg, given s.c. 45 min after sporozoite inoculation. In non-immune volunteers, infected with the drug-sensitive Uganda I isolate of *P. falciparum*, radical cure was obtained with WR 38,839 given orally at the dose of 0.6 g daily for 3 days, but approximately double their dose failed to cure infections with the resistant Malaya Camp and Vietnam Marks isolates [101].

In partially immune African children, WR 39, 839 showed significant schizontocidal activity against a pyrimethamine-sensitive strain of *P. falciparum* [102], which was potentiated by sulphadimethoxine and sulfafurazole [103].

Other compounds, synthesized later, WR 99,210, BRL 51,084 and BRL 6231, showed no cross-resistance with pyrimethamine [104].

The problem to be solved with these compounds is their poor bioavailability after oral administration. Further investigations may be justified to fully exploit their causal prophylactic action and to assess the potentiating effect exerted by sulfonamides.

Other antimalarial compounds of interest

There are many compounds which have shown activity against malaria parasites, but investigations are often at a

preliminary phase. Most of these compounds should actually be considered as lead chemical groups for further studies.

We shall mention only a few of these new leads. The development of "floxacrine", an acridandione derivative, which showed causal prophylactic as well as schizontocidal activity against *P. berghei*, *P. vinckei* and *P. cynomolgi* [105], has been discontinued following the occurrence of endarteritis in chronic toxicity studies in dogs.

New less toxic acridandione derivatives have been synthesized, however, one of which, S 825,455, has exhibited an antimalarial activity greater than floxacrine [106].

Among the antibiotics, the tetracyclines, especially minocycline and doxycycline have shown to act on the tissue forms and markedly against blood stages of malaria parasites.

A chlorinated derivative of lincomycin, "Clindamycin" is much more active than lincomycin and has been used in conjunction with quinine, but is associated with a high risk of inducing pseudo-membranous colitis. "Salinomycin" was found to exert significant activity against *P. falciparum* and *P. berghei in vitro* and *P. berghei* in rats [107].

Amphotericin B and 2 other antifungal agents, ketokonazole and miconazole, were strong inhibitors *in vitro* at non-toxic concentrations of chloroquine-sensitive and chloroquine-resistant *P. falciparum* isolates [108].

A metabolite of the fungus *Tolypocladium inflatum*, cyclosporin A has been shown to possess a potent effect against *P. berghei* and *P. yoelii* in mice and *P. falciparum in vitro* [109]. It would appear that it may be very useful in the treatment of severe and complicated falciparum malaria if given simultaneously with intravenous quinine.

Among the plant substances, "Yingzhaosu A" from *Artabetrys hexapetolus* seems to possess a significant antimalarial effect in the *P. berghei*/mouse model [110], and substances derived from the *Simaroubaceae* family, the "quassinoids" completely inhibited *P. falciparum in vitro* at concentrations considerably lower than those utilized to inhibit tumor growth, for which they were initially synthesized.

Chemoprophylaxis

The selection of drugs for malaria chemoprophylaxis has become increasingly difficult in recent years for travelers visiting or residents living in areas where *P. falciparum* resistant to the most commonly used antimalarials is a widespread phenomenon.

The current epidemiological situation of malaria and the lack of completely safe and effective drugs for prophylaxis have highlighted the role that protective measures against mosquito-bites play in malaria protection.

When drugs must be used to protect individuals who cannot avoid a high risk of exposure, chloroquine remains the drug of choice for prophylaxis against *P. vivax*, *P. ovale*, *P. malariae* as well as chloroquine-sensitive *P. falciparum*.

The problem arises in *P. falciparum* chloroquine-resistant areas. The substitution of chloroquine with amodiaquine had to be abandoned in view of cases of neutropenia, agranulocytosis [111-113] and liver damage [114] which have been recently reported in the literature in subjects under regular amodiaquine prophylaxis.

The weekly administration of the combination sulfadoxine/pyrimethamine (Fansidar) or sulfalene/pyrimethamine(Metakelfin) given alone or in combination with chloroquine, may expose to the risk of severe side-effects such as agranulocytosis, epidermal necrolysis, drug fever and photodermatitis, jaundice and Steven-Johnson syndromes which may be fatal [115-118].

Cases of agranulocytosis have been reported following the weekly utilization of the combination of pyrimethamine/dapsone (Maloprim), given alone or in combination with chloroquine [119, 120], with deaths occurring especially when the drug was taken twice a week [121].

The combination of chloroquine with proguanil or chlorproguanil has not conferred the protection than one would have expected, apparently due to the short half-life of the DHFR inhibitors at the doses and intervals so far utilized [122, 123].

For the reasons mentioned above, it is generally accepted that protection in areas with widespread chloroquine-resistant *P. falciparum* should rely on the utilization of chloroquine, provided that a therapeutic dose of Metakelfin, or Fansidar, or Mefloquine be available and be taken in case of a breakthrough. The utilization of Fansidar for the protection of the individuals who are not sensitive to sulfonamides is still advocated from some quarters, although international organizations are no longer in favor of it [124, 125]. Our knowledge on the toxicity of Metakelfin and Maloprim when utilized for malaria suppression is limited, and further studies may be justified.

New approaches

Mefloquine. - Mefloquine is already being used and will be certainly used, especially by non-immune travellers to areas with high malaria transmission and with prevalent chloroquine-resistant *P. falciparum* populations. There are already indications that adequate and steady concentrations of the drug are mantained with a weekly dose of 125 mg base if a loading dose of 250 mg base weekly for four weeks is given.

Longitudinal studies, however, aiming at assessing the eventual effects of its prolonged administration and its accumulation in body tissues, as well as pharmacokinetic and treatment studies in pregnant women and children below 2 years, have not yet been completed. For these reasons, Mefloquine should not be given for chemoprophylaxis for periods longer than 6 weeks.

Tetracyclines. - Tetracyclines, especially doxycycline for its longer half-life in the blood as compared to other tetracyclines, have been recommended for chemoprophylaxis in combination with chloroquine. The doses recommended are 100 mg doxycycline daily and 300 mg base of chloroquine weekly [126]. Although further studies are required to determine the drug efficacy, there are some reservations: tetracyclines, including doxycycline, cannot be used in pregnant women or children under 8 years of age due to their potential danger of damaging bones and teeth and are not free from side-reactions even in adults [127-129].

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