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THE CHALLENGE OF DRUG-RESISTANT MALARIA*

PAUL E. THOMPSON
Department of Parasitology, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602

Malaria was formerly a serious endemic disease in most of the habitable areas of the world. Geographically, the endemic regions extended from roughly 45° North to 45° South and had mean summer isotherms of at least 60° F. The specific antimalarial action of quinine was widely recognized by the middle of the 17th century. Moreover, the cause of malaria was discovered in 1880 and its transmission by mosquitoes was demonstrated in 1898. These three advances pointed to rational methods of controlling the disease, particularly in the more highly developed areas of the world. Actually, great reduction in malaria was accomplished during the early part of the 20th century in the temperate, more affluent areas, particularly where transmission was of short duration. Such reduction was accomplished largely via the application of a variety of antimosquito measures including the use of larvicides and short-acting insecticides. Even as late as 1943, however, Russell estimated that malaria still caused annually at least 3,000,000 deaths and 300,000,000 cases of fever.[1]

More diligent efforts to control malaria were instituted in the 1940's. The major factors responsible for this increased effort were the discovery of the residual insecticide DDT in 1939, elucidation of the identity and biology of the major anopheline vectors, and the development of synthetic suppressive drugs during the 1940's. Consequently, tremendous progress was made during the next 15 years with the result that by the mid-1950's malaria had been virtually eradicated in the United States and Western Europe and had been greatly reduced in most of the relatively advanced temperate and subtropical areas.

Malaria has persisted, however, as a major health problem in the rural areas of many tropical, underdeveloped regions. It still occurs as an endemic disease in countries where more than one billion people live.[2]

Furthermore, it is entirely possible for malaria to become re-established as an endemic disease in areas where it has been eradicated. This is true because the control of malaria is rarely based upon the complete eradication of the vectors capable of transmitting it. The removal of malaria as a health problem usually is followed by diminished programs of vector control. In addition, the reduction or eradication of malaria also leads inevitably to a progressive loss of immunity in the population. Consequently, all that is needed for the re-establishment of malaria is reintroduction of the parasites. Moreover, the possibility of such reintroduction is proportionally related to the progressive increase in travel between the endemic areas and those that were formerly endemic.

Owing to the continuing presence of malaria in vast areas of the world, and because of the possibility of either its resurgence in countries where it is being controlled or reintroduction into regions where it has been eradicated, it is mandatory that we continue to consider malaria as an important real or potential health problem for much of the world.

The earlier great progress of the 1940 to 1955 period in malaria control and eradication has not been extended during the past 15 years for a variety of reasons. Foremost among these are the development of resistance by anophelines to residual insecticides, the inefficiency of domicile spraying in dealing with mosquitoes that bite mainly outdoors, the nomadic habits and lack of permanent housing of some population groups, a shortage of funds and lack of public health programs in primitive areas and, finally, the emergence of drug-resistant parasites.

Historically, research on antimalarial drugs has been pursued at a remarkably inconsistent pace. Contributing factors were the early development of quinine and the progressive diminution of malaria as an important health problem in the affluent countries prepared to sponsor chemo-

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therapeutic research. Regrettably, determined efforts to develop antimalarial drugs have been largely tied to military and political considerations. Thus, concern regarding supplies of quinine following World War I stimulated research on synthetic antimalarial drugs in Germany; this research led to the development of pamaquine, quinacrine, and chloroquine. Similarly, a shortage of quinine for the Allied Forces in World War II provided the stimulus for intensive chemotherapeutic research programs in the United States and England. These programs elucidated the importance of a loading dose in the use of quinacrine and chloroquine. They also resulted in the development of such additional 4-aminoquinolines as amodiaquine and amopyroquine and several 8-aminoquinolines—notably primaquine. These programs also pointed to several new families of antimalarial drugs and to the discovery of chlorguanide. The success of chlorguanide led to the development of pyrimethamine in 1948.

Now, I wish to disgress a moment to the World War II program. One could say with justification that the long range effects were highly beneficial; but this program, implemented on a crash basis, actually helped during World War II only in one respect, namely, discovering how to use the old drug quinacrine.

Except for programs dealing with the administration of antimalarial drugs in table salt,[3] and efforts to develop repository drugs,[4] little emphasis was given to malaria chemotherapeutic research during the 1950's.

In the mid-1960's military considerations again resulted in a resurgence of research on antimalarial drugs, particularly in the United States under the sponsorship of the Department of Defense. The most recent program is directed primarily at the development of alternative drugs for use against drug-resistant parasites. This program also was launched on a crash basis but despite several years of intensive efforts by all concerned it has not yet provided a superior new drug that is clearly ready for use in all segments of a population. Rather than an implied criticism of the program, this point is mentioned to emphasize the difficulties of developing new drugs quickly.

Drug resistance in malaria. This subject has been so widely publicized and reviewed recently[5-16] that it is appropriate here to deal only with its major facets in outline form. The first point to emphasize is that as in other areas of chemotherapy drug resistance in malaria is qualitative as to type of drug and etiologic agent. Resistance to such antifolic drugs as chlorguanide and pyrimethamine has been recorded among strains of all four types of human malaria parasites. Such resistance is easily induced and occurs roughly proportionately to the extent these drugs are used. It has been observed most frequently in Africa and Southeast Asia.

In contrast, resistance to chloroquine in human malaria is limited to *Plasmodium falciparum.* Such resistance was first recognized in 1961 in Colombia and soon thereafter in Brazil and Southeast Asia. Despite repeated suspicions, chloroquine-resistant *P. falciparum* has not yet been found to occur in Africa. It appears highly probable that in time, however, chloroquine-resistant strains will be disseminated to the various areas where *P. falciparum* is endemic. Strains showing resistance to chloroquine typically show substantial cross-resistance to other 4-aminoquinolines and to quinacrine. Some of the chloroquine-resistant strains of *P. falciparum* also exhibit decreased sensitivity to quinine.[17,18] Current thoughts regarding resistance of *P. falciparum* to quinine are somewhat complicated by one's definition of resistance. It has been known for many years that the total amount of quinine required to eradicate infections caused by various strains of *P. falciparum* varies as much as 5-fold.[19-21] The difficulty here is in establishing a reference point. Recent experience in dealing with falciparum malaria acquired in Southeast Asia has, however, discouraged reliance on quinine alone for radical cure.[22] Better cure rates have been obtained by using various drug combinations, notably quinine plus pyrimethamine,[22,23] or pyrimethamine plus sulformethoxine with or without quinine.[23] The sulfones dapsone[24] and diformyl diaminodiphenyl sulfone[25,26] have been found to be useful supplements to chloroquine in prophylaxis against chloroquine-resistant *P. falciparum.*

Multiple resistance, that is resistance to both the antifolic drugs represented by pyrimethamine and to the 4-aminoquinolines represented by chloroquine, is a major problem with *P. falciparum* in Southeast Asia. New drugs to deal with this problem in troops and other non-immune expatriots have been the primary goals of the current large program on antimalarial drugs sponsored by the U. S. Department of Defense.

A second general point to be emphasized is the quantitative aspects of drug resistance in malaria. This is particularly important in resistance to chloroquine and related compounds characterized by steep dose-response curves. A further complication arises from the well-known fact that lower doses of a drug commonly are sufficient in semi-immune than in non-immune subjects. These two factors led to much early confusion in the assessment of resistance to chloroquine. Consequently, the WHO has defined four levels of response to the conventional full course of treatment with chloroquine consisting of 1.5 gm of drug base during three days.[6] Based primarily on the asexual parasitemia, the responses range from sensitivity—clearance within 7 days without recrudescence, to RI resistance—temporary clearance, to RII resistance—marked reduction but not clearance, and finally to RIII resistance—no marked reduction. The occurrence of such a wide range of responses is an important and complicating factor in the assessment of alternative drugs for use against chloroquine-resistant malaria. Thus the apparent promise of a new drug could be unduly affected by the degree of resistance represented by the strain of parasite used to evaluate it. A good example of this situation is provided by data on the phenanthrenemethanol WR 33,063. Against *P. berghei* in mice WR 33,063 exhibited a 4-fold cross resistance to a moderately chloroquine-resistant line but >27-fold resistance to a completely resistant line.[27] Moreover, WR 33,063 was effective in man against a strain of *P. falciparum* that was moderately resistant to chloroquine but it was only suppressive against a more resistant strain.[28]

A third area worthy of consideration is the origin and mechanisms of drug resistance. The basic events leading to drug resistance in plasmodia evidently are the same as those leading to other types of microbial resistance, namely, mutations.[7, 29] It is reasonable to assume that mutations occur spontaneously favoring either an increase or a decrease in sensitivity to a particular drug, but that only the latter is apt to catch our attention.

The emergence of a resistant population of parasites is most likely to occur with drugs that have a flat dose-response curve, act slowly, and affect primarily only certain stages in the asexual blood life cycle.[8] Examples of such drugs are chlorguanide, pyrimethamine, cycloguanil, sulfones, and sulfonamides. Experience has shown that resistance to each of these drugs can be either induced easily or occurs readily under ordinary conditions of use.

In contrast, resistance has been more difficult to induce and has been recognized in the field much later with drugs that have steep dose-response curves, act rapidly, and affect all asexual blood stages. Such drugs include 4-aminoquinolines, 9-aminoacridines, and quinine. Indeed, the deliberate induction of resistance to chloroquine is difficult and has been achieved only with the rodent parasites *P. berghei*[16] and *P. vinckei*,[30] although several attempts are known to have been made with other species of plasmodia.

It is of interest to mention the three known possible mechanisms for microbial drug resistance generally and to note which of these are known to operate in malaria parasites. One possible mechanism is inactivation of drug by the microorganism; this has not been demonstrated in malaria. A second mechanism is decreased penetration into the cell; this has been demonstrated in chloroquine-resistant *P. berghei*[31] and *P. falciparum*.[32] The third mechanism is alteration of microbial metabolism; this has been demonstrated in pyrimethamine-resistant *P. berghei* which has as much as 10-fold greater dihydrofolate reductase activity as drug-sensitive *P. berghei*.[33]

A fourth point to be emphasized is that resistance has been noted to at least 14 specific drugs. These include amodiaquine, chloroquine, hydroxychloroquine, quinacrine, quinine, naphthalene 377-C-54, chlorguanide, cycloguanil, pyrimethamine, pamaquine, primaquine, dapsone, sulfadiazine, and metachloridine. Moreover, this list could be greatly extended if one added to it the many compounds that show cross-resistance with one or more of the above drugs. Furthermore, at least 11 species of plasmodia are known to show drug resistance. These include *P. berghei*, *P. vinckei*, *P. gallinaceum*, *P. fallax*, *P. lophurae*, *P. knowlesi*, *P. cynomolgi*, *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. The important message from such compilations is that drug resistance in malaria is an inescapable present reality and must be given foremost consideration in the appraisal of new agents.

Development of new drugs for dealing with drug-resistant malaria. The biggest single problem in the development of alternative drugs is an increasing insistence that a new therapeutic agent

be devoid of side effects. Almost all of our older useful drugs, both antimalarials and other types of drugs generally, do have side effects and our standards for acceptance have so increased that most of them would be rejected if introduced in the United States today. Although emphasis on a lack of side effects is a commendable goal its rigid application constitutes a much greater hurdle than the discovery of compounds having specific efficacy with respect to the known types of drug resistance in malaria. The situation in malaria is further complicated by such factors as the following: 1) the drug should be suitable for use in all age groups including children and pregnant women in the various stages of health typical of field conditions, and 2) it must be effective under simple conditions of administration suitable for use in mass chemotherapy. Consequently, a program directed toward the development of alternative drugs, barring exceptional luck, must be of long duration allowing the systematic evaluation of many compounds. Plans to prepare and evaluate many compounds are necessary because only a rare drug will meet all requirements. We also must realize that it takes several years to develop a new drug and that it would be realistic to anticipate that the agent in turn may well eventually become less useful because of drug-resistant organisms. This, of course, is the situation in most areas of chemotherapy and should be fully anticipated in malaria. Therefore, continuing efforts to provide an array of new antimalarial drugs are clearly warranted.

A drug development program begins largely with the synthesis of new compounds variously related to existing drugs or from the follow-up of leads encountered from almost random screening. Only a few compounds have been synthesized on the basis of information pertaining to the biochemistry and physiology of parasites, and this approach has not yet led to a useful new antimalarial drug. This situation apparently is due to the fact that the available information has rarely pointed to an attractive parasite target that is suitably separated from the biochemical and physiological features of the host.

The customary first biological test is one for activity against a drug-sensitive species of plasmodia in a small animal—usually either *P. berghei* in mice or *P. gallinaceum* in chicks. The manner of testing varies in thoroughness according to the objectives and philosophy of the investigator and his sponsors.

Compounds showing activity in a primary test are then examined for efficacy against drug-resistant lines of *P. berghei.* An example of how this is done is illustrated from current work under my direction. The test infections are comprised of the drug-sensitive KGB-173 strain of *P. berghei* and derivatives from it that have been rendered resistant to chloroquine, cycloguanil, and dapsone, respectively. These resistant lines were selected because they encompass the known types of resistance among suppressive drugs in use. Experimental procedures have been devised to test a drug for: 1) its comparative potency as a suppressive agent against sensitive and resistant parasites—such testing determines whether or not the compound shows cross resistance with established drugs and, if so, the degree of cross resistance; 2) its potency relative to quinine against sensitive parasites; 3) its approximate therapeutic index; 4) whether or not it is active by oral administration; and 5) the rate and duration of action when given in one oral dose. Information on the rate of action is needed to determine whether or not the drug is likely to act fast enough for use in the treatment of severe falciparum malaria. Data on the duration of action are useful in the selection of a treatment regimen —one dose, a few doses, or multiple daily doses for several days.

The foregoing procedures using *P. berghei* in the mouse comprise useful secondary assessments of efficacy but may be inadequate for several reasons. The most important source of uncertainty is that both the parasite and host are artificial relative to human malaria. *P. berghei* responds to most established drugs well and somewhat more similarly to *P. falciparum* than to *P. vivax.* For instance, sulfones and sulfonamides are much more effective against *P. berghei* and *P. falciparum* than against *P. vivax.* An example of how differences in drug metabolism between the mouse and man may occur is illustrated by chlorguanide. This useful drug has such poor action against *P. berghei* in mice that it would not have been selected for further consideration if its initial testing had been against *P. berghei* in mice. The activity of chlorguanide in man is due, however, to a metabolite. This metabolite is cycloguanil and it is highly effective against *P. berghei* in mice. Such deficiency in the *P.*

berghei-mouse system is not offered to condemn it but rather to illustrate the hazards of any one artificial system.

The next most important recognized deficiency of *P. berghei* resides in the degree of induced resistance to chloroquine with *P. berghei*. It has been difficult so far to develop and maintain lines having moderate, stable resistance to chloroquine. Therefore, most of the cross-resistance testing with *P. berghei* has been done with lines that are extremely resistant (>100-fold) to chloroquine. The toxicity of chloroquine for man and lower primates does not permit the recognition of more than a few-fold resistance to the drug. Hence there is no evidence indicating the *P. falciparum* develops the high degree of resistance to chloroquine characteristic of typical chloroquine-resistant *P. berghei*. Furthermore, efficacy is inversely related to the degree of resistance of the parasites used for the assay.[27,34] Consequently, undue reliance on extremely resistant *P. berghei* may lead to an underestimation of the value of some compounds for use against chloroquine-resistant malaria in man. Such risk, however, appears to apply only to certain structural types, represented for instance by the quinolinemethanol WR 30,090 and the phenanthrenemethanol WR 33,063.[27] The use of drug-resistant lines of *P. berghei* has not, however, proved to be a liability in assessing the potential value of such other types of drugs as triaminoquinazolines, sulfones, and sulfonamides. It appears, therefore, that the battery of resistant lines of *P. berghei*, including those that are highly resistant to chloroquine, remain quite useful in assessing new compounds provided due precaution is taken in assessing the results.

Fortunately, recent developments have provided important new procedures to bridge the gap between the testing of drugs against chloroquine-resistant parasites of rodents and those of man. Strains of *P. falciparum*[35] and *P. vivax*[36] have been established in the owl monkey (*Aotus trivirgatus*). The strains of *P. falciparum* adapted to owl monkeys include a spectrum of parasites ranging from high sensitivity to varying degrees of resistance to chloroquine and pyrimethamine, and extensive chemotherapeutic testing using these strains is under way.* The resistance of these strains appears to be stable and representative of field conditions. It is almost needless to mention that such infections have at least three important advantages over the *P. berghei*-mouse systems, namely, the use of human plasmodia, the use of a host that is closer physiologically to man than is the mouse, and the use of strains having resistance more typical of field conditions.

I have dealt so far only with the evaluation of efficacy but it cannot be emphasized too strongly that this is only a part of the biological work and investment required to develop new drugs. Formal toxicity studies in several species of animals, teratogenicity and carcinogenicity studies in animals, and tolerance studies in normal human subjects all are essential steps prior to clinical trial for efficacy. Information on the physiological disposition, stability, and optimal formulation of a drug also are needed and can contribute greatly to its best use.

The capstone of developing alternative antimalarials is clinical evaluation under controlled conditions. Fortunately, it has been possible in this country to conduct such studies in healthy non-immune volunteers using selected resistant strains. It is difficult to exaggerate the value of this arrangement in the precise evaluation of drugs.

SUMMARY AND CONCLUDING STATEMENT

The challenge of drug-resistant malaria stems from the following basic facts. First, malaria is still a foremost tropical disease in much of the world and it is possible for it to recur with devastating effect in many areas where it formerly was endemic but has been either eradicated or effectively controlled. Second, drug resistance operates as a present and future problem in malaria comparable to the situation with other infectious diseases. Third, we have an excellent sequence of biological methods for the evaluation of candidate compounds, both as to efficacy and safety. We are most handicapped, however, by the wishful thinking that a new drug can be developed on a crash basis. Some of us may want to blame this philosophy on politicians but I believe that the blame lies more with ourselves —too many of our biomedical research leaders accept the crash program approach. Based on more than 25 years of personal research on drugs and on close observation, I am convinced that a crash approach is much less efficient and much

* L. H. Schmidt, Southern Research Institute, Birmingham, Alabama. Personal communication.

more costly than an orderly, sustained program. It is our obligation and responsibility, individually and as a Society, to correct this error of the crash approach in chemotherapy. Therefore, I would like to close with the plea that all investigators and administrators in a position to allocate either private or public research funds give this problem its proper support on a long-range basis, not only to cope with present deficiencies but also to prepare for new problems in drug resistance that history should teach us to expect in the future.

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