

Chapter 17 FUTURE PERSPECTIVES IN DRUG RESEARCH

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BACKGROUND

Treatment of malaria disease with chemotherapeutic agents remains the cornerstone of patient management and will likely remain so for the foreseeable future. But, albeit much can be done with existing drugs if properly used, there is an urgent need for new drugs with mode of actions different from existing ones. The reason is that resistance to antimalarials is emerging and spreading, and there are few effective drugs in the pipeline (1).

Drug research should be led by, and adjusted to, the needs of malaria treatment in the field so that research products can meet and satisfy the diverse practical requirements of case management in different situations. Needs for malaria drugs include life-saving therapy and drugs for broad use for uncomplicated malaria -many of the drugs for current use are restrained by resistance, cost or toxicity in target groups. It is equally important that the cost-benefit and the acceptability of new interventions receive careful evaluation at an early stage of development.

There are short-term and long-term research objectives. A reasonable objective in the short term would be to optimize the use and effective life-span of available drugs: exploring drug combinations would be critical to improve dosing regimens and minimize the development of resistance. Longer term, though unavoidable, objectives are the identification of chemotherapeutic targets and the discovery of new leads, as well as a better understanding of the mechanisms of drug action and resistance.

THE NEEDS

Treatment of severe malaria

Mortality due to cerebral malaria is still high; case-fatality rates with standard treatment range 12-25% depending on prevailing standard of care, even in areas of full quinine sensitivity (2). Quinine is safe but its efficacy is fading in Thailand (3).

The needs in connection with the management of severe malaria are three fold: developing new antimalarials unrelated to quinine to overcome resistance; assess alternative approaches to prevent progression to severe malaria; better understanding of the pathogenesis of severe malaria to identify adjunct treatment.

Artemisinin derivatives (artemether, arteether, artesunate, dihydroartemisinin) show no cross-resistance with other antimalarials and have rapid activity (4,5). With the exception of artesunate they are all insoluble in water and therefore cannot be administered intravenously. At present, no conclusive comparative study is available to enable to opt for one derivative or another. The use of these drugs has traditionally been limited to China and areas of Southeast Asia because the available pre-clinical and clinical data did not satisfy stringent regulatory requirements for international marketing authorization. Intramuscular artemether has subsequently received further development and is now being commercialised in other parts of the world.

It is worthwhile noting that a clear clinical advantage in terms of mortality and sequelae of artemisinin derivatives over quinine is so far apparent only in the presence of parasites with decreased susceptibilities to quinine (6,7,8). This finding highlights the need for alternative approaches to prevent the occurrence of the serious complications of falciparum malaria. Ideally, prompt diagnosis and treatment preferably of uncomplicated cases would settle the problem, but in practice very often patients -mostly children- are already comatous when they reach the hospital. That is why it has been suggested that control strategies should be directed against the disease rather than killing the parasite since children in endemic areas develop immunity to the serious complications of malaria several years before their parasitaemias start to fall (9). Better understanding of mechanisms involved in the pathogenesis of severe malaria (cytokines, cytoadherence and rosetting) is therefore needed (10,11).

Treatment of uncomplicated malaria episodes

The therapeutic armamentarium for treating malaria is limited, and drug cross-resistance is facilitated by the fact that most of the available compounds belong to a restricted collection of chemical structures. Chloroquine has been for decades the mainstay of malaria treatment and prevention, but resistance to this drug is nowadays widely spread (12,13). Second-line treatment in Africa is pyrimethamine/sulfadiazole combinations, but resistance is emerging after a relatively short time from introduction. Toxicity (particularly severe cutaneous adverse events) may also limit its use. In fact, the blessing and the drawback of the in-use antifolates has been their long half-lives, which can generate resistance and be responsible for drug adverse reactions (14,15). In certain areas of Southeast Asia, multidrug resistant parasites have emerged and there is evidence for reduced efficacy of almost all known antimalarials. Resistance is already established to mefloquine and halofantrine in Southeast Asia, particularly Thailand (12,13,16-18) and sporadically elsewhere (19,20). The use of halofantrine is limited by cross-resistance with mefloquine and possible cardiac toxicity (21).

Although artemisinin derivatives rapidly reduce parasitemia, parasite recrudescence occurs frequently unless given for 5 days or longer or used in combination with mefloquine (22-26). But concerns are expressed over the use of oral formulations of artemisinin derivatives for the treatment of uncomplicated malaria in areas where other first-line medications could be used: unregulated use of these drugs may exert drug pressure that can lead to resistance; moreover, the effect of repeat dosing is not known yet and might lead to cumulative (neuro- or cardio-) toxicity so far observed only in experimental animals (27,28). The long-term implications of their use need to be assessed, and new derivatives with better disposition, oral bioavailability and toxicological profile need to be sought.

Thus, there appear to be two sets of priorities in connection with these patterns of resistance: one is for a first-line, safe, oral, preferably cheap medication for broad use to replace chloroquine, particularly for Africa. In addition, drugs effective in multidrug-resistant malaria are badly needed in limited areas of the world nowadays. For the time being the numbers of people in need of such drugs are comparably small and relatively high priced medications can be afforded. But the world may also face the appalling prospect of multidrug resistant strains spreading to high transmission areas of Africa and this would be a crisis of immense proportion (19,20). Waiting for new drugs with novel mechanisms of action to become available, a rationale assessment of combination chemotherapy regimens should be carefully done in order to prevent or delay parasite resistance (29).

The reduced efficacy of drugs against blood stages makes it increasingly important to develop causal prophylactic or radical curative agents, thus preventing blood stages from emerging and causing clinical diseases.

PERSPECTIVES

The previous section has highlighted the compelling need for novel antimalarials. But, although

chemotherapeutic targets and new lead compounds have been identified, the drug development pipeline is drying off. Interesting though they are, the new avenues in research appear to have little impact on the ultimate availability of new drugs on the pharmacy shelves. Moreover, the costs and risks of drug development, particularly in the face of the perceived limited profits and the long pay-back period of drugs for the developing world, are seen by the pharmaceutical industry as disincentives for antimalarial drug development.

New Drugs Under Development (30)

Two new injectable artemisinin derivatives are currently being developed: one, arteether, closely related to artemether, is water-insoluble and such injected intramuscularly, is in Phase II-III clinical development; the other, sodium artelinate, water-soluble, is being developed for intravenous administration. Also existing artemisinin derivatives are receiving further study, including potential combinations with other antimalarial drugs (22-26), and alternative formulations. Studies are being initiated to assess whether the availability and use at the peripheral level of the health system of artesunate suppositories for patients unable to swallow "en route" to the hospital can prevent severe malaria or improve its outcome.

Atovaquone (hydroxynaphthoquinone), is a new antimalarial with a broad spectrum of activity to opportunistic pathogens (*T.gondii*, *P.carinii*). It shows high intrinsic activity against erythrocytic stages of *P. falciparum* *in vitro* and also primary liver stages. As an inhibitor of electron transport, it has a novel mode of action and is not cross resistant with other antimalarials. In contrast to previous members of the naphthoquinone series, it is metabolically stable with an elimination half-life of approximately seventy hours. However, resistant parasites emerge readily after single-agent treatment with atovaquone in approximately 30% of cases; atovaquone is therefore now combined with proguanil to prevent or delay emergence of resistance, and is currently in advanced phases of international clinical development for the treatment of uncomplicated *P.falciparum* malaria. The combination appear to be very effective in the various settings where it was tested (31,32).

The combination of chlorproguanil and dapsone proved to have the best therapeutic index among available drugs investigated *in vitro* to assess the activity and toxicity of antifolate drug combination, (33). This combination was then chosen for clinical trials, also based on preliminary clinical results and good track of safe use of the two individual drugs (34): the results of a Phase II trial in Kenya comparing a three-day and a one-day regimen with chlorproguanil/dapsone vs pyrimethamine/sulfadoxine are being analysed. The rationale for using alternative antifolates with shorter residence in the organism is two fold. First, due to their short half life they are believed to exert lesser selective pressure on the parasites and to be less prone to toxicity. Second, pyrimethamine and sulphonamide resistance are unlinked in *P.falciparum* (35,36). But this approach, which may be valid in areas of no or just emerging antifolate resistance, may not be enough in areas of Southeast Asia with high levels of resistance to both DHFR and dihydropteroate synthase (DHPS) inhibitors. There, newer drugs are required, such as the triazine WR99210, administered as the prodrug PS-15 (or WR250417) (37).

There are also other drugs under development that share common chemical structures with in-use drugs. Despite common chemical features, Mannich bases (eg. quinoline-type: amodiaquine, amopyroquine; acridine-type: pyronaridine, pyracrine) differ substantially from chloroquine in their activities and in the rate and stability of resistance (38,39). The development of amopyroquine was abandoned because of high cost of production, minimal advantage over amodiaquine, and poor oral bioavailability. Amodiaquine -which has long been underutilised after reports of severe and fatal haematological toxicity in prophylaxis- has still a place in the treatment of uncomplicated malaria as it

is comparatively more active and no more toxic than chloroquine (42). Pyronaridine stands out as the more active molecule of the whole lot (43). It has been used clinically in China since the 1970s and is now marketed in that country, but is not widely available and has been only used experimentally outside China. Two recent studies in Cameroon and Thailand have shown the drug is effective against chloroquine-resistant parasites and retains activity in areas of multidrug resistance (44,45). Pyronaridine is a potentially valid addition to the therapeutic armamentarium for the treatment of malaria. Nevertheless, a larger accessibility of the drug is conditional to substantial investments in the studies required to meet international standards for registration outside China in light of the possible life-span of the compound. Also, the current price makes it by far too expensive for extensive use: dosing regimes need to be optimised and Phase I studies with more suited oral formulations are scheduled to begin presently (30).

Another Chinese synthetic compound benflumetol, a fluoromethanol, is currently under development for concurrent use with artemether, but limited efficacy data are available so far.

Primaquine replacements under investigation are WR238 605 and CDRI 80/53. WR238 605, an 8-aminoquinoline compound which was originally designed as a primaquine-replacement for prevention of relapsing malaria, shows promising activity for prevention and treatment of *falciparum* malaria. It is currently in early phase II clinical testing. Its potential advantages over primaquine are: a longer half-life, a better therapeutic index, a greater blood schizontocidal activity in addition to its activity as a tissue schizontocidal (44). It is hoped that this drug can be used for causal prophylaxis and as single-dose radical curative for *P.vivax*. CDRI 80/53 has comparable activity with primaquine but is claimed to have lesser haemolytic potential.

Some alternate approaches

As pointed out before, several other factors beyond «parasite susceptibility» contribute to malaria mortality, hence the need for adjuvant treatment and «antidisease» therapy to prevent progression to severe malaria. Antagonists of Tumor Necrosis Factor (TNF) are being developed and tested by several pharmaceutical companies to combat septic shock and autoimmune diseases and some of them have been tested clinically in severe malaria, but the results are not encouraging so far (B.Greenwood, personal communication). Preliminary data indicate pentoxifyllin may be a valid adjunction to standard quinine treatment (45,46). Putative receptors and ligands on the surface of infected cells which are involved in cytoadherence and in rosetting have been identified. These could lead to novel interventions for the management of severe malaria. It is hoped that ongoing research can lead to drugs that, combined with anti-parasite therapy, would reduce malaria-associated morbidity and mortality.

Restoring susceptibility to antimalarials to which parasites have become resistant could also be a valuable solution. Various drugs, including calcium-channel inhibitors (eg. verapamil) and tricyclic compounds (eg. desipramine) that are known to reverse the mdr phenotype in human cells have been shown to "reverse" or "modify" chloroquine resistance *in vitro* (47,48). Others, like penfluridol, modulate resistance to mefloquine and halofantrine. Unfortunately, no compound has yet proved effective *in vivo* and there is some concern for potential host cell toxicity (49). The availability of such resistance modifiers or chemosensitizing compounds would have great practical repercussions by restoring the effectiveness of present first-line antimalarial drugs in areas where parasites are no longer susceptible. This would be particularly desirable for chloroquine, which was shown to be valuable also in inhibiting cytokine production (50).

Long-term goals: Drug discovery

Achieving short-term objectives by «getting extra-mileage» from existing drugs or their combinations can momentarily ease the struggle for tools to control malaria, but will only partly offset the trend to further expansion of resistance. In fact, the current armamentarium of drugs against malaria is limited, and this appears to be one of the factors which have contributed to the development of drug resistance. Therefore, long-term objectives of research must focus on the discovery of compounds with new structures acting on new chemotherapeutic targets.

More research needs to be done in order to identify and validate targets, and to use them for testing compound activity. But we cannot expect all targets to be suited; a chemotherapeutic target must be an essential feature of the parasite life-cycle (involved in a rate limiting biochemical process with no alternative pathways which circumvent the target), and either parasite-specific (eg. food vacuole functions, the mitochondrion/plastid) or differ significantly from any analogous process in the host (eg. DHFR, signal transduction). A number of putative chemotherapeutic targets have been identified for malaria.

Examples of validated targets already amenable to compound screening are: proteinase inhibition in the plasmodium food vacuole (plasmepsin I, II, and cystein proteinase), inhibition of folate metabolism (DHFR, DHPS), and phospholipid metabolism. Other drug targets are at earlier stages of investigation or have not yet led to the discovery of new leads. Chemotherapeutic targets for malaria are summarised in Table 2 and are reviewed in Vial & Olliaro, 1995 (51).

Ultimately, the main motor for change will have to come from technology, and already recent discoveries and developments open new avenues of research for drug discovery. There are already several examples of new technologies which can be increase our capability to discover new compounds: recent advances in genetic engineering now allow to identify and confirm targets; the malaria genome is being sequenced, and this has the potential for leading to new targets identification; huge numbers of compounds can now be tested by *robotic high throughput screening* is possible against selected enzyme targets. The capability to generate new structures is also now tremendously improved by new techniques such as *combinatorial chemistry*. This consists of a systematic assembly of molecular "building blocks" in multiple combinations and generates collections of compounds named *combinatorial libraries*. These molecules can be either biological (DNA/RNA, peptides/proteins) or chemical (peptides, polymers or peptoids, and now also organic). In essence, the rationale for combinatorial chemistry is twofold. Firstly, many believe that our difficulty in discovering novel effective molecules stems from the limited numbers of chemical structures explored so far - combinatorial chemistry aims at generating chemical *diversity*. Secondly, compound screening and lead optimisation by medicinal chemistry work has traditionally been a bottleneck in drug discovery; now many would hope that chemical libraries may lead to a drug with minimal medicinal chemistry. By coupling combinatorial chemistry and robotic high throughput screening it is now hoped that huge numbers of compounds in produced in minimal quantities can be screened in very limited times. This new technique is now being adopted by several pharmaceutical companies as an alternative to more classical approaches to drug discovery.

But, since parasites appear to invariably get round any human intervention, research should also focus on understanding the mechanisms of resistance, if we are ever going to keep pace with the development of resistance.

The Research and Development (R&D) process for drugs

The rate at which such new drug entities will become available for clinical use depends on the available resources. Over the past ten years, there has been a significant cut in funds allocated to drug discovery in the drug industry, and the number of new chemical entities has dropped substantially. This affects primarily low-priority areas for the pharmaceutical industry such as tropical diseases. The burden for development is therefore on the public sector, however, governmental efforts also are being reduced significantly.

New chemical entities discovered through the process mentioned above cannot be expected to become drugs available on the market soon. The drug discovery and development process is lengthy and expensive. The timescale now averages 12 years, and can fail at any stage. Full R&D costs for each new chemical entity on the market average US\$ 395 million -costs inclusive of projects prematurely terminated; a commonly accepted figure is that 1 in 10,000 molecules studied is ultimately put on the market. Review of dossiers by regulatory authorities also needs time: the review process by the Food and Drug Administration in the United States of America takes on average 32 months (52).

CONCLUSIONS

Much is being done for malaria but this is obviously not enough. The dominating theme in antimalarial use and research is resistance, which is the key factor in determining the duration of a drug effective lifespan.

The efficacy and safety of existing antimalarials could be "protected" if we could use them better and

combine them rationally. But these would represent only momentary solutions, and there are obvious difficulties in regulating the use of antimalarials when these are freely available in the market-place. New drugs need to differ in their chemical structures from existing ones and to target different processes of the parasite, and as such artemisinin derivatives and atovaquone/proguanil represent recent breakthroughs. But will they be accessible and used properly? How long will their efficacy last? When can we expect other drugs to become available? The duration, costs and risk of the drug R&D process work against having new antimalarials available soon. There are no many antimalarial compounds in the pipeline, and although there appear to be new interesting prospects, these are most often at very preliminary stages, and one should not forget that the risk of failure is highest in the early phases of the drug development process. Although several new targets have been identified, we cannot expect all of them to eventually prove essential and specific. The ultimate need is for basic research to discover new targets and lead compounds and to elucidate the mechanisms of resistance.

Finally, people should realise that development and implementation of antimalarial drugs needs a concerted venture, and that efforts are doomed to failure if a partnership is not created between public and private sector.

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