Editorial

Probing for Antiparasitic Drugs

Every person on earth is infected by at least several parasites. The most dangerous parasites are transmitted by vectors and cause the death of millions annually. In order to reduce mortality resulting from such infections there is a need to interrupt the life cycle of the parasites. A parasitic disease will not be eradicated unless efforts to contain it below a "critical mass" succeed. The critical mass may be defined as a combination of a minimum number of infected human, infected reservoir animals (when relevant, e.g. leishmaniasis), and a minimum amount of vectors (e.g. *Simulium* in river blindness). A failure to control the vectors and insufficient treatment of human patients originates from the increasing resistance of the vectors to insecticides and from parasite drug resistance. In addition, economic considerations are highly responsible for the prevalence of the diseases.

In this issue our aim is to consider novel and recent approaches to chemotherapy of vector-born parasitic diseases. Recent advances regarding the biology, physiology and biochemistry of the parasites have led to the recognition of new targets for chemotherapy. These targets must be specific in order to increase the therapeutic value of the drug. Lead optimization may help in designing new drugs such as enzyme inhibitors. For example, cysteine proteases of various parasites could be an important target; in malaria parasites they are involved in hemoglobin degradation and in Leishmania parasites in digestion within lysosomes/endosomes. A second approach to drug discovery is based on the screening of traditional drugs. Some of these compounds were used long before the understanding of their mechanism of action and some are still in use despite their unknown mechanism (e.g. quinine). A third approach aims at improvement of existing drugs by derivatization, which enables better transport to the target cell/organ (e.g. diamidine derivatives against sleeping sickness) or slow release in order to allow for sufficient stable amounts of the drug.

Despite the continuous, extensive studies being carried out, the amount of newly approved effective anti-parasitic drugs in the last 5 years, is frustrating and can be counted on the fingers of one hand. A main reason for the limited success of developing new drugs is the lack of understanding of mechanisms of drug action which are additional to the direct anti-parasitic effects. While it is relatively easy to examine drug effects on *in vitro* cultures of the parasite, the actual activity, efficacy and safety of drugs depend on various factors. These include the direct anti-parasitic effect, and the effect on immune and physiological functions. These considerations could be demonstrated by artemisinin and its derivatives. Artemisinin is a prodrug which is transformed to the active derivative dihydroartemisinin (DHA). It is a common dogma that it is active because it contains a peroxide bridge which interacts with iron to form a reactive free radical. Despite its accumulation in the parasitized erythrocyte its high anti-malarial therapeutic index can not be solely attributed to its radical activity. DHA *in vitro* kills bacteria, various protozoa (e.g. Leishmania) and animal cells at μM concentrations. However, the ID_{50} for malaria parasites is lower than 1 nM. This low ID_{50} has been attributed to DHA's activity against the plasmodial SERCA (Ca-ATPase). There are additional effects which are not anti-parasitic but affect the fate of the malaria infected patient: DHA reduces vascular endothelial growth factor (VEGF) which consequently may affect the pathogenesis of *in vivo* infection. Moreover, treatment with high concentrations of DHA may suppress both humoral and cellular immune responses. Low concentrations may stimulate T-lymphocyte cell mediated responses.

The therapeutic index of a drug would be increased if large amounts could be targeted to the affected host organ and parasite molecule. The outmoded former drug chloroquine was a most valuable drug because it was accumulated in the parasitized erythrocyte and has a specific target which is related to hemoglobin degradation and hemozoin formation. Chloroquine is not in use any more due to plasmodial drug resistance. It took more than 20 years until chloroquine resistance spread throughout all endemic areas. However, sometimes it is possible to predict the probability of induction of drug resistance by exposing the parasites to increasing quantities of the drug and by using molecular markers. J. Clos and K. Choudhury use functional cloning as a means to identify *Leishmania* genes involved in drug resistance. Resistance to established anti-leishmanial drugs is a mounting problem in high-endemicity regions and in the context of HIV-*Leishmania* coinfections. The molecular basis for clinical drug resistance is still largely unknown. It is important, however, to identify all relevant drug resistance markers for further drug development and for epidemiological surveys. An elegant and powerful method to identify such drug resistance markers without bias is functional cloning, using cosmid-based genomic DNA libraries. The review of Clos and Choundury discusses the merits and caveats of this approach.

Many similarities exist between cancer cells and parasites. A potentially lucrative starting point for the discovery of novel drugs to combat parasites is to examine available compounds developed against cancers for new anti-parasitic properties. Here, M.-Q. Klinkert and V. Heussler review the use of current and promising anti-cancer agents for the treatment of major human parasitic

diseases. The paper by J.D. Berman describes the clinical considerations that eventually enabled the approval of miltefosine as an anti-leishmanial drug. This may be the best example of a successful anti-parasitic drug that originally was planned as an anti-tumor drug. It is also the last anti-parasitic drug that was approved for human use (2002).

The clinical treatment of leishmaniasis is based on a limited number of drugs which are associated with adverse effects and have already induced resistance. Amphotericin B (AmB) is the only anti-leishmanial drug which has not induced clinical resistance. The limiting factor in the use of AmB is its toxic effects, mainly nephrotoxicity. The mode of action of AmB is associated with its toxicity: it selectively binds to parasite membrane ergosterol but also, to a lesser extent, to human cholesterol. AmB also has immunomodulatory effects, some of them deleterious. J. Golenser and A. Domb discuss the current efforts to improve AmB by production of AmB aggregates in liquid solutions, encapsulation with lipid components, and solubilization by binding to soluble polymers. The expected improved treatment is based on better pharmacokinetics, reduced toxicity and an altered pattern of immune responses. Of particular importance are the attempts to produce derivatives for oral treatment, which will decrease costs of hospitalization and improve applicability.

The plasmodium-erythrocyte unit is specifically sensitive to oxidant stress that is inserted by the growing parasite, some labile iron that is released by the parasite and may participate in radical inducing reactions, and possibly the host immune response. Parasite enzymes involved in antioxidant defence are representing interesting target molecules for rational drug development. S. Rahlfs and K. Becker summarize the currently available data on structural, biochemical, and functional properties of these proteins in an attempt to evaluate and compare their potential as drug targets. I. Yeh and R. B. Altman present a comprehensive study of anti- malarial drugs. In their “Drug Targets for *Plasmodium falciparum*: a post-genomic review/survey” they discuss drug targets by broad biological functions and emphasize the evidence for each drug target. The authors emphasize that the list of targets has blossomed because of the efforts of scientists who have taken advantage of the information provided by the sequenced genome. The current set of drug targets is heavily weighted toward metabolic pathways, but this may change with increased understanding of parasite signalling mechanisms and the parasite’s interactions with host cells.

In order to cure or alleviate a parasitic disease it is common to use drugs that reduce parasite burden or minimize deleterious host responses to the parasite. Another approach which could decrease the prevalence of the diseases is to kill parasites by infecting them with their own parasites. This kind of treatment has been successfully used for various insect vectors. Evolution goes in various directions - in the case of filariasis it is possible to reduce the prevalence of the disease by using biological agents like *Bacillus thuringiensis israeliensis* (Bti) which kills the vectors. K. M. Pfarr and A. M. Hoerauf discuss a different approach: the use of antibiotics against *Wolbachia* endosymbionts of filariae. *Wolbachia* are essential to the biology of filarial worms and appear to have a major role in the development of filarial pathology. Therefore, *Wolbachia* are targets for the development of new antifilarial chemotherapy: the drugs which deplete *Wolbachia* from the worm have demonstrated the feasibility of this strategy and have provided a new chemotherapeutic tool. Recent research shows that depleting *Wolbachia* will also lessen pathology, and lessen adverse reactions to traditional anti-filarial drugs.

It is impossible to include in one issue all of the new approaches to parasite chemotherapy but we present here a selection of reviews which could demonstrate new approaches that might lead to better treatment of parasitic diseases.

Jacob Golenser
The Hebrew University of Jerusalem
Jerusalem
Israel
E-mail: golenser@md.huji.ac.il

Nicholas H. Hunt
University of Sydney
Sydney
Australia

Shalom Sarel
The Hebrew University of Jerusalem
Israel