Drugs to Combat Tropical Protozoan Parasites

Michael H. Gelb and Wim G. J. Hol

T he 130 scientists from 20 countries who gathered at a Keystone Symposium (1) to discuss development of drugs to combat tropical protozoan parasites were inspired by alternate waves of desperation and hope. Desperation stemmed from the fact that tropical protozoan diseases such as malaria, leishmaniasis, and Chagas’ disease affect 3 billion people (see the table), most of whom survive on less than $2 a day. Every minute, two people, usually children, die from malaria, and every year, more than 300 million persons suffer at least one malaria attack. In Latin America, millions of people are infected with the protozoan Trypanosoma cruzi, which causes Chagas’ disease and kills 10 to 20% of the people it infects. Meanwhile, Kala-azar, the most deadly form of leishmaniasis, is epidemic in the Bihar and Uttar Pradesh states of India. For most tropical diseases caused by protozoan parasites, there are either no safe efficacious drugs or, as in the case of malaria, once effective and affordable drugs like chloroquine are less widely used because the Plasmodium parasites that cause malaria have become resistant to them (2, 3).

Yet a wave of hope arrives with efforts to sequence the genomes of these protozoan parasites. As David Roos (Univ. of Pennsylvania) and Peter Myler (Seattle Biomedical Research Institute) discussed, the genome of Plasmodium falciparum, the most deadly of the malaria parasites, is essentially complete, and those of Leishmania major, Trypanosoma brucei, T. cruzi, and Plasmodium vivax are progressing rapidly with completion slated for 1 to 2 years’ time. Of course, functional annotation of the parasite genomes will take considerably longer, but is already under way. Clearly, the complete genome sequences of protozoan parasites will accelerate efforts to develop cheap, effective drugs for treating the tragic diseases that they cause.

There was much discussion about different approaches to developing antiprotozoan drugs. Gary Posner (Johns Hopkins Univ.) discussed analogs of artemisinin, a natural antimalarial derived from Chinese traditional medicine. The analogs have improved solubility compared to the parent compound and a simpler structure; they decreased parasitemias in primates infected with malaria. Donald Krogstad (Tulane Univ.) demonstrated the efficacy of aminquinolines even against chloroquine-resistant malaria, in vitro and in rodent models of malaria. Protease inhibitors received considerable attention, with inhibitors of the enzyme cruzipain (Jim McKerrow, UCSF) eliciting much enthusiasm, thanks to their fortunate property of being taken up selectively by T. cruzi. Compounds synthesized by Phil Rosenthal (UCSF) target cysteine proteases in the malaria parasite’s food vacuole. Richard Tedwill (Univ. of North Carolina) disclosed the promise of pentamidine-type compounds as antiprotozoan agents. Henri Vial (CNRS, Montpellier) described inhibitors of choline metabolism (required for phospholipid synthesis) that appear remarkably effective against plasmodial parasites (4).

Exciting results were also obtained by “redirecting” compounds developed for other diseases toward tropical protozoa (also called “therapy switching” or “piggybacking”). Protein farnesyltransferase inhibitors, under vigorous development as anticancer agents, show promise (Fred Buckner, Wes Van Voorhis, Mike Gelb, Univ. of Washington, Seattle; Andrew Hamilton, Yale; Bill Windsor, Schering-Plough). As always, serendipity is a crucial player in drug discovery—Buckner reported his accidental discovery of an extremely potent inhibitor of the T. cruzi enzyme lanosterol 14-demethylase, which is essential for sterol biosynthesis in the parasite. The sterol biosynthetic pathway was also the topic of talks by Eric Oldfield (Univ. of Illinois) and Julio Urbina (IVIC, Venezuela), who demonstrated that inhibitors of isoprenoid and sterol biosynthesis were effective against T. cruzi in vitro and in rodent models of Chagas’ disease (5).

Vern Schramm (Albert Einstein College of Medicine, New York) discussed highly potent inhibitors that target the purine nucleoside phosphorylase of P. falciparum. Clearly, almost all of these promising compounds still need to undergo extensive testing for safety and efficacy before they will be useful in the field. However, Simon Croft (London School of Hygiene and Tropical Medicine) described the first orally active compound against cutaneous and visceral leishmaniasis (developed by Zentaris in Germany).

THE HUMAN TOLL OF TROPICAL PROTOZOAN PARASITES

<table>
<thead>
<tr>
<th>Disease</th>
<th>Major parasite</th>
<th>Insect vector</th>
<th>Regions affected</th>
<th>Estimated number of cases</th>
<th>Estimated number of annual deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>Plasmodium falciparum</td>
<td>Anopheline mosquitoes</td>
<td>Tropics</td>
<td>300 million/ year</td>
<td>&gt;1 million</td>
</tr>
<tr>
<td>Sleeping sickness</td>
<td>Trypanosoma brucei</td>
<td>Tsetse flies (Glossina spp.)</td>
<td>Sub-Saharan Africa</td>
<td>300,000+</td>
<td>66,000</td>
</tr>
<tr>
<td>Leishmaniasis</td>
<td>Leishmania spp.</td>
<td>Phlebotomine sandflies</td>
<td>Tropics and subtricops</td>
<td>1.5–2 million</td>
<td>57,000</td>
</tr>
<tr>
<td>Chagas’ disease</td>
<td>Trypanosoma cruzi</td>
<td>Reduvid bugs (triatomines)</td>
<td>Latin America</td>
<td>16–18 million</td>
<td>50,000</td>
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Source: WHO, CDC

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covalent inhibitors of trypanothione reductase are stacked one on top of the other in the active site, each covalently bound to the enzyme in different ways (Bill Hunter, Univ. of Dundee). Gerhard Klebe (Philippines Univ., Marburg) reported his collaborative work with Jomaa Pharmaceuticals (8) to synthesize inhibitors of deoxyxylulose phosphate (DOXP) reductoisomerase. This essential enzyme is part of the nonmevalonate isoprenoid pathway of the apicoplast, a unique organelle in the malaria parasite. Klebe's group has just solved the crystal structure of Escherichia coli DOXP reductoisomerase, along with that of glutamate dehydrogenase from P. falciparum. Glaucio Olliva (Univ. of Sao Paulo, Brazil) reported the crystal structures of T. cruzi phosphoenolpyruvate carboxykinase and glyceraldehyde-3-phosphate dehydrogenase in a complex with a natural product inhibitor derived from a plant in the Brazilian Atlantic forest. A recently created network of centers in Brazil is set to tap the rich biodiversity of this country's flora for the discovery of lead compounds.

Another encouraging development is NIH funding for the recently formed Structural Genomics of Pathogenic Protozoa (SGPP) consortium (www.sgpp.org, sgpp@u.washington.edu). This consortium aims to develop and apply high-throughput methods to express large numbers of genes and to elucidate 3D crystal structures of proteins from P. falciparum, T. brucei, T. cruzi, and Leishmania species. This initiative is likely to have a significant impact on drug design by making 3D crystal structures available to all researchers.

Functional genomics is crucial for identifying protein targets for structural genomic and drug development. David Roos—who oversees the P. falciparum genome database (PlasmoDB) (9)—has used sophisticated computer analysis to unravel all of the metabolic pathways in the plasmoidal apicoplast (10). Terry Gaasterland's group (Rockefeller Univ., New York) is combining powerful bioinformatics techniques to annotate protozoan genes. State-of-the-art mass spectrometry is being used to analyze collections of proteins expressed in the various life cycle forms of malaria (Daniel Carruci, Naval Medical Research Center; John Yates, Scripps).

Several talks revealed the intricate biochemical pathways of these devastating yet ingenious protozoa. Dan Goldberg (Washington Univ., St. Louis) described how the earlier discovery of one plasmoidal aspartyl protease, plasmspsin—a key player in hemoglobin degradation in the plasmoidal food vacuole—was followed by the identification in the P. falciparum genome sequence of nine other plasmspsins. One of these plasmspsins is a most unusual histidyl-aspartyl protease that may be amenable to selective inhibition; meanwhile, a unique plasmoidal enzyme, the maturase, which processes the plasmspsins into their active forms, may be an exciting new drug target. The complexities of the folate pathway in trypanosomatids discovered by Beverley's group (Washington Univ, St. Louis) explains why dihydrofolate reductase inhibitors have disappointingly little effect on Leishmania species. Buddy Ullman (Oregon Health Sciences Univ.) revealed the sophistication of the trypanosomatid purine salvage pathway. Meanwhile, studies on purine transporters are unveiling crucial new proteins. Sanjeev Krishna (St. George's Hospital Medical School, London) described several new hexose transporters in P. falciparum. The complexities of the malaria parasite's metabolism became clear in Akhil Vaidya's talk (MCP Hahnemann, Philadelphia). He pointed out the remarkable synergy required between the two components (atovaquone and proguanil) of the new antimalarial drug malaron, which collaborate to cause the collapse of the mitochondrial membrane potential of the parasite. Vaidya postulates that plasma membrane proton pumps and pyrophosphate are crucial for maintaining the energy metabolism of the parasite. Aloysius Tieiens (Univ. of Utrecht) elaborated on the nonclassical biochemical pathways of trypanosomatid mitochondria and listed several specific drug targets. Paul Michels (Catholic Univ. Louvain, Belgium) discussed the properties of a unique trypanosomatid organelle, the glycosome, and indicated potential new pathways in this organelle that regulate glycolysis. Ching-Chung Wang (UCSF) described the fascinating and essential ubiquitin-proteasome pathway of T. brucei.

Talks from pharmaceutical company scientists made clear that large chemical libraries and experienced medicinal chemists (rarities outside the world of big pharma) are absolutely essential for drug development (11). This led participants to propose establishing a network of high-throughput synthesis centers that would synthesize chemical libraries and lead compounds against tropical protozoa. Each center would prepare chemical libraries in response to requests from scientists working on promising drug targets or lead compounds. Proposals would be solicited and ranked by review panels, and then the power of the synthetic teams would be made available for the high-priority projects. Such centers would be of immense benefit for translating the results of functional and structural genomics into drug candidates.

The conference also addressed the fascinating yet tragic phenomenon of drug resistance. Point mutations provide resistance to a number of antimalarial folate inhibitors (3). Dyann Wirth (Harvard) described multidrug resistance in P. falciparum due to efflux protein pumps residing in the complex multivesicular tubule system of this malaria parasite. There seems to be no end to the array of tricks that these parasites use to combat drugs. Pradip Rathod (Univ. of Washington, Seattle) provided evidence for a specialized molecular machinery in P. falciparum that increases the mutation rate in highly specific areas of the malaria genome, thereby allowing rapid escape of the parasite from drug pressure without endangering its survival.

No wonder that participants ardently discussed ways to maintain the power of precious current (and future) drugs that have passed tests for safety and efficacy. The only way to safeguard the value of new drugs will be to bring them into the field in a well-controlled manner—possibly in paired combinations. A key requirement will be to maintain a large number of candidate drugs in the pipeline. Fortunately, several new funding sources have recently been created to achieve this end. Solomon Nwaka discussed the Medicines for Malaria Venture (www.mmv.org), and Victoria Hale described the Fledgling nonprofit Institute for One World Health (www.iowh.org), which aims to fill gaps in the development of new drugs for neglected diseases.

We calculate that 20 to 30 new drugs will be needed for long-term control of the protozoan diseases rampant in the tropics. As it may take $200 million to bring each successful compound to patients, we will need $4 billion to $6 billion spread out over 10 to 20 years to achieve a goal of 20 to 30 effective new antiprotozoan drugs (a mere 10 cents per world citizen per year for a few decades). It is primarily a matter of organization, vision, and bringing together the right people and organizations to ensure that today's wealth of genomic knowledge will be smoothly translated into the new therapies of tomorrow.

References and Notes
1. Drugs Against Tropical Protozoan Parasites; Target Selection, Structural Biology, and Rational Medicinal Chemistry, Keystone Symposium, 3 to 8 March 2002, Keystone, Colorado.
11. Supplementary online figure depicting different approaches to drug development can be found at www.sciencemag.org/cgi/content/full/297/5580/341/DC1.
12. We thank F. Buckner, S. Croft, P. Michels, G. Oliva, P. Rathod, A. Vaidya, and W. Van Voorhis for valuable comments.