PERSPECTIVES: PARASITOLOGY

Drugs to Combat Tropical Protozoan Parasites

Michael H. Gelb and Wim G. J. Hol

The 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*).

Enhanced online at www.sciencemag.org/cgi/content/full/297/5580/343

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 aminoquinolines 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 Tidwell (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 drug, a phospholipid analog called miltefosine, originally developed as an anticancer agent, has passed phase III clinical testing and was registered in March this year for treating visceral leishmaniasis patients in India (*6*, *7*).

Other groups talked about compounds that are not as far along the development pathway—for example, the xanthones that reduce the protective formation of hemozoin by *Plasmodium* (Michael Riscoe, Oregon Health Sciences Univ.), and adenosine analogs that block energy generation in trypanosomatids (Gelb, Hol, Van Voorhis, Buckner, Michels). Meanwhile, availability of the crystal structure of *T. brucei* ornithine decarboxylase has assisted in the generation of inhibitors that block this enzyme (Meg Phillips, Southwestern Medical Center). Also, crystal structures reveal that

THE HUMAN TOLL OF TROPICAL PROTOZOAN PARASITES

Disease	Major parasite	Insect vector	Regions affected	Estimated number of cases	Estimated number of annual deaths
Malaria	*Plasmodium falciparum* *Plasmodium vivax*	Anopheline mosquitos	Tropics	300 million/year	>1 million
Sleeping sickness	*Trypanosoma brucei*	Tsetse flies (*Glossina* spp.)	Sub-Saharan Africa	300,000+	66,000
Leishmaniasis (cutaneous, visceral, mucocutaneous)	*Leishmania* spp.	Phlebotomine sandflies	Tropics and subtropics	1.5–2 million	57,000
Chagas' disease	*Trypanosoma cruzi*	Reduviid bugs (triatomines)	Latin America	16–18 million	50,000

Source: WHO, CDC

M. H. Gelb and W. Hol are in the Departments of Chemistry and Biochemistry, respectively, University of Washington, Seattle, WA 98195, USA.

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 (Philipps 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 nonmevolanate 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*. Glaucius Oliva (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 genomics 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 plasmodial 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 plasmodial aspartyl protease, plasmepsin—a key player in hemoglobin degradation in the plasmodial food vacuole—was followed by the identification in the *P. falciparum* genome sequence of nine other plasmepsins. One of these plasmepsins is a most unusual histidyl-aspartyl protease that may be amenable to selective inhibition; meanwhile, a unique plasmodial enzyme, the maturase, which processes the proplasmepsins 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 malarone, 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 Tielens (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.
2. H. A. Portillo *et al.*, *J. Infect. Dis.* **183**, 1653 (2001).
3. C. H. Sibley *et al.*, *Trends Parasitol.* **17**, 582 (2001).
4. K. Wengelnik *et al.*, *Science* **295**, 1311 (2002).
5. J. A. Urbina, *Curr. Opin. Infect. Dis.* **14**, 733 (2001).
6. S. L. Croft *et al.*, *J. Antimicrob. Chemother.* **38**, 1041 (1996).
7. T. K. Jha *et al.*, *N. Engl. J. Med.* **341**, 1795 (1999).
8. H. Jomaa *et al.*, *Science* **285**, 1573 (1999).
9. A. Bahl *et al.*, *Nucleic Acids Res.* **30**, 87 (2002).
10. D. S. Roos, *Philos. Trans. R. Soc. London Ser. B* **357**, 35 (2002).
11. Supplementary online figure depicting different approaches to drug development can be found at www.sciencemag.org/cgi/content/full/297/5580/343/DC1

12. We thank F. Buckner, S. Croft, P. Michels, G. Oliva, P. Rathod, A. Vaidya, and W. Van Voorhis for valuable comments.