Invited Article

Recent highlights in anti-protozoan drug development and resistance research

Frederick S. Buckner a,⁎, Norman C. Waters b, Vicky M. Avery c

a Department of Medicine, University of Washington, Seattle, WA 98195-7185, United States
b Department of Chemistry and Life Science, United States Military Academy, West Point, NY 10996, United States
c Discovery Biology, Griffith University, Nathan, Queensland 4111, Australia

Abstract

This article summarizes the highlights of research presented in January, 2012, at the Keystone Symposium on “Drug Discovery for Protozoan Parasites” held in Santa Fe, New Mexico. This symposium which convenes approximately every 2 years provides a forum for leading investigators around the world to present data covering basic sciences to clinical trials relating to anti-protozoan drug development and drug resistance. Many talks focused on malaria, but other protozoan diseases receiving attention included African sleeping sickness, Chagas disease, leishmaniasis, cryptosporidiosis, and amoebiasis. The new research, most of it unpublished, provided insights into the latest developments in the field.

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1. Introduction

This special issue of IJPDDR provides a sampling of the research presented in January, 2012, at the Keystone Symposium on “Drug Discovery for Protozoan Parasites” held in Santa Fe, New Mexico. The symposium on this topic has taken place approximately every 2 years since its inception in 2002. As in previous years, the meeting provided a forum for leading investigators around the world to present data covering basic sciences to clinical trials relating to anti-protozoan drug development and drug resistance. As would be expected, many talks focused on malaria, but other protozoan diseases receiving attention included African sleeping sickness, Chagas disease, leishmaniasis, cryptosporidiosis, and amoebiasis. This editorial summarizes many of the highlights of the new research reported at the meeting.

2. Advances in antimalarial drug discovery and development

The need for low cost and effective anti-malarials against multidrug resistance Plasmodium falciparum has highlighted the need for more diverse chemotypes and validated drug targets. Throughout this meeting we heard of the approaches and results which have been achieved since the last meeting in 2009.

Emily Derbyshire (Harvard Medical School) gave a comprehensive account of the collaborative efforts of her work in Jon Clardy's lab (Harvard Medical School) with Maria Mota's group (University of Lisbon, Portugal) in disrupting liver stage development of malaria parasites. A luminescence-based liver stage malaria assay was developed and used to screen a library of bioactive compounds. Based on known targets of the compounds, various cell receptors, proton pumps and CDKs were predicted to have potential as drug targets. The commercially available blood pressure drug, telmisartan, was demonstrated to reduce parasitemia in a Plasmodium berghei mouse model. The benefits of re-purposing commercially available drugs as liver stage specific therapeutics or research tools were discussed.

The non-mevalonate pathway (MEP) pathway of isoprenoid biosynthesis in P. falciparum, which is required for malaria parasite growth, is the focus of the research interests of Audrey Odom (Washington University, St. Louis). Isoprenoids are a diverse class of biomolecules with multiple cellular functions, including roles as co-factors and signaling molecules. Evidence supporting the essentiality of the plastomidal enzyme IspD (methylerythritol phosphate cytidytransferase) [PF-IspD], and thus suitability of PF-IspD as a target, was provided and illustrated that it plays a key role in metabolic control of Plasmodium parasites. The non-mevalonate isoprenoid biosynthesis has been demonstrated to be important in trophozoite and schizont stages in intraerythrocytic parasites and in liver stage parasites. The antibiotic, fosmidomycin, has been shown to block IspD in P. falciparum. A malachite green-based PF-IspD pilot screen of 1256 compounds was performed and novel PF-IspD inhibitors identified will be validated to confirm activity against the enzyme PF-IspD, as well as confirmation of activity against the whole malaria parasite.

The development of the sexual stages, namely the gametocytes, is required for transmission of malaria and thus an inability to commit and progress to sexual development may mediate transmission blockade. Andy Waters (University of Glasgow) indicated that the production of gametocytes is an investment and a selective disadvantage for the asexually propagating parasite. He discussed how sub-telomeric deletion on the species specific region of Chr9 was...
associated with loss of gametocytogenesis (Pfgig). Characterization of mutations of the ANKA line 820 of *P. berghei* that were unable to produce gametocytes identified a gene that plays a central role in gametocytogenesis. He also discussed ApiAP2 transcription factors that are thought to drive the intraerythrocytic development cycle gene expression cascade.

Dominic Hoepfner (Novartis) described the use of haploid insufficiency profiling (HIP HOP) for chemogenomic analysis in yeast. Cladosporin is a potent antiplasmodial agent with 60 nM activity against *P. falciparum*. The HIP HOP approach identified lysyl-tRNA synthetase as a target. In the identification of point mutations, resistance-conferring mutations are involved in the ATP–lysine interaction. He also indicated that cladosporin showed the same inhibition profile as many established protein synthesis inhibitors. Results from enzymatic assays confirmed key residue specificity and cladosporin was shown to have a more potent IC\textsubscript{50} on the lysyl-tRNA synthetase than on the corresponding human homolog.

In addition, observed resistance was moderate and occurred at low frequency, thus suggesting cladosporin has potential as a starting point for novel anti-protozoan drugs.

Understanding host–parasite interactions in malaria is a focus of the Greenbaum lab. In this context, Doron Greenbaum (University of Pennsylvania) discussed screening of small nonpeptidic host molecules of host defense proteins (HDP mimics) developed by PolyMedix Inc., against *P. falciparum*. Lead compounds were found to lyse the parasite digestive vacuole, but not the plasma membrane of the parasite. Two compounds were tested in mice infected with *Plasmodium* parasites and were able to significantly lower parasitemia.

Michael Riscoe (Oregon Health Sciences University) provided a historical review of the discovery of chloroquine. This included the research activities in the 1930s to identify an improved molecule (sontochin) and the ultimate return to chloroquine due to its superior qualities. The need for low cost antimalariales, especially against drug resistant forms of malaria, led Riscoe and colleagues to re-evaluate the original chloroquine replacement drug. Finding that sontochin retains significant activity against chloroquine resistant strains, he described their strategy to create sontochin analogs. One of the highlighted analogs, PH-203, was at least 10 times more effective than chloroquine *in vivo* in treating infected mice. Experiments suggest that enhancements in the metabolic stability of this molecule will be needed to optimize the antimalarial performance of this chemothpe. This work focused attention on the potential for development of low cost antimalariales using sontochin as a guide.

Anacor Pharmaceuticals (San Francisco, CA) has a unique boron-containing compound library which they are exploiting in their quest for new anti-infectives. Dickon Alley presented several compounds exhibiting potent antiparasitic and antifungal activity currently in preclinical development or clinical trials. One such compound is AN3661, which has an IC\textsubscript{50} value of 26 nM against *P. falciparum* and is in preclinical development. Using AN2690 as an example, he described how the unique mode of action of boron-containing compounds has been applied to drug discovery.

Margaret Phillips (UT Southwestern Medical Center, Dallas) described the dihydroorotate dehydrogenase (DHODH) inhibitor, DSM74, which has been demonstrated to reduce parasitemia in a *P. berghei* mouse model with no evidence of toxicity. These data provided the first proof-of-concept that DHODH inhibitors could block parasite growth *in vivo*. It was indicated that the X-ray structure of PjDHODH in complex with representative triazolopyrimidines was then used to facilitate the identification of compounds with improved potency and ADME properties. DSM265 was shown to be a potent and selective inhibitor of the malaria enzyme, effective against both drug sensitive and resistant parasite strains, with the compound having similar efficacy to chloroquine in the SCID mouse model of *P. falciparum* infection. Validation that DHODH was the specific target for DSM265 and is an ideal antimalarial target was obtained through genetic rescue experiments. The synthetic scheme for DSM265 is simple and inexpensive to synthesize, and the compound has excellent oral bioavailability and a long plasma half-life and low clearance. DSM265 is currently in the preclinical development stage, and is part of the MMV portfolio.

Geoffrey Dow (WRAIR) described the research program in which he has led for some years on Next Generation Quinolone Methanols (NGQM) as potential intermittent preventative treatment (IPT) for malaria in children and pregnant women, and prevention in travelers. The neurological related side effects of mefloquine have limited the use of this drug (Dow et al., 2006). Geoff Dow presented an overview of the project leading to WR621308 which, despite heroic efforts, continued to retain too many of the adverse attributes of the parent compound, ultimately resulting in the termination of the program (Milner et al., 2011).

Geoff McFadden (University of Melbourne, Australia) described the metabolic pathways in the mitochondrion and apicoplast of the malaria parasite as potential drug targets. In a genetic knockout of *P. berghei*, the transmission of malaria to mice is blocked and the ATPase-negative strain does not produce oocysts, ookinetes and zygotes. However, the β subunit appears not to be essential in *P. berghei* blood stage hence it was concluded that ATPase synthase is not an optimal target. PfACCase knockout in the blood stages of the parasite results in lack of malonyl-CoA production. Given that neither PfAChase nor PfACCase have been demonstrated as essential for the blood stages, it was concluded that these were both poor therapeutic targets.

Thierry Diagana (NITD, Singapore) discussed the outcome of projects from the NGBS consortium which had 2 goals (1) to discover a one dose cure for *P. falciparum* and (2) a curative modality for *Plasmodium vivax*. A phenotypic SYBR green based HTS assay for *P. falciparum* was used to undertake a screening campaign. Following extensive lead optimization, NITD609 and GNF156, which are both effective against *P. falciparum*, were identified as candidate drugs. NITD609 has shown single dose cure in *P. bergheli* and both compounds have been demonstrated to block parasite transmission in a mouse model by pre-incubation of the compounds with mature gametocytes. GNF156 also reduced parasitemia in a humanized mouse model. NITD609 has been well described and pharmacologically characterized, and is now entering phase II clinical trials (Rottmann et al., 2010). It is anticipated that GNF156 will start phase I trials in human by the end of 2012.

The challenge with achieving the second goal of a curative modality for *P. vivax* was the availability of a suitable assay for the identification of compound activity on the hypnozoite liver stage. An overview of the development of the technology platform required to support this aspect of the program was presented, and the subsequent identification of a novel antimalarial effective against *P. vivax*.

A success story of the ozonide, OZ439, a peroxide based antimalarial currently in clinical development, was presented by Susan Charman (Monash University, Australia). Previous first generation oxonides such as OZZ277 displayed potent antimalarial activity, yet still had a relatively short half life *in vivo*. When OZZ277 was progressed to clinical trials, reduced plasma concentrations were evident in malaria patients compared to concentrations seen in healthy volunteers. This finding was believed to be caused by rapid blood-mediated clearance in the presence of infection. Structural modifications led to the discovery of new generation oxonides such as OZ439 that maintained potent antimalarial activity and exhibited cures with a single dose in animal models of malaria. Compared to the earlier oxonides, OZ439 is considerably more stable in non-infected and infected blood, and displays a much longer half
life and prolonged plasma exposure profile in healthy volunteers and malaria patients. OZ439 is currently in Phase II clinical trials (Charman et al., 2011).

3. Advances in the understanding and impacts of drug resistance by the malaria parasite

Any discussion on malaria drug development would be incomplete without addressing the concerns of drug resistance. The efficacy of every antimalarial drug in use today has been jeopardized by the development drug resistance (Petersen et al., 2011). In fact, the history of malarial drug developments parallels the history of drug resistance development. Fears deepen as reports now indicate that resistance is developing against the last remaining class of antimalarial drugs, artemisinins. This urges the developing of new antimalarial candidates currently in the pipeline and highlights the importance of expanding our knowledge of the molecular mechanisms associated with drug resistance. Future drug candidates that are developed with the aim to circumvent or stall resistance will help produce the next generation of therapies that prevent or reduce mortality.

Thomas Wellems from National Institutes of Health provided an excellent overview of malaria drug resistance which highlighted the hurdles that must be overcome with developing novel antimalarial drugs. In particular, consideration should be given to drugs that circumvent the drug-resistant transporters that are responsible for limited efficacy of numerous antimalarial agents. Two transporters that have been implicated in diminished efficacy of several antimalarial drugs are Pfcrt and Pfmdr1. Different resistance mutation patterns in these transporters have been reported in different part of the world reflecting differences in the history of drug use. Laboratory engineered parasites by David Fidock (Columbia University) and field studies conducted by Philip Rosenthal (University of California, San Francisco) support the importance of evaluating drug transporters for drug efficacy studies. David Fidock reported on the drug sensitivity of genetically engineered P. falciparum clones that express geographically-defined Pfcrt haplotypes from the Philippines and Cambodia. A correlation exist between chloroquine resistance and a loss of parasite fitness in several areas, however an allele from Cambodia conferred high level chloroquine resistance without diminishing fitness suggesting a strong transmission potential. Interestingly, biochemical analysis suggests that although chloroquine efflux is a factor in chloroquine resistance, it is not the sole explanation for Pfcrt-mediated chloroquine resistance. These studies highlight the geographic-specific evolution of Pfcrt haplotypes. The genetic lines used in this study were resistant to amodiaquine but sensitive to lumefantrine. Field studies conducted by Philip Rosenthal and colleagues in Uganda tracked diminished drug efficacy via ex vivo sensitivity testing and surveillance for acquisition of polymorphisms in Pfmdr1 and Pfcrt. Particular emphasis was placed on the selective pressure induced by prior treatment with drug combination regimes. It was observed that prior use of amodiaquine-containing therapies conferred a decrease in in vitro sensitivity and that amodiaquine selected for the Pfmdr1 86Y and 1246Y, and Pfcrt 76T polymorphisms. This selection was not observed however if prior therapy was with the artemether–lumefantrine combination, which selected for Pfmdr1 N86 and D1246, and also for Pfcrt K76. Thus, parasites are responding differently to the drug pressure exerted by different artemisinin-based combinations.

An additional implication of transporters involved in artemisinin combination therapy was presented by Leah Mwai (Kenya Medical Research Institute). In this study, P. falciparum parasites were induced and selected for in vitro lumefantrine resistance and monitored the expression profile of selected transporters. In these lines, 18 putative transporters, to include pfmdr1 and pfmrp, displayed a differential expression pattern. Future studies aim to characterize these transporters.

Artemisinin based drug combinations are commonly used throughout the world as first line treatment for uncomplicated P. falciparum infection. Although highly effective at killing parasites, recrudescence is a common problem associated with treatment. Qin Cheng (Australian Army Malaria Institute) and in this issue of IJPDDR, presented an evidence-based model as a possible explanation for the high rate of recrudescence and relate recrudescence to a stress-induced dormancy state. The model suggests that exposure of ring-stage parasites to artemisinin, induces a dormant growth stage that allows the parasites to tolerate the drug. Once the drug is removed, parasites emerge from dormancy and continue to develop into mature parasites. Upon repeated exposure to artemisinins, parasites may develop tolerance that neglcts the need to exist in the protective dormant state. In this regard, tolerance to artemisinin may result in fewer dormant parasites or faster recovery rates. In a clinical setting, this may translate to a delay in parasite clearance times and/or an increase in the rate of recrudescence. Dennis Kyle (University of South Florida) during his keynote address reported the in vivo observation of artemisinin induced dormancy. Additionally, the phenotype associated with artemisinin resistance was reported. The induction of dormancy appears to be linked to the artemisinin sensitivity of early ring-stage parasites. Unfortunately the molecular mechanism responsible for dormancy induction and recovery are unknown however it is believed that a cell cycle mechanism may be involved. Norman Waters (United States Military Academy) presented evidence to support a G1 cell cycle checkpoint during the early ring-stage development. The checkpoint appears regulated by cyclin-dependent protein kinases. A growth-retarded phenotype was observed only when parasites were exposed to CDK inhibitors before the breach of the checkpoint. In this regard, the G1 checkpoint may control the ring to trophozoite transition. It remains to be determined if this checkpoint is responsive to environmental stress and drug pressure.

The artemisinin mechanism of action is not fully understood but oxidative stress mediated by hemoglobin digestion is believed to play a key role (Meshnick, 1998). Leann Tilley (University of Melbourne, Australia) reported evidence that artemisinin activity is dependent on hemoglobin uptake and digestion. Hemoglobin digestion begins 10–14 h following red blood cell invasion and artemisinin sensitivity increases as the rate hemoglobin degradation increases. Treatment with artemisinin was shown to increase the level of oxidative stress in the parasite cytoplasm. Disruption of the hemoglobin degradation pathway by gene-knockout or enzymatic inhibition of the hemoglobinase falcipain-2 resulted in a decrease in the level of oxidative stress and a decrease in parasite killing. Sub-lethal doses of artemisinin retarded parasite growth and reduced the rate of hemoglobin uptake. The evidence is indicative that P. falciparum growth and metabolism are responsive to artemisinin treatment, although the signaling pathways and molecular machinery responsible for these artemisinin-induced responses are unknown. An enhanced ability to suspend hemoglobin digestion could lead to artemisinin tolerance. Oxidative stress is believed to play a key role in the antimalarial activity of artemisinins. As reported by Warangkhana Songsunthong, disruption of the glutathione (an intracellular antioxidant) biosynthetic pathway in P. berghei increased sensitivity to artesunate and pyrimethamine. The consequence of oxidative stress provides insight and rationale that should be considered for the development of antimalarial drug combinations.

Progress reported by Dylan Pillai (University of Calgary, Canada) continued the theme of characterizing and inhibiting enzymes that may potentiate the efficacy of existing antimalarial drugs and
circumvent the development of drug resistance. PfHsp90, a stress induced chaperone and cell cycle regulator in the malaria parasite was shown to interact with PfCRT. The PfHsp90 inhibitor, PU H71, is a potent antimalarial agent that synergizes with chloroquine and was able to reverse chloroquine resistance. Similar results were obtained from either a cell based assay or in the P. berghei mouse model. These findings support PfHsp90 inhibitors as a viable partner for the development of drug combination therapies.

Two independent groups reported efforts to elucidate the molecular mechanisms for the acquisition of drug resistance. Pradipsinh Rathod (University of Washington) presented new data from his team on how parasites with ARMD (Accelerated Resistance to Multiple Drugs) phenotype may initiate drug resistance. Resistance to the PfDHODH inhibitor, DSM1, invariably resulted in amplification of a fragment on chromosome 6 which contained the PfDHODH gene. Dr. Rathod illustrated how this system could be used to study these early steps of the initiation of drug resistance and the various factors that may determine whether the outcome would be stable or not, with and without drug pressure. Geoffrey Siwo (University of Notre Dame) reported studies to exploit the genomic alterations acquired by chloroquine resistant malaria parasites in predicting vulnerable parasite pathways. Using a systems biology approach to test the hypothesis that genomes are “rewired” in response to drug pressure, comparison between chloroquine resistant and sensitive genomes predicted targets that would respond to inhibitor treatment. The essence of the approach is to compare the differential co-expression patterns of the selected target to that of PfCRT. In proof-of-concept examples, accurate predictions were made for the effectiveness of inhibitors on ferrochelatase (heme biosynthesis) and a MutS homolog (DNA repair). Collectively these studies demonstrate that genomic tools, alongside tailored in vitro growth inhibition assays, can provide a fuller understanding of the mechanism of drug resistance and predict drug susceptibility.

The development of drug resistance and the associated implications for morbidity and mortality caused by P. falciparum is apparent; however there are other species of parasites causing malaria that have been neglected. Kevin Baird (Eijkman-Oxford Clinical Research Unit, Indonesia) highlighted P. vivax as “the neglected malaria parasite” even though this parasite is responsible for 100–400 million infections per year, of which an as yet unknown proportion are severe or fatal: 24% and 8% in one study in Indonesia. P. vivax resistance to chloroquine (blood schizontocide) and primaquine (hypnozoitocide) has developed and for chloroquine, appears to be widespread throughout Southeast Asia. Unfortunately, unlike chloroquine resistance in P. falciparum, the mechanism of resistance in P. vivax is unknown. Efforts must be initiated and supported to develop novel therapies for P. vivax. However, any new therapies to replace chloroquine must be evaluated with primaquine since the latter is the only drug available to clear hypnozoites, the source of relapses. Evidence presented suggests primaquine requires an appropriate co-drug to be efficacious. In this regard, new therapies designed for P. vivax must be pursued as a drug combination endeavor, not only due to drug resistance but because of the need to address the diverse parasite forms occurring during the life cycle of the P. vivax infection. In this issue of IJPDDR, Kevin Baird expands on the challenges ahead for chemotherapeutic discovery and highlights the need to develop therapeutic strategies for not only P. falciparum, but also for all malaria species. Furthermore, any discussion concerning malaria elimination and control must include endemic malaria which is often difficult to diagnose and ineffectively treated.

Alyson Auliff (Australian Army Malaria Institute) reported the development of a transgenic system in which wild-type and mutant P. vivax dhfr genes are stably expressed in P. falciparum and used to assess the effect of current and new antifolate drugs. Due to the difficulties of culturing P. vivax, the transgenic expression system has the potential to support drug discovery efforts for specific dhfr targets and provide a system to characterize the molecular mechanisms responsible for drug resistance. Karryn Gresty (Australian Army Malaria Institute Pacific Malaria Initiative) reported the surveillance of P. vivax infection and genotypic studies of samples collected throughout the South Pacific. It is envisioned that the development of molecular tools, assays and culturing techniques will soon complement ongoing surveillance efforts to better understand the evolution and dynamics of P. vivax drug resistance.

4. Advances in drug development for other protozoan pathogens

Human African trypanosomiasis (HAT) remains a serious public health problem in regions of sub-Saharan African and antitrypanosomal drugs are severely limited by poor efficacy, toxicity, and costs. Efforts by the research community to discover new treatments for HAT depend on efficient and effective screening methods of compound libraries. The identification and characterization of “hit” compounds against Trypanosoma brucei has been greatly facilitated by techniques reported by Bowling et al. in this issue of IJPDDR that are amenable to high-throughput screening platforms. Using the fluorometric dye, resazurin, the investigators performed a screen of a nearly 48,000 compound library with excellent Z’ scores in the 0.3–0.9 range. The screen has led to six distinct chemical series for subsequent hit-to-lead development. In addition, the authors describe methods for examining the effects of serum binding on the antitrypanosomal activity of compounds as well as techniques for measuring time-to-kil and reversibility of the compounds’ activities, all of which provide important information for prioritizing compounds for subsequent investigation.

Bakela Nare representing the same group at Scynexis Inc., also presented an update on the development of oxaborole compounds for HAT. The best of these low molecular weight, boron containing compounds demonstrated curative activity in the stringent late-stage mouse model of infection where the parasites were cleared from the central nervous system. By combining excellent pharmacokinetic stability with the ability to cross into the central nervous system, these antitrypanosomal compounds have properties that make them excellent clinical candidates for treating late-stage HAT. The lead compound SCYX-7158 is presently in Phase I clinical trials where human pharmacokinetics and safety will determine the potential to progress the compound into field studies for HAT.

Paul Wyatt from the Drug Discovery Unit at the University of Dundee reported on progress in targeting the N-myristoyltransferase enzyme for anti-T. brucei chemotherapy. RNA interference demonstrates the essentiality of the target, and high-throughput screening followed by lead-optimization led to a sulfonamide compound with highly potent ED50 values of 2 nM. The compound cured mice with early-stage trypanosomiasis and related compounds cured late stage disease in mice lacking PgP drug efflux pumps. However, compounds with better ability to penetrate the CNS will be necessary to cure the late stage of the disease.

Other biochemical targets in T. brucei that were reported to have potential to be exploited for drug therapy include the aurora kinase, methionine aminopeptidase, methylthioadenosine phospholase, nucleotide metabolism, the RNA editing ligase, and the methionyl-tRNA synthetase. Christophe Verlinde (University of Washington) reported new compounds targeting the latter target with evidence for excellent brain permeability, thus having potential for treating late-stage African trypanosomiasis.

American trypanosomiasis, commonly called Chagas disease, is widespread throughout Latin America and has a growing impact.
in North America and Europe where immigrants with chronic infection have settled in large numbers. As with HAT, the anti-parasitic treatments for American trypanosomiasis are unsatisfactory due to poor efficacy and high toxicity. In this issue of JPDDR, Buckner and Urbina summarize developments in exploiting sterol biosynthesis inhibitors as anti-Trypanosoma cruzi agents. The P450 enzyme, sterol 14-demethylase, has been investigated extensively. Antifungal drugs in the azole class target sterol 14-demethylase and have activity on the T. cruzi orthologue. The clinical drug, posaconazole, has sub-nanomolar activity on T. cruzi cultures and cures the stringent chronic mouse model of T. cruzi infection. Clinical trials in Spain and Argentina are currently underway to assess the potential for repurposing this antifungal drug for this protozoan disease. Similarly, another sterol 14-demethylase inhibitor, an oral prodrug of ravaconazole (developed by Eisai Pharmaceuticals through phase II studies for fungal infections) is entering clinical trials for Chagas disease in Bolivia under the auspices of the Drugs for Neglected Diseases Initiative. At the symposium, Buckner (University of Washington, Seattle, WA) presented data on analogs of the anti-cancer clinical candidate, tipifarnib. This class of sterol 14-demethylase inhibitors has subnanomolar activity against T. cruzi in vitro along with the advantage of having low activity against human liver CYP enzymes that improve its potential for safety compared to other sterol 14-demethylase inhibitors.

To facilitate drug discovery against T. cruzi, Melissa Sykes (Griffith University, Queensland, Australia) described a 384-well imaging assay employing the use of cytoplasmic and nuclear markers that allows the simultaneous determination of activity on both T. cruzi-infected and non-infected host cells. The advantage of this technique is that it allows the screeners to assess both the antiparasitic activity and the host-cell cytotoxicity in a single high-throughput assay.

Maria Papadopoulou (Northshore University, Evanston, IL) presented data utilizing luciferase expressing T. cruzi for bioluminescent imaging of infected mice to assess the activity of investigational compounds. This work led to the discovery of novel 3-nitro1,2,4-triazole-based compounds with potent activity in the mouse model of T. cruzi infection. Another area of interest was research reported by Denise da Gama Jaen Batista (Institute Oswaldo Cruz, Rio de Janeiro, Brazil) looking at combination chemotherapy for T. cruzi infection. In particular, the group described combining the standard T. cruzi drug, benznidazole, with diamideine compounds (known for potent anti-T. brucei activity) and showed significantly enhanced the activity of over either compound used alone. The studies reinforce the idea that combination chemotherapy may be crucial for developing optimal treatment regimens for treating Chagas disease.

As with T. brucei, compounds targeting the N-myristoyltransferase were tested for anti-T. cruzi activity as reported by Linda Herrera of Rosa Maldonado’s group (University of Texas El Paso). The unpublished data showed that the compounds have submicromolar activity against intracellular cultures of T. cruzi.

Another kinetoplastid organism, Leishmania, was the topic of several presentations at the meeting. Causing cutaneous, mucocutaneous, or visceral leishmaniasis, species of Leishmania threaten the health of people living in 88 countries. The existing drugs are limited by problems such as widespread resistance, parental routes of administration, toxicity, and/or teratogenicity. To address the shortcoming in anti-leishmanial therapeutics, scientists at The Ohio State University represented by Mark Drew screened a compound library and discovered a potent hit in the berberine class. A semisynthetic berberine analog had potent anti-leishmanial activity in vitro with a therapeutic window >270-fold against mammalian cells. The compounds decrease the liver burden of Leishmania donovani in mice by nearly 50%. The target of action is unclear, but the series appears to have broad spectrum activity against protozoa including other trypanosomases and Plasmodium.

The group at the Sandler Center represented by De Muylder (University of California, San Francisco) has developed a high-content cell-based screening assay for L. donovani and reported results from a pilot screen of 900+ bioactive compounds. Drug discovery for visceral leishmaniasis has been particularly hampered by low-throughput methodologies, so this research represents a significant advance to help accelerated drug discovery against this pathogen.

The enteric protozoan, Entamoeba histolytica, is responsible for almost 100,000 deaths per year worldwide, only second to malaria as a cause of mortality by protozoan pathogens. In this issue of JPDDR, Abhyankar et al. (University of Virginia, Charlottesville, VA) provide new data on a remarkable class of transmembrane kinases of which there are over 100 in E. histolytica. These proteins are believed to serve as signaling molecules especially at the parasite-host interface. By decreasing expression levels of one transmembrane kinase (EhTMKB1-9), the investigators showed lower levels of phagocytosis and endocytosis under experimental conditions. Overexpression of a mutant form of EhTMKB1-9 lacking the kinase domain resulted in a competitive disadvantage with regard to survival in the mouse gut, however, these parasites were still able to form abscesses in the liver abscess model in gerbils. Transmembrane kinases are viewed as potential drug targets for E. histolytica due to their apparent role in virulence and proliferation.

A presentation by Rosa Andrade (University of California, San Diego) described anti-Entamoeba spp in vitro activity of a drug approved for use in rheumatoid arthritis (auranofin). The IC50 of auranofin against E. histolytica trophozoites was 2 μM. Of note, auranofin has much higher cysticidal activity on Entamoeba invadens cysts than the standard amoebicide, metronidazole, providing a potential therapeutic advantage. As an FDA approved drug, auranofin has potential to be advanced into clinical studies for amoebiasis much more rapidly than non-approved compounds.

On a similar note, the strategy of repurposing FDA approved drugs for anti/protozoan applications was described for the enteric pathogen, Cryptosporidium parvum. Kovi Bessof (University of Vermont) developed a high-throughput screening platform for C. parvum then screened a collection of 727 approved or drug-like molecules both alone or in combination with nitazoxanide (an established anti-Cryptosporidium drug). Hits from this screen are being further evaluation alone or in combinations in immunocompromised animals with C. parvum infection.

Finally, the agent of amoebic meningoencephalitis, Naegleria fowleri, was the subject of investigations by Anjan Deb Nath (University of California, San Francisco). An in vitro high-throughput screening platform was developed, and used against a library of FDA-approved drugs and drugs in clinical development. A polynye antifungal drug similar to amphotericin B, called corifungin, was identified in the screen and determined to have excellent activity (better than amphotericin B) in a mouse model of primary amebic meningoencephalitis. Based on these results, the FDA has approved an Orphan-Drug status of corifungin for treatment of primary amebic meningoencephalitis.

5. Concluding remarks

The Keystone Symposium on “Drug Discovery for Protozoan Parasites” provided an extraordinary opportunity to engage individuals in all aspects of protozoan drug development. Parasitologists, medicinal chemists and clinicians from academia, industry and governmental institutions shared a common goal of reducing the burden of protozoan diseases. There are many problems and hurdles to overcome in drug development; however this conference promoted the discussions of obstacles unique to protozoan
drug development. Momentum from this conference will advance drug development efforts as new collaborations were made and unpublished data revealed.

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