REVIEW

Novel Antimalarial Drug Targets as Potent Tools to Accelerate Drug Discovery: A Short Review

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ABSTRACT

Introduction: Malaria is a significant tropical disease and the greatest killer of all time. The molecular pathways of known antimalarial drugs have been extensively elucidated. However, the emergence of resistant plasmodium species, especially that of *P. falciparum*, further threatens the prospects of its eradication. The advancement in proteomics and genomics has taken us a step further. Mere serendipity and pharmacology-based approaches can no longer take the lead in drug discovery. Newer and better antimalarial drug targets need to be sought.
Objectives: This study presents the need and problems in identifying and validating novel antimalarial drug targets to accelerate drug discovery.
Methods: Relevant literature was retrieved from Google Scholar, PubMed, and ScienceDirect. An exploratory search for traditional antimalarial drug targets and their shortcomings were reviewed, and the problems in identifying and validating novel drug targets. Possible solutions were proposed.
Body: Emerging resistance and advances in proteomics drive the need for newer targets. Significant problems include the lack of crystal structure of some targets and determining the essentiality of genes and their cognate proteins. The in-silico approach using phylogenetic comparison can quickly determine the essentiality of genes, and Protein Interference Assay (PIA) is potent in validating newer targets.
Conclusion: Identifying and validating novel antimalarial drug targets will effectively drive the search for and discovery of newer drugs.

Keywords: Malaria; Antimalaria; Drug discovery; Drug target; Target identification; *Plasmoduim falciparum*

INTRODUCTION

The battle against the plasmodial parasite has only seen ups and downs but not resolved till now. Recently, this has been heightened with the emergence of multidrug-resistant species, especially *P. falciparum*, which threatens all available therapies. Previous reviews on the status of parasite resistance clearly show that a constant discovery of new antimalarials needs to be provided[1]. Before now, the pharmacologically based approach was the mainstay in searching for new drugs. The current emphasis is on the target-based approach in identifying targets of known antimalarials and the adaptability of the malaria parasite[2]. This approach has also not been very successful due to the usual focus on

traditional targets. So, in essence, the Harlow-Knapp effect, first described for the human kinases, is already immersed in antimalarial research and drug development[3].

There is now an urgent need for successful combatting of multidrug-resistant malaria parasite, and this requires: (i) use of at least drugs in combination, (ii) use of a multitarget drug. Scientists have lately started scouting for newer targets to meet the challenge. However, this process has been slow due to the unavailability of tools for identifying and validating novel targets[4,5]. In this review, we provide perspectives from published literature on the need for newer targets for antimalarial drug discovery, the bottlenecks and the approaches to overcome them.

METHODS

Research articles were retrieved from Google Scholar, PubMed, and ScienceDirect. Search on these databases were done using keywords like "antimalaria drug target and (or) identification and (or) validation of novel drug targets". Relevant literatures were selected from this search strategy. Quality of the article in relation to the topic of our review was judged by the authors based on the contents of those articles. Previous antimalaria drug targets, their shortcomings and emerging novel targets were explored from those selected articles. The problems facing the identification and validation of novel drug targets were reviewed and possible solutions from the literatures were discussed.

DISCUSSION

Previous antimalarial drug targets

Antimalarial drugs act at specific points in the plasmodium life cycle and on specific targets to elicit their therapeutic effect (Figure 1). This is evident when elucidating their mode of action. Many proteins in the malaria parasite serve as targets for antimalarial drugs.

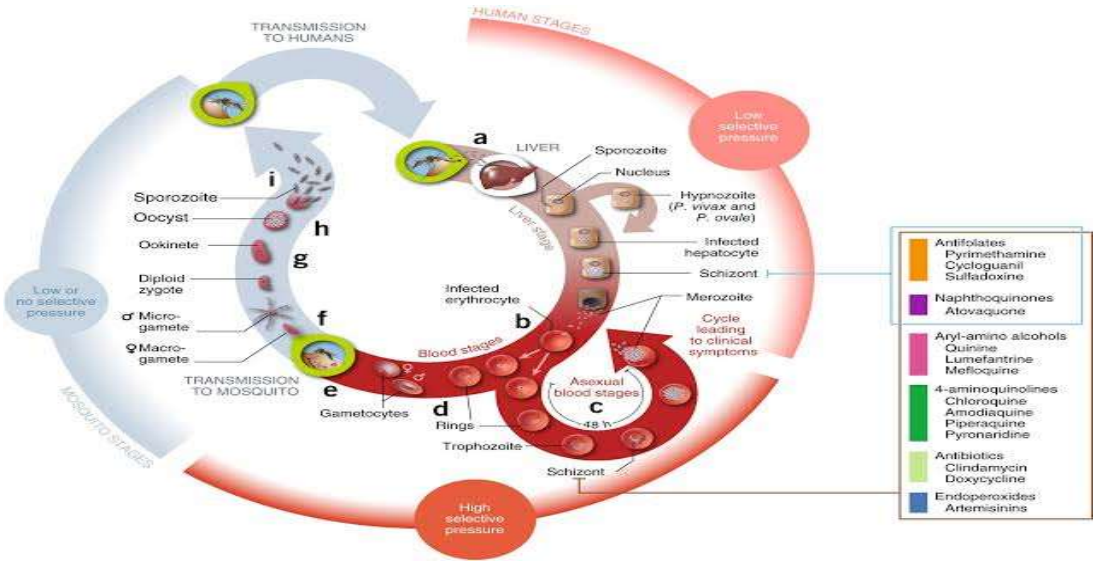

Figure 1: Schematic representation of the asexual lifecycle of malaria parasite (*P. falciparum)* and targets of conventional antimalaria drugs[6].

Dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS)

Mitochondrial dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) have long been identified as antimalarial agent targets. These enzymes are critically involved in synthesizing or acquiring folate cofactors essential in cellular metabolism that concern the methylation of proteins, DNA and various metabolites and the synthesis of nucleic acids and some amino acids by the malaria parasite[7]. Both DHPS and

DHFR are crucial for synthesizing folate cofactors in the malaria parasite. DHFR has an additional role in metabolic processes involving methylation. Folate cofactors are not synthesized in humans but obtained from green leaves and other nutritional sources[8]. Due to that, DHPS is absent in humans, and DHPS inhibitors do not affect humans.

On the other hand, DHFR is present and essential in malaria parasites and human hosts. However, the host enzyme differs in structure from the parasite and is not significantly inhibited by DHFR inhibitor[9]. Malarial DHPS are targets of drugs such as sulfadoxine which are DHPS inhibitors. DHFR is inhibited by pyrimethamine and cycloguanil. DHPS and DHFR inhibitors are generally known as antifolates[10].

Heme breakdown and the detoxification of heme

The breakdown of haemoglobin and the detoxification of heme occur in the food vacuole of the malaria parasite. These processes are unique to the parasite and are not found within the host, hence serving as a target for antimalarial agents. In addition, both processes are essential to the parasite; the breakdown of haemoglobin results in the formation of amino acids and the release of heme, a toxic substance that lyses the parasite's membrane, thus leading to its death. Hence, detoxification of heme by forming hemozoin via biocrystallization in the food vacuole is critical to the malaria parasite's survival. Chloroquine and quinolone derivatives are antimalarial agents; that elicit their antimalarial effect by inhibiting these processes.

Oxidative stress

Toxic substances are produced during metabolic processes in *P. falciparum* as metabolic wastes. Metabolic processes such as haemoglobin digestion release reactive oxygen intermediaries (ROI) and heme. Naturally, the parasite's defence mechanisms enable it to detoxify these substances. Scientists have leveraged this to produce antimalarial agents that increase oxidative stress in the parasite, thereby overwhelming the ROI defence mechanism leading to parasite death. Artemisinin and its derivatives have this as their target[11,12,13].

Dihydroorotate dehydrogenase and Cytochrome C reductase (parasite electron transport chain)

Dihydroorotate dehydrogenase is an enzyme that synthesizes orotate from dihydroorotate for pyrimidine biosynthesis during the intra-erythrocytic stage of the infection. Furthermore, this has served as a target for an antimalarial drug. This enzyme generates electrons disposed of by the electron transport chain of the malaria parasites. Also, cytochrome c reductase, an enzyme actively involved in the electron transport chain of the parasite, has been targeted by the antimalarial agent. Inhibition of these enzymes results in the parasite's death following the interruption of the transport of electrons, and hence the respiratory process of the parasite. Atovaquone is an antimalarial agent that acts through this mechanism[14].

Novel antiplasmodial drug targets

One approach to developing new drugs involves identifying small molecules with an antiparasitic activity using phenotypic screening, testing compounds directly against living parasites. The concerted efforts by many labs to design high-throughput phenotypic screening methods for the entire *Plasmodium* lifecycle and assemble chemical libraries have led to the discovery of many compound candidates effective in parasite clearance[15]. This approach has led to new clinical candidates, such as KAE609 (cipargamin) and KAF156 (ganaplacide), in phase IIb clinical trials[16].

Additionally, as the discovery rate of new antimalarial chemotypes decreases, many are reconsidering target-specific screens like a recent biochemical screen designed to identify inhibitors of lysyl-tRNA synthetase. Such screens can be less expensive to run, do not require specialized access to parasites, and could theoretically give leads with improved potency and selectivity, especially when

combined with knowledge of the target's structure. Using this method, two promising compounds in clinical trials, DSM265 and P218, were found to target DHODH and DHFR, respectively. The current bottleneck lies in identifying high-quality targets, such as lysyl-tRNA synthetase. A bioinformatics approach can predict alternative binding sites in *Plasmodium* aminoacyl tRNA synthetase that have low homology to those in humans, thus making them potential druggable targets[17].

In-vitro evolution and whole genome analysis unveils novel target genes

A promising method for discovering new targets involves *in-vitro* evolution and whole-genome analysis (IVIEWGA). This method comprises exposing *P. falciparum* parasites to sublethal doses of a compound until an upward shift in IC_{50} is observed in the culture, indicative of resistant parasites. Sequenced genomes of the resistant parasites are compared to those of the drug-naive parent to reveal genetic changes such as single nucleotide polymorphisms (SNPs) and copy number variants (CNVs). These genomic changes can point to genes encoding drug targets. However, they can also point to genes encoding multidrug resistance, such as the *P. falciparum* multidrug resistance 2, PfMDR2 or improving the fitness of a drug-selected line[18].

Metabolomic profiling and transcriptomics narrow down the mode of action

P. falciparum parasites with a compound of interest, extracting and analyzing the metabolites via LC-MS (Liquid Chromatography and Mass Spectroscopy hyphenated technique), and comparing the metabolic fingerprint, or meta print, to untreated parasites. This method may not provide the exact target, but it narrows down the mode of action, which is valuable for target validation or characterization. One caveat is that metabolite changes could be attributed to non-specific or non-viable phenotypes, but this can be overcome with testing at various concentrations or over time[19].

The mitochondria as a drug target for *P. falciparum* malaria

Mitochondria are organelles that act as the cell's power plants, as they produce energy for all cellular activities. There are several molecular and functional differences between the mitochondria of *Plasmodium* species and those from the host. It is also known that the plasmodial mitochondria play a critical and essential role in the parasite's life cycle. Previous studies have suggested that oxidative phosphorylation is not an essential pathway for parasites' survival during the blood stage. The parasite depends mainly on glycolysis as an energy source in this stage. The glucose consumption in *P. falciparum*-infected red blood cells (RBC) was 75 to 100-fold higher than in uninfected RBC[20]. Significant glucose uptake during the infection leads to hypoglycaemia, which, together with increased lactate production and resulting lactic acidosis, are significant causes of mortality during severe malaria. Thus, it is generally believed that the role of mitochondria in the parasite is not oxidative phosphorylation but the maintenance of the inner mitochondrial potential. The chemotherapeutic Malarone®, a combination of mitochondrial *bc1* complex inhibitor, Atovaquone and the dihydrofolate reductase inhibitor, Proguanil, collapses the inner mitochondrial potential and induces parasite's growth arrest, confirming the mitochondrial metabolism to be crucial for the viability of the parasite[21].

Other possible antimalarial drug targets

These might include manipulation and use of the following mechanisms and pathways:

1. Electron transport chain (ETC)[22,23,24]
2. Dihydroorotate dehydrogenase (DHODH)[23,25]
3. Cytochrome bc1 (complex III)[26,27]
4. Mitochondrial glycerol-3-phosphate dehydrogenase (mG3DH)[28,29]
5. Succinate dehydrogenase (SDH)[30,31]

6. Malate-quinone oxidoreductase (MQO)[32,33]
7. Pyrimidine biosynthetic pathway[34,35].

Bottle necks for validating drug targets and recent approaches

Having observed the progress so far, we concede that much of what has been left in antimalarial drug research and development is grounded in the availability of research tools to identify and validate new targets. There is a reluctance to seek and validate newer pathways and targets that will eventually allow a constant flow of novel antimalarials. Practical tools to validate candidate drug targets are limited for the malaria parasites[36,37]. Moreover, this is even more critical in all areas of life science. Once tools required for further research are unavailable, progress and advances in that field are halted. Advances in proteomics and phylogenomics have brought newer searchlights but have also seen the limitation of elucidating the 3D structure of many potential targets. Enzymes like the pfNDH2 (Plasmodium falciparum Type II NADH dehydrogenase**)**, whose crystal structure has not been significantly delineated, limit the search for this target. Much reliance on the virtual screening method will not give us headway[3]. The genomic sequence of *P. falciparum* has been elucidated and thus has paved the way for many potential drug targets. Likewise, advances in malaria genetics offer a more efficient way of characterizing potential targets. PlasmoDB, like many other databases, is a great tool that aids rapid search and analysis of the plasmodial genome. Rapid identification of tentative plasmodial targets, which are orthologues of validated target proteins from other systems, can significantly be facilitated by gene sequencing. Previous techniques in identifying and prioritizing proteins as candidate targets include elucidation of an essential step in metabolic pathways reported by Plata and colleagues[37] and Fatumo and colleagues[38] and the use of TDR web resources as in work by Magarinos and colleagues[39].

A significant challenge here is determining the essentiality of genes and thus their cognate proteins. These essential proteins, too, need to be conserved across so many malaria parasite species. The mutation of genes responsible for particular encoding proteins has also been used to determine essentiality. Here the identified/supposed target genes' base-pair sequences are altered to introduce active site mutants with modified activity in vitro and in vivo models. Gene knockout has been a mainstay, but as reported by Triglia and colleagues[40], it is almost absolutely problematic to refer to a gene as essential due to the inability to knock it out. The essentiality of genes can be estimated by the inability to be knocked out. However, this can be very misleading. For example, MQO (Maleate-Quinone Reductase) has been studied as an essential enzyme in the ETC (Electron Transport Chain) and thus a potential target, yet no viable knockout strains of *P. falciparum* could be obtained[3].

In work by Ludin and colleagues[36], a candidate antimalarial drug target should (i) have been conserved in all mammalian pathogenic plasmodial species and (ii) not have a match in *P. falciparum* nor a good match in the human proteome (iii), be functional in the trophozoites or gametocyte stage (iv) perform at least a receptor or enzyme function. Plasmodium species' cost-effective conservation of proteins in all the mammalian pathogenic Plasmodium is vital to ensure the development of drugs that can be used across the parasite species. It also remains a very cost and practical approach. Targets not found in the host provide virtually the best avenues for drug development since selectivity attains its peak here. A target present in the host can also be of great advantage, especially if structural differences abound between it and its counterpart in the host.

An example is the dihydrofolate reductase, the sulfonamide target in the de novo synthesis of purines. It is even best if the counterpart host target has been well studied in the past as a potential therapeutic target[41,42]. These problems, and many others in the various

works cited above, would continue to compound efforts to identify and validate essential genes, their cognate proteins, and thus potential targets unless better approaches are exploited.

Target identification and validation have taken the in-silico approach, involving the phylogenic comparison within a group of related parasites to make inferences about protein essentiality. It is pretty good, though not experimental nor very detailed, as it allows varied quantification without referring to a mouse model and thus is generally applicable. The proteins so identified usually fulfil the criteria for essentiality as outlined previously. To a great extent, this tool is reliable since included in the list of identified proteins are the targets of currently used antimalarial drugs. In work by Lunev and colleagues, a novel tool for validating drug targets was proposed: the Protein Interference Assay (PIA). It is built on Oligomerization, a ubiquitous feature of all biological systems. This process (dimerization and higher orders) determines the protein complex's biological activity. Oligomeric interfaces are less conserved among homologous proteins[43,44]. Small molecules that can bind across active sites of both parasite and host proteins cannot do the same for homologous oligomeric proteins. Thus, direct interference with the proper order of oligomeric structures will offer an opportunity for validating the protein as a valid target.

Research into new proteins and enzymes should be undertaken, especially elucidating plasmodia and human genomics. The vast roles of proteases ranging from the ingress and egress into and from red blood cells to feeding and survival in red blood cells, are known and widely reported[2]. Consideration of scaffolds and other subunits of already validated targets should also stay at the forefront. The instance of research on the bc1 complex—the most cited plasmodia protein—which has only focused on the Qo subunit and left out the Qi site is greatly discouraged[45,46,47]. Thus, exploration and identification of new targets will include but are not limited to the search for entirely new enzymes and proteins[48].

CONCLUSION

A lot has been done, and the progress so far is quite impressive. The MOAs and target proteins of traditional therapies are all elucidated. The fields of genetics, experimental pharmacology, bioinformatics, chemoinformatic, proteomics, and genetics are currently employed to see the eradication of this threat. Despite the many approaches to seeing around-the-clock availability of drugs against the plasmodia parasite, its eradication has remained relatively elusive. To effectively wage war against the seeming invisibility of this parasite, newer and novel targets must be sought. These newer targets would first have to be identified and validated as tools for such accelerated discovery. Studies and research into such identification and validation tools will vigorously promote target discovery and thus push forward antimalarial discovery.

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