JMGCB
ISSN:3032-1085
https://doi.org/10.61796/jmgcb.v1i8.878

MUTATIONS AND DRUG RESISTANCE IN MALARIA, TOXOPLASMOSIS AND GIARDIASIS: A REVIEW OF CURRENT CHALLENGES AND MECHANISMS

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Received: Jun 22, 2024; Accepted: Jul 29, 2024; Published: Aug 18, 2024;

Abstract: Objective: This study addresses the growing threat of drug resistance in malaria, toxoplasmosis, and giardiasis by analyzing the underlying genetic mutations that compromise the efficacy of current treatments. Understanding these mechanisms is crucial for developing more effective therapeutic strategies. **Method:** A comprehensive review of literature was conducted to identify key mutations associated with drug resistance. In malaria, the focus was on mutations in genes like Pfmdr1, Pfcrt, Pfmrp, and Pfnhe1, which are linked to resistance against antimalarials, particularly the K76T mutation in PfCRT for chloroquine resistance. For toxoplasmosis, mutations in the DHFR-TS and DHPS genes were analyzed, particularly the T83N mutation in DHFR-TS, which confers resistance to pyrimethamine. In giardiasis, the study reviewed mutations in the ferredoxin oxidoreductase gene that reduce the efficacy of metronidazole. **Results:** The analysis revealed that specific mutations in these parasites significantly contribute to the development of drug resistance, complicating treatment protocols. In malaria, PfMRP1 mutations are particularly concerning due to their role in resistance to both chloroquine and quinine. Toxoplasmosis resistance is notably influenced by DHFR-TS and DHPS mutations, while giardiasis resistance is linked to alterations in drug transport and enzyme function. **Novelty:** This study synthesizes the most recent findings on genetic resistance mechanisms in these three parasitic diseases, offering insights that could inform the development of next-generation therapies and improve resistance management strategies, ultimately contributing to better health outcomes.

Keywords: Drug Resistance; Giardiasis; Metronidazole Resistance; Malaria; PfCRT; Toxoplasmosis.

Introduction

The complex life cycle of Plasmodium falciparum contributes to a more pronounced random genetic drift and a more efficient purifying selection than what is typically expected in classic population genetics, resulting in a higher occurrence of non-synonymous mutations [1]. The parasite's expansion rate is typically 5- to 10-fold per asexual blood stage (ABS) cycle, allowing parasites with a selective advantage to proliferate rapidly during a typical 1- to 2-week infection period [2]. During the mosquito stages, which include the diploid oocyst stage, genetic diversity is generated through sexual reproduction. Current antimalarial drugs primarily target the intraerythrocytic development of Plasmodium parasites, with key drugs focusing on the food vacuole of ring-stage and trophozoites or enzymes involved in the folic acid biosynthesis pathway during the trophozoite stage [3]. However, to achieve malaria eradication, there is an urgent need for drugs that can prevent parasite transmission and target asymptomatic and hepatic forms of the parasite.

The global strategy for malaria eradication is built upon three major pillars: (a) ensuring universal access to malaria prevention, diagnosis, and treatment; (b) accelerating efforts towards elimination and achieving malaria-free status; and (c) transforming malaria surveillance into a core intervention [4], [5]. These pillars are supported by two crucial elements: ongoing innovation and research, and a robust enabling environment [5]. The lack of effective vaccines and emerging drug resistance necessitate continual adaptation of this strategy to overcome new challenges in malaria control and elimination efforts.

Parasitic diseases, especially malaria and schistosomiasis, represent a significant global health issue, causing nearly 1.1 million deaths annually and leading to high disability-adjusted life years (DALYs) [6]. The continuous threat posed by these diseases is exacerbated by the lack of effective vaccines and the emergence of drug-resistant strains. For example, Toxoplasma gondii, the causative agent of toxoplasmosis, poses a serious risk due to its ability to cause life-threatening complications in immunocompromised individuals and congenital infections [7]. Current treatment options, such as sulfonamides and pyrimethamine, target the parasite's folate synthesis pathway but are increasingly challenged by drug resistance, intolerance, and malabsorption issues [8].

Mutations in the dihydropteroate synthase (dhps) and dihydrofolate reductase (dhfr) genes are associated with resistance to antifolate drugs, a common treatment for toxoplasmosis and other parasitic infections [9]. Research conducted in 2017 identified a novel mitochondrial protein, TgPRELID, in T. gondii, which may be involved in multidrug resistance, underscoring the need for further investigation into the mechanisms of drug resistance in parasitic organisms [10]. Understanding these mechanisms is crucial for developing new therapeutic strategies to combat drug-resistant parasites.

Given the significant impact of parasitic diseases on global health and the growing challenge of drug resistance, this review is essential for synthesizing current knowledge and identifying gaps in the research. By highlighting recent advancements and ongoing challenges, this review aims to guide future research directions and inform the development of more effective interventions. The importance of this review lies in its potential to contribute to the global effort to control and eventually eradicate parasitic diseases, thereby reducing the burden on affected populations.

Methods

This study employs a literature review methodology to investigate genetic mechanisms underlying drug resistance in malaria, giardiasis, trichomoniasis, and toxoplasmosis. The review was conducted by systematically searching academic databases such as PubMed, Google Scholar, and Scopus using relevant keywords and selecting studies published between 2015 and 2024. Articles were chosen based on their focus on gene mutations and drug resistance mechanisms, with data extracted on specific genes, types of mutations, and their impact on treatment efficacy. This approach aggregates findings from primary research to provide a comprehensive overview of genetic and epigenetic changes associated with drug resistance, identifies common themes and gaps in the literature, and serves as a basis for future research and the development of novel treatment strategies.

Results and Discussion

Results

Mutations and Malaria

Plasmodium falciparum parasites progress through an asexual blood stage (ABS) cycle that lasts approximately 48 hours [11]. During this cycle, an invading merozoite matures from the ring stage to the trophozoite stage, then to the schizont stage, and finally ruptures the host red blood cell

(RBC) to release 8–24 daughter merozoites, which can initiate further ABS cycles [12]. Approximately 1%–2% of these ABS parasites will undergo sexual differentiation into male and female gametocytes. These gametocytes are then ingested by a mosquito during a blood meal, where they undergo sexual recombination and develop into sporozoites, ready to infect a new human host [1]. The majority of current antimalarials target the ABS, but recent drug discovery efforts have increasingly focused on compounds that also act on the liver or mosquito transmission stages, as the lower parasite numbers in these stages may reduce the potential for drug resistance development [13], [14]. Additionally, the intra-erythrocytic residence of the parasite limits its exposure to the host immune system, while the parasite further suppresses the immune response through mechanisms such as antigenic variation, cytoadherence to the vascular endothelium to avoid splenic clearance, and induction of immunosuppressive cytokines [2].

The control and elimination of malaria are significantly threatened by the development and spread of resistance to drugs, including artemisinins and their partner drugs in Artemisinin Combination Therapy (ACT), which is the first-line treatment recommended by the World Health Organization (WHO) [15], [16]. Currently, resistance to antimalarial drugs has been documented in three of the five species of malaria that affect humans: P. falciparum, P. vivax, and P. malariae. The intensification of antimalarial drug resistance can also be attributed to cross-resistance between drugs of the same chemical family or those that share similar modes of action [17]. Evaluating drug-resistance biomarkers and conducting therapeutic efficacy studies in malaria-endemic regions are critical for detecting resistant parasites and understanding the degree and extent of resistance within specific populations [18].

There is substantial evidence linking antimalarial drug resistance to genetic factors in the parasite. Mutations—whether simple, double, or quadruple—in different genes enable the parasite to withstand antimalarial drugs [15]. For instance, mutations in the Pfmdr1, Pfcrt, Pfmrp, and Pfnhe1 genes have been established in several parasite populations, facilitating the spread of drug resistance due to the selective advantage conferred by these mutations [19]. The Pfcrt and Pfmdr1 genes encode multiple predicted transporters in the P. falciparum genome. Polymorphisms in these plasmodial protein transporters impact drug sensitivity, with multiple mutations in PfCRT (chloroquine resistance transporter) enabling the efflux of chloroquine (CQ) from the parasite's digestive vacuole, thereby preventing CQ from binding to heme and inhibiting its detoxification. The Pfcrt gene consists of 13 exons localized on chromosome 7, encoding a 424-amino acid transmembrane protein [20]–[22]. The PfCRT protein belongs to the drug/metabolite transporter superfamily, with 10 putative transmembrane domains spanning the digestive vacuole membrane of the parasite [23]. Among these, the K76T mutation plays a pivotal role in determining CQ resistance, where the substitution of a positively charged lysine with a neutrally charged threonine at position 76 facilitates the efflux of diprotonated CQ from the digestive vacuole via active transport [24]. Other mutations, such as C72S, M74I, N75E, A220S, Q271E, N326S, I356T, and R371I, also contribute to resistance, though typically in conjunction with the K76T mutation [25]. Variations in the PfCRT protein also influence susceptibility and resistance to other antimalarials like quinine, amodiaquine, and lumefantrine, with CQ showing cross-resistance with amodiaquine and quinine, predominantly mediated by 76T, while lumefantrine exhibits inverse cross-resistance, with reduced susceptibility in association with the wild-type K76 [26]. In Southeast Asia and Africa, mutations at codons 72 to 76 confer higher resistance to CQ and moderate resistance to amodiaquine, whereas in South America, these mutations are linked with greater amodiaquine resistance, making the K76T mutation a potent molecular marker for antimalarial drug resistance, dependent on prior drug use in the region [27].

The Pfmrp1 gene, which encodes the P. falciparum multidrug resistance-associated protein (PfMRP), is a member of the ABC transporter superfamily and is primarily localized in the parasite plasma membrane [28]. The Pfmrp gene contains one exon, located on chromosome 1, and encodes an 1822-amino acid protein predicted to have two nucleotide-binding domains and two membrane-spanning domains, each consisting of six helical transmembrane domains [29]. PfMRP is involved in the transport of organic anionic substrates such as oxidized glutathione, glucuronate, sulfate conjugates, and possibly in drug transport as well [30]. Mutations at positions Y191H and A437S in PfMRP have been associated with resistance to CQ and quinine. Genetic knockout of the Pfmrp gene in resistant parasites has demonstrated heightened sensitivity to various antimalarial drugs, including CQ, quinine, primaquine, and artemisinin [31]. It is hypothesized that the PfMRP protein effluxes various metabolites and drugs out of the parasite in conjunction with other transporters [32].

In addition to PfMRP1, other transporters such as PfMRP2 and PfMDR5 have been described. PfMRP2, a full transporter belonging to the ABC C family, and PfMDR5, a half transporter from the ABC B family, are located on the plasma membrane across all asexual erythrocytic stages of P. falciparum [33]. The localization of these ABC transporters highlights their potential role as drug exporters. Like PfMRP1, PfMRP2 and PfMDR5 may contribute to the efflux of glutathione, CQ, and quinine, thereby enhancing the parasite's ability to extrude toxic compounds [34]. The Pfnhe and PfATP4 genes are also implicated in antimalarial resistance mechanisms. Pfnhe, encoding the Na+/H+ exchanger (PfNHE), is a transmembrane protein located in the parasite's plasma membrane, comprising 1920 amino acids and predicted to have 12 transmembrane domains [35]. Although the precise role of PfNHE remains unclear, it is hypothesized to be involved in the active efflux of protons to maintain a neutral pH of 7.4 within the parasite, in response to acidification by anaerobic glycolysis, which is the parasite's primary energy source [36].

Mutations and Toxoplasmosis

The primary treatments for toxoplasmosis, pyrimethamine, and sulfadiazine, are known to cause significant side effects, including neutropenia, thrombocytopenia, and hypersensitivity reactions [38]. Severe reactions such as agranulocytosis and toxic epidermal necrolysis, though rare, have also been reported [38]. A study indicated that 62% of patients undergoing this combination therapy experienced toxicity, necessitating a change in treatment for 44% of them [39]. Alternative medications like azithromycin, clarithromycin, and cotrimoxazole, although available, are poorly tolerated and ineffective against the bradyzoite stage of the parasite [40], [41]. Acute toxoplasmosis is commonly treated with pyrimethamine and sulfadiazine, which target the parasite's dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) enzymes, crucial for pyrimidine synthesis [38]. This combination exhibits strong synergistic effects against the parasite's survival and replication. However, due to the presence of DHFR in humans, its inhibition can lead to folate deficiency, causing hematological side effects and embryopathies [38].

Recent research has identified single-point mutations in the Toxoplasma gondii DHFR-TS gene, such as W25R, L98S, and L134H, that contribute to drug resistance in certain strains [42]. The T83N mutation, in particular, has been associated with resistance to pyrimethamine, with resistance further increased when combined with S36R and F245S mutations [42]. Despite these findings, variability in T. gondii strains' susceptibility to pyrimethamine was observed by Meneceur et al., without clear evidence of drug resistance or a direct correlation with specific genotypes or mutations in the drug target genes [43].

Sulfonamides, often used in combination with pyrimethamine for toxoplasmosis treatment, are intolerable for some AIDS patients. Sulfonamide resistance was first noted with sulfamethoxazole after parasites were exposed to sub-lethal doses for extended periods [44]. The R-SulR-5 strain, along with the Swa-20 strain isolated from patients, exhibited sulfonamide resistance, with an IC50 near 5 mM. A mutation at position 407 of the DHPS gene was linked to this resistance, and was also found in laboratory-induced R-SulR-5 strains [45]. However, this mutation was absent in five Brazilian T. gondii isolates from newborns with congenital toxoplasmosis [46].

Silva et al. (2017) identified a Brazilian isolate, TgCTBr11, resistant to sulfadiazine, with numerous DHPS gene polymorphisms but no clear association between SDZ susceptibility and parasite virulence or genotype. Despite known DHPS mutations conferring resistance in T. gondii and showing cross-resistance to various sulfonamides, not all clinical isolates exhibit SDZ resistance due to DHPS mutations [46]. Overall, sulfonamide resistance appears to be more widespread than resistance to pyrimethamine or atovaquone [46].

1-Hydroxyquinolones have been effective in inhibiting T. gondii replication, with TgDHODH identified as a significant target for compounds like HDQ and other 1-Hydroxyquinolone derivatives [47]. Drug-resistant mutants are valuable for identifying new anti-Toxoplasma drug targets. Antibiotics such as clindamycin, spiramycin, and azithromycin are active against T. gondii, but the ClnR-2 (RH) mutant shows cross-resistance to these antibiotics. Resistance is associated with rRNA genes in the 35-kb genome and targets apicoplast protein synthesis [48], [49]. Additionally, T. gondii is susceptible to dinitroaniline compounds, which disrupt microtubules without harming host cells. Mutations in alpha-tubulin, such as G142S or F52Y, confer resistance to these compounds. Ma et al. (2008) identified T. gondii lines with suppressed microtubule defects due to these mutations. Moreover, secondary resistant mutations were found that correct fitness defects in the parasite. These findings suggest that targeting parasite microtubules could be a promising approach for developing new anti-parasitic therapies [50].

Enzymes involved in epigenetic modifications of histones are emerging as potential targets for drug development, as several existing compounds can be repurposed to inhibit these enzymes [51]. These modifications are crucial for gene regulation and parasite growth [52]. Among the various epigenetic modifications, histone acetylation has been a prominent target for inhibiting T. gondii and other parasites [53]. Compounds that potentially inhibit histone acetyltransferases (HATs) and deacetylases (HDACs) have shown activity against T. gondii, indicating that histone acetylation is essential for tachyzoite survival and thus a promising therapeutic target [54], [55].

Mutations and Giardiasis

Giardia lamblia, a microaerophilic gastrointestinal parasite, is responsible for giardiasis, which affects nearly one billion people globally, with an annual incidence of 200-300 million cases [56]. This infection can cause both chronic and acute symptoms, including nausea, diarrhea, and malabsorption [56]. Additionally, giardiasis has been linked to conditions such as type II diabetes, obesity, and irritable bowel syndrome [57]. Current treatments for giardiasis include nitroheterocyclic compounds like metronidazole, nitazoxanide, and furazolidone, as well as benzimidazoles such as albendazole and mebendazole [58]. However, resistance to metronidazole presents a significant challenge. This resistance is often associated with mutations in the ferredoxin oxidoreductase gene, which decrease the enzyme's expression and binding affinity, leading to reduced drug efficacy [59]–[61]. Distinguishing between cure and reinfection or other disorders like lactose intolerance further complicates treatment outcomes.

While resistance has been evaluated in laboratory lines, clinical isolates have not been extensively studied at the DNA replication level [62]. Metronidazole's permeability issues and its numerous side effects underscore the need for alternative treatments [62]. Nitazoxanide, approved in 2004 for pediatric giardiasis treatment in the USA, targets the ventral cell membrane and induces vacuolization [63]. Resistance to 5-nitroimidazoles in Giardia duodenalis is linked to reduced drug susceptibility and altered intracellular metronidazole concentrations [64]. This resistance may be due to defective transport mechanisms across the cellular membrane or insufficient intracellular reduction of the drug to its active metabolite [65], [66]. The latter is attributed to modifications in proteins involved in drug activation, including reduced concentrations of pyruvate: ferredoxin oxidoreductase and downregulation of ferredoxin pathways in the parasite's low-redox-potential anaerobic metabolism, which leads to decreased metronidazole uptake [67], [68].

In a recent study involving three different metronidazole-resistant G. duodenalis cell lines, common resistance mechanisms included the upregulation of genes encoding variant-specific surface proteins, high cysteine membrane proteins, calcium and zinc channels, a Mad-2 cell cycle regulator, and a putative fatty acid α-oxidase. Simultaneously, there was downregulation of genes encoding nitroreductase-1, putative chromate and quinone reductases, and several genes related to pyruvate: ferredoxin oxidoreductase [69]. In contrast, a cell line with increased passive resistance due to a nonsense mutation in nitroreductase-1 transcripts showed increased transcription of nitroreductase-2 and a MATE transmembrane pump system, indicating active drug detoxification and efflux. Lines without this mutation had to manage a higher oxidative stress load caused by metronidazole- and oxygen-derived radicals [69].

Genomic sequencing of various G. duodenalis assemblages A and B strains revealed that genetic variability is common in key genes involved in metronidazole metabolism and the management of oxidative and nitrosative stress. This includes a high number of non-synonymous single nucleotide polymorphisms [70]. Additionally, chromosomal rearrangements and repetitive DNA changes have been observed in metronidazole-resistant strains [71]. Notably, the loss of a 3000-base pair sequence, G6/1, on chromosome 4 of G. duodenalis, which appears to affect the parasite's cell division, has been linked to the onset of metronidazole resistance [72]. Increased expression of the gene for protein disulfide isomerase 2 (PDI2) has been reported in strains resistant to either metronidazole or nitazoxanide, with combined resistance also associated with PDI4 expression. Drastic changes in the expression of genes for variant surface proteins (VSP) have also been observed in strains resistant to these drugs [73]. Interestingly, changes in the expression of stress response-related and heat shock proteins (HSP70 B2, HSP40), major surface antigens like the variant surface protein (TSA417, AS7), and nitazoxanide-binding proteins such as nitroreductase and protein disulfide isomerase PDI4 can disappear after an encystation and excystation cycle, suggesting epigenetic changes rather than permanent DNA sequence alterations [74]. Metronidazole-induced cellular stress can lead to the encystation of resistant G. duodenalis strains, resulting in less susceptibility to metronidazole [75].

Discussion

Drug Resistance in Malaria

The progression of drug resistance in malaria underscores the importance of understanding genetic mutations in parasites. The mutations in genes like PfCRT and PfMRP1 significantly impact drug efficacy and contribute to resistance patterns observed globally. The K76T mutation in PfCRT is a well-established marker for chloroquine resistance and has implications for other antimalarials

[24][27]. Similarly, PfMRP1 mutations and other transporters' roles in drug export highlight the complex nature of resistance mechanisms. Continuous monitoring and the development of new treatment strategies that target different stages of the parasite lifecycle are crucial for effective malaria control.

Drug Resistance in Toxoplasmosis

In toxoplasmosis, the emergence of drug-resistant strains highlights the need for alternative treatments and novel drug targets. The mutations in T. gondii's DHFR-TS and DHPS genes provide insights into resistance mechanisms, but variability in resistance patterns suggests that other factors also play a role [42][44]. The potential of targeting epigenetic modifications for drug development offers a promising avenue for overcoming existing resistance [51][52][53][54][55]. Ongoing research into the molecular basis of resistance and the development of new therapeutic strategies are essential for managing toxoplasmosis effectively.

Drug Resistance in Giardiasis

The resistance of Giardia lamblia to metronidazole and other treatments reflects a complex interplay of genetic and biochemical factors. Mutations affecting drug metabolism, transport, and stress response contribute to the parasite's ability to evade treatment [59][64][69]. The variability in genetic responses and the identification of key resistance mechanisms offer opportunities for developing targeted therapies. Future research should focus on understanding these mechanisms in clinical isolates to improve treatment outcomes and address resistance challenges more effectively.

Conclusion

Highlight Findings: Gene mutations in parasites, including Pfmdr1, Pfcrt, dhfr, and β-giardin, play a significant role in the development of drug resistance across various treatments for diseases such as malaria, giardiasis, trichomoniasis, and toxoplasmosis. These mutations lead to resistance against critical drugs, including chloroquine, antifolates, albendazole, and metronidazole. **Implication:** The emergence of drug-resistant strains due to these genetic mutations poses a serious challenge to the effectiveness of current treatments, making it imperative to understand the underlying mechanisms to maintain therapeutic efficacy. **Further Research:** There is an urgent need for in-depth studies on the molecular and epigenetic mechanisms driving drug resistance, with a focus on identifying novel therapeutic targets and strategies to overcome or prevent the spread of resistant parasitic strains.

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