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OPINION

Therapeutics through glycobiology: an approach for targeted elimination of malaria

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Abstract

The emergence of drug resistance in *Plasmodium* jeopardises worldwide malaria eradication efforts necessitating novel therapeutic approaches and therefore the identification of key metabolic pathways of parasite and human host for drug development garners importance. Enzymopathies like glucose-6-phosphate-dehydrogenase (G6PD) and pyruvate kinase (PK) deficiencies have been shown to protect against the severe consequences of malaria. Glycome profiles and the regulatory mechanisms involving the microRNAs or transcription factors' expression related to the histo-blood group glycogenes may add up to resolve the underlying pathogenesis. The glycan derivatives viz. heparin-like molecules (HLMs) interrupt parasite proliferation that can be exploited as leads for alternative therapies. The *Plasmodium* invasion of erythrocytes involve events of receptor recognition, adhesion, and ligand interactions. Since post translational modifications like N-glycosylation of merozoite surface proteins and several erythrocyte cluster of differentiation (CD) antigens and complement receptor, among others, are crucial to parasite invasion, understanding of post translational modification of proteins involved in the parasite-host interactions should identify viable antimalarial strategies.

Keywords Malaria · *Plasmodium* · Drug-resistance · Glycotherapy

Abbreviations

CAZymes	Carbohydrate-active enzymes
CD	Cluster of Differentiation
CPS	Capsular Polysaccharides
CSP	Circumsporozoite protein
EPCR	Endothelial protein C receptor
GAGs	Glycosaminoglycans
G6PD	Glucose-6-phosphate dehydrogenase
GPIs	Glycosylphosphatidylinositols
HLMs	Heparin-Like Molecules
ICAM-1	Intercellular adhesion molecule
IL-1	Interleukin-1
KLH	Keyhole limpet hemocyanin
PfEMP1	*Plasmodium falciparum* Erythrocyte Membrane Protein 1
PK	Pyruvate Kinase
RBCs	Red Blood Cells
TNF	Tumor necrosis factor
VCAM-1	Vascular cell adhesion molecule

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Malaria after showing a declining trend for the number of infections and deaths from the year 2000 to 2020 had exhibited a sign of reversal as shown by the data for the year 2020-21 that was also significant for the impact of the COVID-19 pandemic (WHO 2021; Prabhu et al. 2022). The host-parasite interactions in the disease have long been explored for the development of ideal antimalarials for therapy, prophylaxis and transmission blockade and yet the challenge of complete malaria control remains. Combination chemotherapy with artemisinin and quinoline-derived compounds have been adopted as the first-line treatment for the parasite infection. Unsurprisingly, over the years, *Plasmodium* had also evolved to resist treatment using

antimalarials thereby diminishing the efficiency of chemotherapy (Mohapatra et al. 2014). Classical malarial susceptibility/resistance factors identified involve G6PD and PK deficiencies that are often unnoticed in affected individuals. By lowering the parasite burden and limiting the binding of infected RBCs to organs, these particular enzymopathies in humans help resist the severe malarial complications. G6PD is involved in the protection of RBCs from oxidative stress, the reduced number of enzyme molecules or instability due to structural alterations causing deficiency in this enzyme (Hedrick 2011). G6PD deficient RBCs are more prone to damage by oxidative stress, thus, infected deficient RBCs have a shorter half-life. PK is involved in the production of ATP by glycolysis, which is the only pathway that provides energy to RBCs. Deficiency in this enzyme makes the RBCs more susceptible to macrophage clearance, increases their sequestration in the spleen, and enables their early destruction by the reticuloendothelial system. The deficiency also interferes in parasite DNA replication indicating the vulnerability in altered host genetic background (López et al. 2010).

Genome-wide studies have identified markers and genes associated with drug resistance and increased severity of the disease. The overwhelming phenomenon of the development of drug resistance by the plasmodial strains have led also to a renewed interest in novel enzyme targets encoded by the parasite genome e.g., phosphatidylinositol 4-kinase, dihydroorotate dehydrogenase etc., often targeted with candidate small molecules that are in various stages of development or clinical trial (Amelo 2021). Advances in plasmodial genomics and transcriptomics have also propelled the vaccine research and development efforts for combating malaria. However, the efficacy of WHO recommended Mosquirix or RTS,S/AS01E vaccine, containing the fusion protein including the central NANP tandem repeat and the carboxy terminal regions of the *P. falciparum* circumsporozoite protein (CSP) and a hepatitis B virus antigen, is being monitored closely. The reported 36% efficacy over four years for use in children in moderate-high *P. falciparum* transmission areas of sub-Saharan Africa, appear to be way below expectations (RTS,S Clinical Trials Partnership (2015). This underlines the need to add fresh perspectives for exploring interactions affecting both the parasite survival and host resistance at molecular levels. One such perspective includes decoding glycobiology and enzymopathy for disease management, providing leads to newer therapeutic targets (Smith and Bertozzi 2021).

Capsular polysaccharides (CPS)-based vaccines have been developed during the 1960s against bacterial pathogens like *Neisseria meningitidis, Haemophilus influenzae*, and *Salmonella typhi* to counter widespread antibiotic resistance (Berti et al. 2018). CPS conjugates with proteins have been deployed to increase immunogenicity and thereafter higher valent formulations covering for a broad spectrum of phenotypes. Efforts went on to develop vaccines against pathogen groups like protozoans, fungi and viruses based on carbohydrates and effective carbohydrate vaccines are included by many countries into their immunisation schedules for a variety of infectious diseases e.g., for preventing meningitis or pneumonia (Berti et al. 2021).

The glycobiology of malarial infection and pathology would involve carbohydrate-active enzymes (CAZymes) as some of the intricate processes and mechanisms require a high degree of specificity that involve complex carbohydrates and glycoconjugates as well as the interactions between the glycans and different biomolecules of both the parasites and the humans. *Plasmodium* glycosylphosphatidylinositols (GPIs) cause macrophages to secrete proinflammatory cytokines like tumor necrosis factor (TNF) and interleukin-1 **(**IL-1) and induce polyclonal activation of lymphocytes. GPI-linked surface proteins and purified GPIs can significantly increase triglyceride lipogenesis and glucose oxidation by adipocytes, indicating that *Plasmodium* GPIs are insulin-mimetic and are responsible for hypoglycemia and hypertriglyceridemia observed in malaria pathogenesis (Schofield and Hackett 1993). Schofield et al. (1993) synthesized a non-toxic *Pf* GPI glycan coupled to a carrier protein like keyhole limpet hemocyanin (KLH). They have evaluated the effectiveness of the compound on various clinical parameters of malaria to determine its efficacy as an anti-toxic malaria vaccine. Both purified GPI and synthetic GPI induced TNF-α production in mice. The antibodies of mice treated with KLH-GPI could neutralize the TNF-α produced by macrophages as a response to the introduction of crude *Pf* extracts. In contrast to their sham-immunized or naive counterparts, mice immunized with the KLH-glycan had significantly lower death rates from severe malaria, though the levels of parasitemia were comparable across all groups indicating that the synthetic GPI does not affect parasite replication. Similarly, the immunized mice were protected from *P. berghei*-induced pulmonary edema, acidosis, and cerebral malaria and the effects of GPIs in the infected host from the experiment's findings prompted the development of a synthetic GPI-based anti-toxic malaria vaccine (Schofield et al. 2002). Due to their ability to enhance immune response in mice, GPIs are being used as adjuvants for the Pfs-25 transmission-blocking vaccine (Kapoor et al. 2018). Yet another glycan, α-gal could be used as a vaccine candidate, the gene for which is expressed in *Plasmodium* and human gut microbes. The α-gal antibodies found spontaneously in the human serum have been identified as detecting *Plasmodium* surface antigen conferring protection against infection. Lesser susceptible populations residing in malaria endemic regions presented themselves with higher levels of

α-gal antibodies, though below the threshold levels required for complete protection. This could further be addressed by the development of α-gal vaccines (Yilmaz et al. 2014).

Oligosaccharide structures conjugated to lipids or proteins such as the histo-blood group antigens have been recently identified as disease biomarkers and vaccine targets for their significant role in onco-developmental pathways and disease mechanisms. The role of histo-blood group antigens (ABO) of humans have been established in the susceptibility to malarial disease. The variable expression levels of ABO antigens in normal and pathophysiological states and the regulatory mechanisms involving the microRNAs or transcription factors' expression related to the histo-blood group glycogenes may underlie disease conditions. The recent developments in glycobiology of cancer and other diseases indicates that it could be a viable approach in combating diseases like malaria (Loscertales and Brabin 2006; Dotz and Wuhrer 2016). Host blood antigens A and B comprise trisaccharide structures on the red cell surface contrary to the O blood group that carries the disaccharide H antigen. These trisaccharides act as essential co-receptors for rosetting, facilitating *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) binding to the neighbouring blood cells. The rosettes formed in blood group O are smaller and comparatively weaker, thus developing resistance to severe malaria (Carlson et al. 1994; Barragan et al. 2000). Glycosaminoglycans (GAGs) found in the extracellular matrix are also involved in rosetting, and along with other antigens, in the adhesion of infected red blood cells (RBCs) to the placenta (Rogerson et al. 1997). PfEMP1 is a major rosetting ligand (Chen et al. 1998), which also binds to administered GAGs like heparin (Skidmore et al. 2008). However, GAGs present in the human glycocalyx have protective functions by preventing the parasites from adhering to the endothelial cells. This is because the removal of the GAGs allowed the infected RBCs to interact with adhesion molecules like CD36, intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1), and endothelial protein C receptor (EPCR) that are found in the deeper layers, which in turn causes cytoadherence leading to severe malaria (Introini et al. 2018). Also, the soluble GAG heparin inhibits cytoadherence and disrupts rosettes, making heparin-like molecules potential anti-malarial agents (Rogerson et al. 1994; Vogt et al. 2006). The N-glycosylation patterns of red blood cells play a crucial role in determining the infection severity, as is evident in the case of sickle-cell disease. Despite *Plasmodium* parasites infecting both healthy and sickle-shaped cells equally, higher levels of mannose N-glycans on the latter's surface makes them a more recognizable target for phagocytosis. Interestingly, *Plasmodium* reduces the N-glycosylation of infected RBCs, thereby suppressing the host immune response and increasing disease severity (Egan et al. 2015; Wang et al. 2021).

The HLMs are attractive antimalarials and can be used to combat drug resistance in the parasites and can supplement the benefits of vaccines when used in parallel. Sulfation increases the antimalarial properties, and other modifications to specific sites decrease the anticoagulant activity of heparin (Clark et al. 1997; Leitgeb et al. 2011). It is known that they disrupt the receptor-ligand interactions involved in the binding of merozoites to the RBCs. One of the main targets of HLMs is the merozoite protein MSP1, and the binding of HLMs to MSP1 prevents erythrocyte invasion by the parasites (Boyle et al. 2010). Certain structural characteristics of HLMs provide additional protection against malaria and also inhibit schizont rupture. The drawbacks of HLMs include low potency and short half-lives. They are being further developed and investigated to increase their half-lives and oral bioavailability, and they may eventually take the place of parenteral heparin administration for antimalarial treatment (Boyle et al. 2017; Neves et al. 2016).

The differential glycosylation between the *Plasmodium* and human host has been tested in experiments and the basis of these variations have been identified, that may be useful for therapeutic targeting in *Pf* infection. Key differences in post translational modifications between the *Pf* parasite and the erythrocyte stages being invaded have been identified in the proteomes of both. Since *Plasmodium* lacks glycosylating enzymes, most of the glycosylation pathways are triggered in the erythrocytes during parasite invasion (Samuelson et al. 2005). The parasite invasion of erythrocytes involves extensive post translational modifications in their proteins during events of receptor recognition, adhesion and ligand interactions. Post translational modifications especially N-glycosylation of merozoite surface proteins and several erythrocyte CD antigens, complement receptor and others have been crucial to parasite invasion. Thus, deeper understanding of post translational modification of proteins in the parasite-host interactions should eventually lead to several candidate antimalarials (Wang et al. 2021). The malaria pathogen specific targeting of glycans were demonstrated in synthetic experiments with lipidated GPI and GPI glycans of the *Pf* in mice immunization, achieving a better response for a GPI hexasaccharide against cerebral pathology in murine *P. berghei* model through induction of antibodies in serum (Lepenies and Seeberger 2010).

Thus, the glycoengineering of therapeutic proteins like antibodies or synthetic fusion proteins can add therapeutic efficacy. Tools to exploit glycosylation exist based on efficient glycoCRISPR approaches targeting glycosyltransferase genes and cell based glycome displayed libraries. Genetic glycoengineering can also deliver custom designs of glycosylation useful in recombinant glycoprotein therapeutics for

reprogramming processes (Narimatsu et al. 2021; Zhong et al. 2022). Essentially, the knowledge obtained from studying glycobiology can be exploited for reducing the burden of the disease worldwide.

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