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Adaptation of targeted nanocarriers to changing requirements in antimalarial drug delivery

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Abstract

The adaptation of existing antimalarial nanocarriers to new *Plasmodium* stages, drugs, targeting molecules, or encapsulating structures is a strategy that can provide new nanotechnology-based, cost-efficient therapies against malaria. We have explored the modification of different liposome prototypes that had been developed in our group for the targeted delivery of antimalarial drugs to *Plasmodium*-infected red blood cells (pRBCs). These new models include: (i) immunoliposome-mediated release of new lipid-based antimalarials; (ii) liposomes targeted to pRBCs with covalently linked heparin to reduce anticoagulation risks; (iii) adaptation of heparin to pRBC targeting of chitosan nanoparticles; (iv) use of heparin for the targeting of *Plasmodium* stages in the mosquito vector; and (v) use of the non-anticoagulant glycosaminoglycan chondroitin 4-sulfate as a heparin surrogate for pRBC targeting. The results presented indicate that the tuning of existing nanovessels to new malaria-related targets is a valid low-cost alternative to the *de novo* development of targeted nanosystems.

Key words: Glycosaminoglycans; Malaria; Nanomedicine; *Plasmodium*; Targeted drug delivery

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Antimalarial drugs can potentially target a suite of pathogen life stages inside two different hosts: humans and the insect vectors. Infection starts when a parasitized female *Anopheles* mosquito inoculates sporozoites of the malaria parasite, the protist *Plasmodium* spp., into a person while taking a blood meal. Within a few minutes, sporozoites have migrated through the skin and bloodstream to the liver, where they invade hepatocytes. Sporozoites develop into merozoites,[1] which enter the circulation, invade red blood cells (RBCs),[2] and replicate asexually to produce daughter cells that invade new RBCs to perpetuate the blood-stage cycle unfolding through ring, trophozoite, and schizont stages. Some parasites eventually differentiate into sexual stages, female and male gametocytes that are ingested by a mosquito from peripheral blood. When an infected bloodmeal reaches the insect's midgut, micro- and macrogametocytes develop into male and female gametes. Following fertilization, the zygote differentiates into an ookinete that moves through the midgut epithelium and forms an oocyst, which releases sporozoites. The malaria transmission cycle is restarted when sporozoites migrate to the salivary glands and are injected into a human with the mosquito's next bite.

With malaria elimination now firmly on the global research agenda, but resistance to the currently available drugs on the rise, there is an urgent need to invest in research and development of new therapeutic strategies.[3] Encapsulation of drugs in targeted nanovectors is a rapidly growing area with a clear applicability to infectious disease treatment,[4] and pharmaceutical nanotechnology has been identified as a potentially essential tool in the future fight against malaria.[5,6] Nanoparticle-based targeted delivery approaches can play an important role for the treatment of malaria because they might allow (i) low overall doses that limit the toxicity of the drug for the patient, (ii) administration of sufficiently high local amounts to minimize the evolution of resistant parasite strains,[7] (iii) improvement of the efficacy of currently used hydrophilic (low membrane trespassing capacity) and lipophilic antimalarials (poor aqueous solubility), and (iv) use of orphan drugs never assayed as malaria therapy, *e.g.* because of their elevated and wide-spectrum toxicity. In the very nature of nanovectors resides their versatility that enables assembling several elements to obtain chimeric nanovessels tailored to fit the requirements for different administration routes, particular intracellular targets, or combinations of drugs.

One of the limitations of liposomes as carriers for drug delivery to *Plasmodium*-infected RBCs (pRBCs) is that because of the lack of endocytic processes in these cells, a relatively fluid liposome lipid bilayer is required to favor fusion events with the pRBC plasma membrane. As a result, these liposomes are leaky for small drugs encapsulated in their lumen,[8] and when membrane fusion occurs, only a relatively small fraction of the originally contained drug is delivered into the cell. On the other hand, liposomes made of saturated lipids have less fluid bilayers that retain drugs with high efficacy,[8] although fusion events with pRBC membranes are greatly diminished, which might also reduce the amount of luminal cargo delivered to the target cell. The so-called combination therapies, where several drugs are simultaneously administered,[9] significantly improve the antimalarial effect of the individual compounds. Liposomes are particularly adept structures in this regard because they allow the encapsulation of hydrophobic molecules in their lipid bilayer and of water-soluble compounds in their lumen, thus being a potentially interesting platform for combination therapies where lipophilic and hydrophilic drugs are delivered together.

One of the main pRBC-binding molecules are glycosaminoglycans (GAGs), some of whose members include heparin, heparan sulfate (HS), and chondroitin sulfate (CS). Chondroitin 4-sulfate (CSA) has been found to act as a receptor for pRBC binding in the microvasculature and the placenta,[10] and adhesion of pRBCs to placental CSA has been linked to the severe disease outcome of pregnancy-associated malaria.[11] pRBC adhesion to the endothelium of postcapillary venules is mediated by the parasite-derived antigen *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1),[12] whereas CSA has been identified as the main receptor for PfEMP1 attachment to placental cells.[10,13] Single-molecule force spectroscopy data have revealed a complete specificity of adhesion of heparin to pRBCs *vs.* RBCs, with a binding strength matching that of antibody–antigen interactions.[14] Heparin had been used in the treatment of severe malaria,[15] but it was abandoned because of its strong anticoagulant action, with side effects such as intracranial bleeding. It has been shown that heparin electrostatically bound to liposomes acts as an antibody surrogate, having a dual activity as a pRBC targeting molecule but also as an antimalarial drug in itself acting mainly on trophozoite and schizont stages.[16] Because heparin is significantly less expensive to obtain than specific (monoclonal) antibodies, the resulting heparin-liposomes have a cost about ten times lower than that of equally performing immunoliposomes. A question that remains open is whether the heparin-mediated targeting of liposomes to pRBCs could be extended to other glycosaminoglycans, to different *Plasmodium* stages, and to new nanoparticle types.

Through modification of its component elements, the nanovector design is susceptible to improvement and adaptation to new targets such as different *Plasmodium* species or infected cells other than the erythrocyte. Of particular interest here is the targeting of the transmission stages that allow transfer of the parasite between human and mosquito and *vice-versa*, which represent the weakest spots in the life cycle of the pathogen.[17] Heparin and HS are targets for the circumsporozoite protein in sporozoite attachment to hepatocytes during the primary stage of malaria infection in the liver.[18] CS proteoglycans in the mosquito midgut and synthetic CS mimetics have been described to bind *Plasmodium* ookinetes as an essential step of host epithelial cell invasion,[19,20] whereas ookinete-secreted proteins have significant binding to heparin.[21] This body of accumulated evidence suggests that GAGs might be adequate to target antimalarial-loaded nanovectors to *Plasmodium* mosquito stages, either through a direct entry into ookinetes and sporozoites, or indirectly through delivery to pRBCs for those pRBCs that will eventually differentiate into gametocytes.

Here we have explored whether the heparin- and antibody-mediated targeting of drug-containing liposomes to pRBCs could be adapted in a straightforward way to other GAGs as targeting molecules, to different *Plasmodium* stages as target cells, and to new nanoparticle and drug types.