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Immunoliposome-mediated drug delivery to *Plasmodium*-infected and non-infected red blood cells as a dual therapeutic/prophylactic antimalarial strategy

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Abstract
One of the most important factors behind resistance evolution in malaria is the failure to deliver sufficiently high amounts of drugs to early stages of *Plasmodium*-infected red blood cells (pRBCs). Despite having been considered for decades as a promising approach, the delivery of antimalarials encapsulated in immunoliposomes targeted to pRBCs has not progressed towards clinical applications, whereas *in vitro* assays rarely reach drug efficacy improvements above 10-fold. Here we show that encapsulation efficiencies reaching >96% are achieved for the weak basic drugs chloroquine (CQ) and primaquine using the pH gradient loading method in liposomes containing neutral saturated phospholipids. Targeting antibodies are best conjugated through their primary amino groups, adjusting chemical crosslinker concentration to retain significant antigen recognition. Antigens from non-parasitized RBCs have also been considered as targets for the delivery to the cell of drugs not affecting the erythrocytic metabolism. Using this strategy, we have achieved unprecedented complete nanocarrier targeting to early intraerythrocytic stages of the malaria parasite for which there is a lack of specific extracellular molecular tags. Immunoliposomes studded with monoclonal antibodies raised against the erythrocyte surface protein glycophorin A were capable of targeting 100% RBCs and pRBCs at the low concentration of 0.5 μM total lipid in the culture, with >95% of added liposomes retained on cell surfaces. When exposed for only 15 min to *Plasmodium falciparum in vitro* cultures of early stages, free CQ had no significant effect on the viability of the parasite up to 200 nM, whereas immunoliposomal 50 nM CQ completely arrested its growth. *In vivo* assays in mice showed that immunoliposomes cleared the pathogen below detectable levels at a CQ dose of 0.5 mg/kg, whereas free CQ administered at 1.75 mg/kg was, at most, 40-fold less efficient. Our data suggest that this significant improvement is in part due to a prophylactic effect of CQ found by the pathogen in its host cell right at the very moment of invasion.

Keywords: immunoliposomes; malaria; nanomedicine; *Plasmodium*; targeted drug delivery.

Chemical compounds studied in this article: Chloroquine (PubChem CID: 2719); Primaquine (PubChem CID: 4908); DSPC (PubChem CID: 94190); DOPC (PubChem CID: 6437081); Cholesterol (PubChem CID: 5997); Maleimide (PubChem CID: 10935); SATA (PubChem CID: 127532); Pyranine (PubChem CID: 61389); Hoechst 33342 (PubChem CID: 1464).