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

Ernest Moles [a,b,c], Patricia Urbán [a,b,c], María Belén Jiménez-Díaz [d], Sara Viera-Morilla [d], Iñigo Angulo-Barturen [d], Maria Antònia Busquets [c,e], Xavier Fernàndez-Busquets [a,b,c]

[a] Nanomalaria Group, Institute for Bioengineering of Catalonia (IBEC), Baldiri Reixac 10-12, ES-08028 Barcelona, Spain
[b] Barcelona Institute for Global Health (ISGlobal, Hospital Clínic-Universitat de Barcelona), Rosselló 149-153, ES-08036 Barcelona, Spain
[c] Nanoscience and Nanotechnology Institute (IN2UB), University of Barcelona, Martí i Franquès 1, ES-08028 Barcelona, Spain
[d] Tres Cantos Medicines Development Campus, GlaxoSmithKline, Severo Ochoa 2, ES-28760 Tres Cantos, Spain
[e] Departament de Fisicoquímica, Facultat de Farmàcia, University of Barcelona, Av. Joan XXIII, s/n, ES-08028 Barcelona, Spain

emoles@ibecbarcelona.eu

Abstract

Bearing in mind the absence of an effective vaccine against malaria and its severe clinical manifestations causing nearly half a million deaths every year, this disease represents nowadays a major threat to life. Besides, the basic rationale followed by currently marketed antimalarial approaches is based on the administration of drugs on their own, promoting the emergence of drug-resistant parasites owing to the limitation in delivering drug payloads into the parasitized erythrocyte high enough to kill the intracellular pathogen while minimizing the risk of causing toxic side effects to the patient. Such dichotomy has been successfully addressed through the specific delivery of immunoliposome (iLP)-encapsulated antimalarials to *Plasmodium falciparum*-infected red blood cells (pRBCs). Unfortunately, this strategy has not progressed towards clinical applications, whereas *in vitro* assays rarely reach drug efficacy improvements above 10-fold [1].

Here we show that encapsulation efficiencies reaching >96% can be achieved for the weakly basic drugs chloroquine (CQ) and primaquine using the pH gradient active loading method [2,3] in liposomes composed of neutral charged, 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 intracellular delivery of drugs not affecting the erythrocytic metabolism [4]. Using this strategy, we have obtained unprecedented nanocarrier targeting to early intraerythrocytic stages of the malaria parasite for which there is a lack of specific extracellular molecular tags. Polyethylene glycol-coated liposomes conjugated with monoclonal antibodies specific for the erythrocyte surface protein glycophorin A (anti-GPA iLP) were capable of targeting 100% RBCs and pRBCs at the low concentration of 0.5 μM total lipid in the culture (Figure 1), with >95% of added iLPs retained into the cells (Figure 2). When exposed for only 15 min to *P. falciparum in vitro* cultures synchronized at early stages, free CQ had no significant effect over parasite viability up to 200 nM drug, whereas iLP-encapsulated 50 nM CQ completely arrested its growth. Furthermore, when assayed *in vivo* in *P. falciparum*-infected humanized mice, anti-GPA iLPs cleared the pathogen below detectable levels at a CQ dose of 0.5 mg/kg (Figure 3). In comparison, free CQ administered at 1.75 mg/kg was, at most, 40-fold less efficient. Our data suggest that this significant improvement in drug antimalarial efficacy 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.

References

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Figures

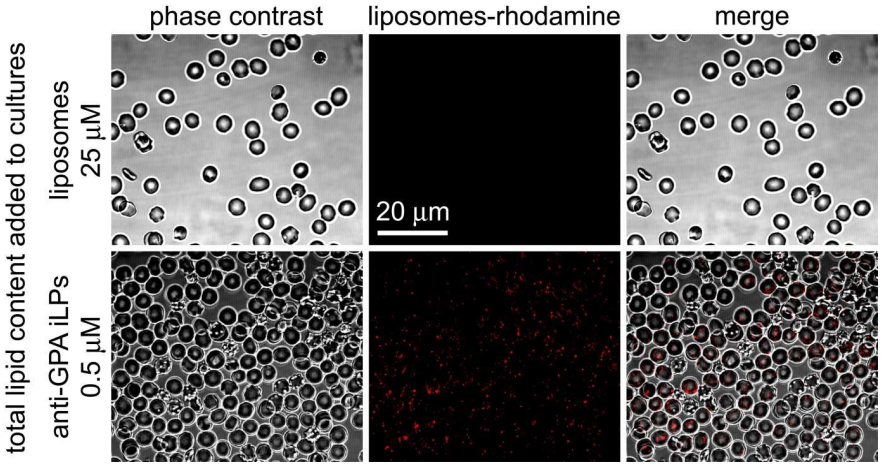

Fig. 1. Confocal fluorescence microscopy assay of live *P. falciparum* cultures showing the fraction of cells targeted by small amounts of monoclonal anti-GPA iLPs (LP-PEG-Mal-NH_2-MAb model). Liposomes contained 0.5% of the rhodamine-labeled lipid DOPE-Rho in their formulation, and the samples were incubated for 30 min under orbital stirring before microscopic examination.

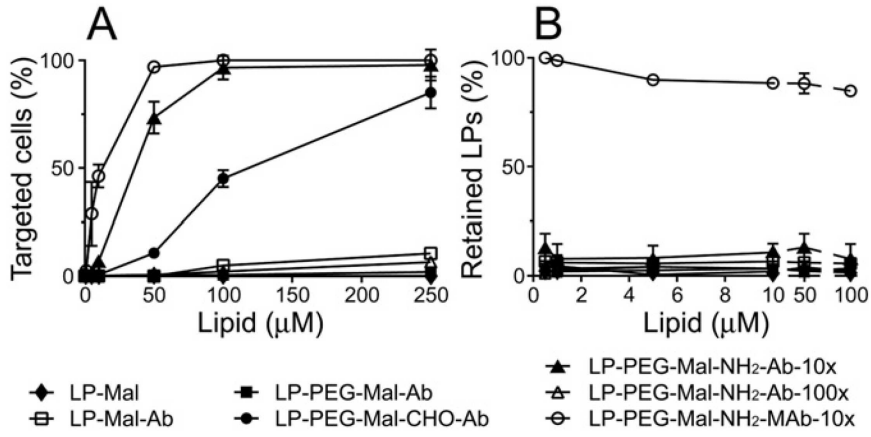

Fig. 2. RBC targeting analysis after 30 min incubation with anti-GPA iLPs loaded with 30 mM pyranine and prepared through different antibody conjugation methods. (A) Flow cytometry results showing the fraction of RBCs positive for pyranine signal. (B) Determination by pyranine fluorescence quantification in the culture supernatant of the iLP fraction bound to cells. All samples were prepared with a polyclonal antibody except LP-PEG-Mal-NH_2-MAb-10× (primary amines conjugation, 10× crosslinker/antibody amount), where a monoclonal antibody was used.

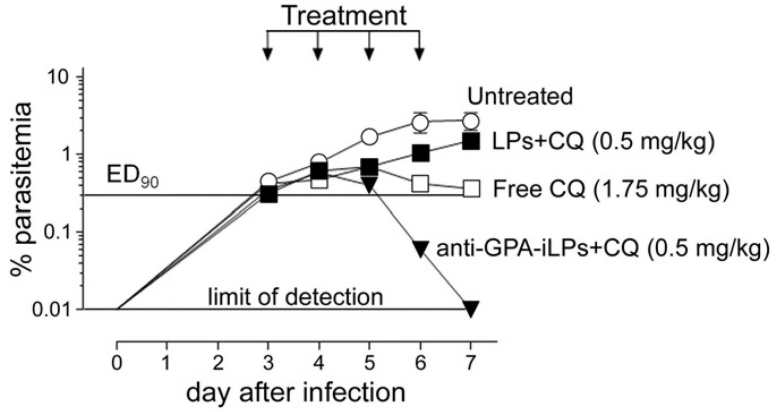

Fig. 3. 4-day test in female immunodeficient mice engrafted with human RBCs (humanized mice) and infected i.v. with *P. falciparum*. The animals were treated with the indicated drug preparations at days 3 to 6 after infection. The anti-GPA-iLP+CQ sample contained 48 mmol CQ/mol lipid, whose administered dose corresponded to ca. 100 iLPs/erythrocyte, assuming 1×10^{10} human RBCs in the mouse blood circulation.