Future MICROBIOLOGY

EDITORIAL

Antimalarial drug delivery to the mosquito: an option worth exploring?

"The history of malaria control shows us that defeating *Plasmodium* will not be an easy task, and we need to consider all options in our fight against the disease."

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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 the research and development of new therapeutic strategies [1]. Drugs can potentially target a suite of pathogen life stages inside two different hosts: humans and the insect vector. 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 [2], which enter the circulation, invade red blood cells (RBCs) [3], and replicate asexually to produce daughter cells that invade new RBCs to perpetuate the blood-stage cycle. Some parasites eventually differentiate into sexual stages, female or male gametocytes that are ingested by a mosquito from peripheral blood. When an infected blood meal reaches the insect's midgut, micro- and macro-gametocytes 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.

Current chemotherapeutic approaches against malaria infections are targeted at the asexual blood-stage parasites that are responsible for all symptoms and pathologies of the disease [4]. However, as there can be several hundred billion *Plasmodium*-infected RBCs (pRBCs) in the bloodstream of a malarious patient, multiple-dose administrations of antimalarials are required to clear infections. Continuous exposure of parasites to drugs increases the likelihood that resistance will evolve, which will rapidly decrease treatment efficacy. The threat of resistance-driven treatment failure is prompting research oriented toward developing drugs that target the weakest spots in the parasite life cycle represented by smaller populations, which are less likely to

KEYWORDS
• *Anopheles* • malaria • mosquito
• nanomedicine • *Plasmodium*
• targeted drug delivery
• transmission stages

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contain resistant individuals that would benefit from the removal of susceptible parasites [5]. The two main bottlenecks in the malaria transmission cycle are found where the parasite is in one of the stages that move between hosts [6,7]. A few thousand sporozoites can be packed inside the mosquito salivary glands, but only approximately 100 will be transferred to the human when *Anopheles* bites; this is several orders of magnitude fewer parasites than are found in an active blood-stage infection. However, the short time that free sporozoites remain in the circulation is a serious obstacle to targeting them before they reach the liver, and, given the low *in vitro* invasion rates of hepatocytes by sporozoites – rarely above 4% and often below 1% [8] – targeting the liver stage will be difficult. A second bottleneck occurs during sexual development, when approximately 0.2–1% of the intraerythrocytic parasites may develop into gametocytes per round of schizogony. Although this still leaves an estimated 10^8–10^9 parasites to be cleared from the blood circulation, targeting gametocytes can ease exposure of the pathogen to drugs and reduce the likelihood of resistance emerging [7].

A largely unexplored avenue in antimalarial drug development is targeting *Plasmodium* stages in the vector itself. While the innate immune system of mosquitoes is capable of completely clearing a malaria infection [9], it is far from the sophisticated arsenal providing long-term protection in mammalian adaptive immunity. This might result in parasite stages with reduced defenses because they only need to survive for a few weeks inside the insect, facing an immune surveillance not as demanding as in the human host. Drugs targeting early *Anopheles* stages must kill only approximately 5×10^3 parasites to free a mosquito from *Plasmodium* infection [10], and the absolute low corresponds to oocysts, of which there are only two to five in a single insect [7], being around for over a week. However, the advantage of few cells to be reached per individual is counterbalanced by a large population. Although at first sight delivering drugs to mosquitoes seems impractical as a potential method of control in the field, we believe that it can provide important advantages if one can avoid the obvious drawbacks. Strategies that control malaria using direct action against *Anopheles* are not new, but most of them aim at eliminating the vector, either by killing it with pesticides [11] or through the release of sterile males [12,13]. Since eradicating an insect species might have a consequence of unpredictable disruptions of ecosystems with potential undesirable side effects, mosquito-friendly antimalarial strategies should be favored whenever possible. Such an ecologically minded and fascinating alternative is the recent proposal of using *Wolbachia*, a maternally transmitted symbiotic bacterium of arthropods, to induce in *Anopheles* refractoriness to *Plasmodium* infection [14]. Thus, administration of drugs to mosquitoes to free them of malaria with the objective of blocking transmission of the disease might not be so far-fetched. Two approaches seem feasible:

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First, it is known that natural plant sugars are an essential pool of energy for female mosquitoes [15]. This requirement could be exploited to develop a sugar meal trap that attracts and drugs them while they feed, although this would only be effective as a control measure if the insects rely on other nutrient origins in between blood meals. The pathophysiology of the malaria parasite might facilitate attraction of *Plasmodium*-infected mosquitoes to alternative feeding places. To keep blood fluid and prevent quick coagulation, *Anopheles* synthesizes an antihemostatic armamentarium containing, among others, the enzyme apyrase. *Plasmodium* inhibits apyrase [16], and in this way entices its carrier to bite more because blood coagulates faster and *Anopheles* has to probe longer to get its full dinner, thereby increasing potentially infective host contacts. It can be expected, then, that infected insects will have a larger probability of searching for nonhuman sources than those that are not parasitized. A trap emitting human volatiles [17] and consisting of a blood-like substance could contain highly concentrated drugs against *Plasmodium* gametes, ookinetes, oocysts or sporozoites without fear of overdoses or toxic side effects for people. These 'fake human' traps, hung on the walls of dwellings in malaria endemic areas, could likely be made at an affordable cost. Such a strategy, because it is not designed for administering antimalarials to humans, will bypass clinical trials that often delay the development and deployment of new medicines for years. An additional advantage of direct delivery from a fixed-volume container is that the drug does not become diluted with time.

Second, as gametogenesis, fertilization and zygote differentiation into an ookinete occur in the insect's midgut, in an environment essentially consisting of human blood, any compound affecting these processes can be delivered through the blood meal on which the mosquito feeds [11].

Thus, we can consider administering to humans antimalarials with a blood half-life sufficiently long to increase their probability of being picked up by *Anopheles*. Preliminary estimations suggest that the drugs need to remain in the bloodstream of people living in endemic areas for 3–4 weeks. Additional challenges being confronted are patient compliance (we are delivering a medicine that has no direct visible effect), and developing a compound that escapes spleen and liver clearance and kidney filtration for a long time span, finally reaching inside the vector at therapeutic concentration. With the current efforts to identify new antimalarials [18] we can reasonably expect the discovery in the near future of new drugs acting against *Plasmodium* mosquito stages. Some of these new chemicals might have a good gametocytocidal activity that however should not be unleashed before being ingested by *Anopheles*, lest their activity be wasted with just a small fraction of gametocytes while still in the human circulatory system. Recent exciting developments in optopharmacology can provide the necessary tools to limit the gametocytocidal activity of drugs in the human body. Light-regulated photoswitches inserted into drug molecules [19] could be activated by movement from the relatively dark human bloodstream into the virtually transparent mosquito; the resulting conformation change would expose the handful of gametocytes inside the insect to an active antimalarial.

This last example hints at the vast potential of new disciplines such as nanomedicine to slip past the formidable defenses of *Plasmodium* parasites. Nanotechnology can also provide useful therapeutic tools against malaria in the form of nanovectors for the delivery of encapsulated drugs against specific parasite stages [20], including those present exclusively in *Anopheles*. Containers of nanometric dimensions can be designed and engineered with components that impart long residence times in biological fluids, have large capacity for cargo transport, and are studded with targeting molecules binding receptors exclusive to the selected target cells, for an efficient and fast elimination of the pathogen in its hiding places. The history of malaria control shows us that defeating *Plasmodium* will not be an easy task, and we need to consider all options in our fight against the disease. The approaches described above can only be developed and tested with a truly multidisciplinary team. An old African proverb says, "*If you want to go fast, go alone; if you want to go far, go together.*" We urge physicians, entomologists, environmentalists, biochemists, evolutionary biologists, nanotechnologists and experts from other disciplines to work together toward the goal of erasing malaria once and for all.

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Acknowledgements

The authors would like to thank Katey Glunt for editing their manuscript.

Financial & competing interests disclosure

This work was supported by grants BIO2011-25039 from the Ministerio de Economía y Competitividad, Spain, which included FEDER funds, and 2009SGR-760 from the Generalitat de Catalunya, Spain. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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