Journal of Infectious Diseases and Therapy
ISSN: 2332-0877

Dennis et al., J Infect Dis Ther 2015, 3:4
DOI: 10.4172/2332-0877.1000229

Review Open Access

Utilizing Nanotechnology to Combat Malaria

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Received date: July 21, 2015; **Accepted date:** August 21, 2015; **Published date:** August 25, 2015

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Abstract

Despite the many advances that have occurred recently in the treatment of infectious diseases the morbidity and mortality associated with malaria remain major burdens. Children in tropical and subtropical climates are the most affected by this disease, especially those in Africa. Nanotechnology has taken the route of improving accepted formulations of therapeutics by increasing the active range of the treatments as well as decreasing the negative side effects. In this review we examine the efforts and advancements that have been made in the treatment of malaria that are based on nanomaterials.

Keywords: Malaria; Nanoparticle; Anti-malarial; *Plasmodium*; *Anopheles*

Introduction

Malaria is a potentially fatal disease caused by *Plasmodium* parasites. In humans disease is due to *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, or *P. knowlesi*. Infection is spread through the bite of the female *Anopheles* mosquito. While person-to-person transmission is not described for malaria the life cycle of the parasite in the blood allows the pathogen to be transmitted from mother to child, and through blood transfusions and organ transplants.

Malaria thrives in tropical and subtropical regions. The World Health Organization estimates that in 2013 there were almost 200 million cases malaria and more than 500,000 deaths [1]. Most of these occurred among African children. P. falciparum is the major causative agent in this region. The high incidence of infection is due, in part, to the lack of effective control of the *Anopheles* vector. When a mosquito bites a human infected with malaria, the human blood has thousands of Plasmodium gametocytes that travel to the midgut of the insect and produce hundreds of zygotes. About 50 to 100 of these develop into ookinetes, which then migrate into the hemolymph or blood and give rise to oocysts. Each oocyst releases about 50,000 sporozoites that migrate to the mosquito's salivary gland. When the mosquito bites another human, about 15 to 18 sporozoites enter the bloodstream and the cycle begins anew [2]. Vector control techniques including insecticide-treated mosquito nets (ITNs) and indoor spraying with residual insecticides (IRS) have been used as preventative measurements to help control outbreaks of malaria, especially in countries with a high rate of malaria contraction such as Africa. Indoor spraying is most efficacious when at least 80% of the houses in an area are treated. The downside of indoor spraying is that the treatment must be repeated several times a year. Genetically engineered mosquitoes that are resistant to colonization by the malaria parasite have been produced using transposons and P elements approaches [3].

Additionally, contributing to the high incidence of infection, malaria parasites are becoming resistant to the current proposed drugs (chloroquine, pyrimethamine, aretesunate, sulfadoxine, etc.), therefore the development of a new and effective antimalarial agent is needed. The World Health Organization has recommended a combination of anti-malarials and aretesunate to treat uncomplicated malaria cases. An endemic is possible if malaria builds resistance to these drug combinations [4-6].

Currently, the pharmaceuticals that are utilized to treat malaria have low bioavailability and adverse side effects. This adds to the need for new prophylaxis and treatments. Treatment for malaria can be complicated one several fronts [4].

- *Plasmodium* parasites are gaining resistance to anti-malarials which increases the need for combination therapy.
- Current malaria drugs are impaired for delivery due to intrinsic characteristics including solubility, route and complexity of administration.
- Patient non-compliance and limited access to treatment further reduce the efficiency of current therapeutic strategies. These factors and many others highlight the need for changes and modifications in treatment that ease the burden that sometimes accompanies malaria treatment.

One possible solution to the problems associated with anti-malarial pharmaceutical is the use of nanomaterial. The use of colloidal drug carriers (liposomes and micro/nanoparticles) provide versatility in site specific or targeted drug delivery along with controlled optimal drug release [7,8]. Nanoparticles have added advantages over microparticles such as bioavailability, the ability to improve drug encapsulation, pharmacokinetics, and therapeutic therapy [9].

Nanoparticles are particulate dispersions or solid colloidal structures ranging from 1 – 1000 nm in diameter [10]. They are composed of synthetic, semi-synthetic and natural polymers in which the active therapeutic molecule has the capability of being entrapped, encapsulated, dissolved, absorbed, or chemically attached [8,11]. Due to their biodegradability, biocompatibility, and versatility in

application, natural hydrophilic polymers have also been extensively investigated [7,12]. Natural polymers are classified as proteins (gelatin, albumin, lectin, legumin, and vicillin) and polysaccharides (alginate, dextran, pullulan, and chitosan).

This review discusses advances in malaria treatment that utilize nanomaterials to prevent and improve the therapeutic outcome of Plasmodium infection.

Nanosuspensions

An estimated 40% of new drugs are water insoluble [4] and consequently, have a short biological half-life, poor bioavailability, and undesirable side effects [5]. Techniques such as the addition of co-solvents, pH adjustment, micellar dispersions, and microionization have been employed to increase the aqueous solubility of poorly water-soluble drugs [4], but these techniques are limited in their efficacy, in part, because large amounts of excipients are required which can cause safety problems [6]. Therefore, there is a need for new methods to improve the efficacy of hydrophobic drugs.

Nanosuspensions are biphasic systems composed of submicron-sized drug particles dispersed in an aqueous solution. There are two methods for formulating nanosuspensions which are "bottom-up" and "top-down" technology [8]. In the bottom-up technology, nanoparticles are formed through precipitation, chemical synthesis, and emulsion methods. Top-down technology involves "nanosizing" or reducing larger sized particles by high pressure homogenization and milling techniques [9,10]. The advantages of utilizing nanosuspensions are the reduction in size which increases the surface area and lowers the toxicity of the drug [11]. Currently, the drugs that are utilized to treat malaria have low bioavailability and adverse side effects. Therefore, there is a need for new drug delivery systems. Several recent studies have demonstrated that nanosuspensions of antimalarial drugs increase the efficiency and bioavailability of antimalarial therapy [12-14].

Lumefantrine is an anti-malaria drug that is used to treat multidrug resistant malaria and cerebral malaria [15]. Lumefantrine kills the P. falciparum parasite by inhibiting it's ability to convert heme to non-toxic hemozoin. Increased toxic levels of heme result in death of the parasite and stop the infection [16,17]. Because of lumefantrine's low aqueous solubility, it has impaired oral bioavailability [18]. When taking lumefantrine, dietary fat intake is suggested to improve bioavailability [19, 20], however, this can pose a problem for people who have decreased appetite because of their illness. Therefore, reducing the size of lumifantrine can possibly improve its bioavailability. Research conducted by Gahoi et al., showed that reducing the size of lumefantrine improved it's antimalarial activity in vivo and in vitro against *P. Yoelii nigeriensis* and P. falciparum respectively. Lumefantrine was reduced from 72 μm of coarse powder to 0.251μm nanosuspension by the DYNO MILL milling technique. This technique is one of the most efficient wet-milling techniques to generate nano-sized drug crystals with high dissolution rates and oral bioavailability [18]. In this study, the half maximal inhibitory concentration (IC50) value for the nanosuspension was 175 times lower than the coarse powder [18].

Dihydroartemisinin (DHA) is an antimalarial drug used for the treatment of uncomplicated malaria [21,22]. DHA is a derivative of artemisinin and has poor water solubility as well [23, 24]. In a study by Chingupitak et al, dihydroartemisinin nanoparticles were formed by using binary and ternary mixtures. The binary mixtures were formed by using polyvinyl pyrrolidone (PVP)/DHA and sodium deoxycholate (NaDC)/DHA. The ternary mixtures were formed by using DHA/PVP/NaDC. The results showed that DHA nanosuspensions had better in vitro antimalarial activity against P. falciparum than the microsuspensions, and that nanosuspensions prepared by ternary mixtures had increased stability compared to the binary mixtures.

The results from the experiments conducted by Chingupitak et al and Gahoi et al show that nanosuspensions have increased antimalarial activity against Plasmodium [18, 25]. Decreased concentrations of the drugs can also decrease the adverse side effects. Due to the increased activity of the nanoformulations at decreased concentrations, they have potential as a drug delivery system for combating the decreased bioavailability of current malaria drugs [27].

Chitosan

Chitosan is the second abundant polysaccharide and is one of the most extensively studied hydrophilic polymer [12]. Chitosan nanoparticles offer many advantages due to their stability, low toxicity, simple and mild preparation methods, provide versatility in of administration, excellent drug deliverer, and provide optimal, controllable drug release [7, 13]. Criteria for ideal polymeric carriers for nanoparticles and nanoparticle delivery systems are as follows: Polymeric carriers-easy to synthesize and characterize, inexpensive, biocompatible, biodegradable, non-immunogenic, non-toxic, water soluble; nanoparticle delivery system-simple and inexpensive to manufacture, no heat, high shear forces or organic solvents involved in preparation methods, reproducible and stable, applicable to a broad category of drugs, small molecules, proteins, and polynucleotides, ability to lyophilize, stable after administration, non- toxic [14, 15]. Chitosan has gained interest in malarial treatment due to its biodegradability, biocompatibility, reduced toxicity, reduction in dose and dose frequency and carriers for controlled site-specific drug delivery [16, 17]. Chitosan bares the ability to conjugate with the age-old drug chloroquine and avoid affecting the liver and reducing mitochondrial dehydrogenase activity [19]. Pathology of malaria depends on the species of Plasmodium and strain of parasite. This determines how the host will respond and indicates the antigen/nature of the parasite [6].

Primaquine (PQ) is a widely used antimalarial drug and is the only antimalarial drug available to combat the relapsing form of malaria, especially from infection by Plasmodium sp. parasites P. vivax and P. ovale [18]. PQ is able to kill Plasmodium parasites that have infected organs. Among the negative effects of PQ are the side effects including nausea, vomiting, upset stomach, abdominal cramps and hemolytic anemia. The drug has side effects, especially dose- limiting side effects, partly due to nonspecific targeting and its short half-life [19-21]. PQ oral bioavailability is also limited. Reformulating PQ into chitosan is a promising strategy to overcome these limitations. Omwoyo et al prepared PQ-loaded solid lipid nanoparticles using a modified double-emulsion solvent evaporation technique [19]. The loaded particles showed superior efficacy in parasite reduction and an extended survival time when compared to conventional PQ. The authors did not directly address potential alterations in dosing that would be expected to decrease the toxicity associated with PQ.

Liposomes

Liposomes are synthetic structures up to several hundred nanometers in diameter containing one or several phospholipid

bilayers enclosing an aqueous core [22]. The concept of using liposomes as vesicles for drug delivery was introduced in the 1970s, and more recently the use of liposomes has been extended to immunological adjuvants and as delivery vehicles for vaccines to specific target cells [23]. Both hydrophilic and lipophilic particles can be incorporated into liposomes and delivered to target sites within a host. Hydrophilic particles including proteins, peptides, and nucleic acids can be entrapped within the inner aqueous phase while lipophilic drugs including lipopeptides and adjuvants can be incorporated onto the outer phospholipid layer. Liposomes are immunologically advantageous due to their targeting and uptake by professional antigen presenting cells, and additionally antigens, antibodies, and adjuvants can be attached to the outer surface of liposomes to facilitate delivery into infected cells [23]. Optimal combinations of antigens, antibodies and adjuvants give liposomes plasticity and allow the opportunity for optimization of different drug regimens.

Liposomes have shown significant promise as nano-carriers for the prophylaxis and treatment of malaria, as well as for vaccine delivery for malaria prevention [24]. Currently, effective therapy for malaria is limited due to toxic drug side effects and the development of resistance to current drug regimens. Encapsulation of therapeutic agents within liposomes can favorably alter the dose and distribution of drugs within the body, which may significantly reduce unwanted toxic side effects, reduce the risk of drug resistance, and increase treatment efficacy [25].

Current malarial vaccine strategies suffer from the development of resistance to recombinant antigens and the need for frequent re-boosting. The use of live-attenuated parasites is limited mainly because high doses of *Plasmodium* are needed and because a clinically appropriate route for inoculation has not been found [26]. Liposomes are advantageous over other vaccine delivery systems because the carrier vesicle protects it's contents from degradationwithin the host are non-toxic, biocompatible and selective. Novel gel core liposomes which use a combination of polymer and lipid based delivery systems have been recently developed and tested for the controlled delivery of malarial antigen Pfs25 combined with CpGODN, a potent immunostimulatory vaccine adjuvant [27]. Gel core liposomes increase liposome stability by incorporation of a polymer into the internal aqueous phase of the liposome that allows for slower drug delivery. The rate of release is controlled by slow diffusion through the polymer gel and through the phospholipid bilayers which enables manipulations of drug concentrations within liposome vesicles to enable the ability of long-term antigen persistence which would decrease the need for boosting. Additionally, novel RTS,S-based vaccine formulations that utilize a liposome based adjutant are currently undergoing clinical trials [26]. The RTS.S/AS01B vaccine induces high antibody responses and at the same time improves T cell responses to the circumsporozoite protein (CSP) in mice and in non-human primates [28].

Antimalarial drugs show different degrees of toxicity, which limits their use. Current therapeutic administration strategies release free drugs into the blood and offer little specificity regarding infected cells. Early studies have shown, liposomalization of the antimalarial agent chloroquine increases its maximal tolerable dose and its efficacy against murine malarial infections greater than just chloroquine alone [29-31]. Moreover, the ability to increase the doses of chloroquine per injection after liposome encapsulation allowed successful treatment of infections with chloroquine-resistant *P. berghei* which could not be cured by a 7-day course with the maximum tolerable dose of free chloroquine [31]. More recently, antibody coated liposomes loaded with antimalarial drugs chloroquine and primaquine completely arrested human-infecting parasite, *P. falciparum* growth in vitro and cleared infections [32]. The success in this study was attributed to dual therapeutic and prophylactic effect achieved with the use of liposome vesicles targets to both infected and non-infected erythrocytes. Resistance to current antimalarial therapy is attributed to large genetic diversity of *Plasmodium* strains, specific mutations in the P. *falciparum* chloroquine transporter gene and in the *P. falciparum* multi-drug resistance gene [33]. Liposomes circumvent drug resistant malaria because they are targeted for intracellular delivery which bypasses chloroquine transporters and pass through cell membranes by alternative mechanisms such as membrane fusion or entrapment of chloroquine in pH-sensitive liposomes [34].

Directing liposomes to parasite-infected erythrocytes is another strategy that would allow for selective drug distribution and allow for exposure of lethal doses directly to the pathogen. Ligands conjugated to the surface of liposomes can be used to target and specifically bind *Plasmodium*-infected cells. Because the blood-stage of *Plasmodium* infection is responsible for all symptoms and pathologies of malaria, *Plasmodium*-infected erythrocytes are the main antimalarial therapeutic target. The targeting of liposomes to erythrocytes using heparin and monoclonal antibodies to erythrocyte surface proteins have been studied in vitro and have shown promise towards targeted drug delivery. Marques et al. 2014 encapsulated primaquine in heparin-coated liposomes, this formulation was demonstrated to have antimalarial activity and specific binding affinity for *Plasmodium*-infected erythrocytes in vitro via heparin targeting of heparin-binding proteins in erythrocyte membranes.

Antibody-mediated erythrocytes targeting using liposomes is another promising strategy for targeted drug release. Recently, drugs carried by liposomes were shown to be specifically targeted in vitro to *P. falciparum* infected erythrocytes relative to non-infected erythrocytes likely by docking to infected cell surfaces to facilitate membrane fusion [35]. This demonstrates the feasibility of constructing a carrier able to completely discriminate infected from non infected erythrocytes. The fast, specific targeting of antibody-labeled liposomes towards *Plasmodium*-infected cells can facilitate adjusting the amount of encapsulated drugs to a low overall concentration that however guarantees a localized delivery of highly toxic doses only to infected cells. This, in turn, opens perspectives for the use in antimalarial therapy of already existing drugs that are not being tested because of their high toxicity and/or elevated specificity.

Recently, liposomes have also been targeted towards hepatocytes to determine their ability to combat liver parasites in a murine model of *P. berghei* infection [36]. In this study, the targeting of liposomes to the liver was achieved by expressing a 19 amino acid sequence of a protein expressed by the *P. berghei* circumsporozoite which was chemically bound to the surface of PEGylated liposomes. Peptide-targeted liposomes were 100 times more selective to hepatocytes than to cells of other organs which present a great approach for targeting antimalarial drugs to the liver. Targeting antimalarial drugs to *Plasmodium* infected erythrocytes and hepatocytes using liposomes reduces toxicity, improves therapeutic efficiency, and prolongs drug release compared to conventional approaches.

Polymeric nanoparticles

Polymeric nanoparticles (PNPs) are biocompatible solid colloidal particles, ranging from 1 to 1000nm in size [37, 38]. Biodegradable synthetic polymers such as poly (D, L,-lactic-co- glycolic acid)

(PLGA), poly (D, L-lactic acid) (PLA), poly (Ɛ-caprolactone) (PCL), and polyalkylcyanoacrylates (PACA) are commonly used to generate PNPs [39]. Co-polymers di- block, tri-block, multi-block, or radial block with poly (ethylene glycol) (PEG) are used during preparation and to encapsulate therapeutic compounds [37, 40, 41]. Nevertheless, macromolecules like albumin, chitosan, and gelatin are used during the synthesis of PNPs as well.

Chloroquine is the treatment of choice for all five malaria species, except for P. vivax cases acquired in Papua New Guinea or Indonesia, where 10% or more of the patients are resistant [42-44]. Its mode of action is to disable the intraerythrocytic stage of infection and releasing into the parasite's acidic digestive vacuole (DV) [6]. Unfortunately, chloroquine becomes less stable (deprotonated) once inside the DV, resulting in high inactive accumulations. Pharmacokinetics differ between mice and humans, henceforth drug sensitivity remains synonymous for all compatible life cycles of mammalian *Plasmodium* species [45].

Nanomedicine is very promising, offering precise delivery systems and posing the ability to restore chloroquine by reducing its toxicity, improving bioavailability, and distribution [24, 44, 46]. The effects of biopolyaminosaccharide chitosan nanoparticles were examined to verify the antimalarial effect to the liver and spleen in vivo against *P. berghei* NK65b. Tripathy et.al. prepared nanochloroquine (Nch) particles by inotropic gelation ranging from 150-300nm [45]. Treatment with Nch at 250 mg/kg-bw for 15 days restored cellular function in damage tissues and eliminated parasites [44]. Nch reduced liver damage by ~ 29% and in the spleen by ~ 37%, three fold decreased compared to chloroquine alone [6]. It was discovered at 25 days that *P. berghei* infection parasitemia decreased ~ 67% and Nch showed inhibition at ~ 92%. Nch particles showed a higher recovery rate of damage tissue in the spleen and liver compared to the conventional drug chloroquine. This data suggests that Nch is more beneficial than naked chloroquine and the nanoparticle alone.

Nanocarriers have the ability to improve drug solubility, reduce toxicity, prolong drug release, and increase drug stability. Therefore, indicating nanoformulations of drugs is a more efficient method for designing innovative therapeutics. Bhardwaj et.al. developed an antimalarial combination drug polymer of primaquine (quinolone analogue) and dihydroartemisinin (artemisinin analogue) to conquer targeted drug delivery and amplify the uptake by hepatocytes [47].

PQ is highly toxic in high doses and can cause excessive tissue damage [19]. PQ is used as a tissue schizontocidal targeting malaria parasites at the sexual and hepatic stages [47]. On the other hand, dihydroartemisinin (artemisinin derivative) functions as a blood schizontocidal, active potent metabolite [48]. This data illustrated that this combination drug is an effective method for curing resistant malaria. The polymer-drug conjugate combination showed evidence of rapid parasite clearance in *P. berghei* (NK65 resistant strain), by disrupting varies stages of the malarial parasite cycle and preventing recrudescence [47].

Metallic nanoparticles

Controlling the vector (*Andopheles*) that is responsible for the spread of malaria has long been acknowledged as a viable means to lessen the spread of disease. Insecticide-treated mosquito nets (ITNs) and indoor spraying with residual insecticides (IRS) have been used as preventative measurements to help control outbreaks of malaria, especially in countries with a high rate of malaria contraction such as Africa [1]. The drawback to these is the need for reapplication. While success has been seen with these methods, much attention has been given to the use of nanoparticles to control the mosquito population.

Of particular interest is the potential use of silver nanoparticles (AgNP) in mosquito control. Numerous groups have reported synthesizing AgNPs that can restrict the growth of larvae and or pupae forms of *Andopheles* [49-57]. This area is particularly attractive because "green" or plant based synthesis methods were utilized. While the methods of synthesis for the nanoparticle varied with regard to the plant or organic molecule used in production of the nanoparticle the products all possessed insecticide activity that was effective against *Andopheles.*

Conclusion

Evolution, proposed by Charles Darwin, is a natural process that contributes to the maintenance of the living system on any change. This has been a major reason that drives for tools that combat against drug and insecticide resistance to malaria pathogens and mosquitoes respectively. To overcome these problems, genetically modified mosquitoes (transgenic) and drug cocktails (multi-drugs) have been proposed and trialed. These last solutions are now facing serious challenges, which is the destruction of the transgenes by mosquitoes and drugs by the body. Nanomedicine has been proposed and could serve as major advantages to finally cure malaria. A multidrug combination utilizing nanoparticles as the/a protector could be a future ultimate way to cure this disease. As mentioned elsewhere, particular attention should be given to biodegradable nanoparticles-multidrug combination drugs to slow down the evolutionary process. Moreover, transgenes used for mosquito engineering could be stabilized by nanoparticles and reduce the transmission of this and some other major diseases. Carefully selected biodegradable nanoparticles. Drugs, and biodrugs could help eradicate malaria in developing countries. This approach could represent a strategy and approach to control other mosquito-borne diseases.

Acknowledgment

MT Coats acknowledges support from the National Science Foundation-CREST(HRD-1241701), NSF-HBCU-UP(HRD-1135863), NSF-AGEP(HRD-1432991) and National Institutes of Health-MBRS-RISE (1R25GM106995-01) grant.

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