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Review Article

A Novel Approach for Malaria Treatment

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ABSTRACT:

Malaria eradication is still away from reach due to the development of multiple drug resistance and the side effects associated with the use of conventional antimalarial chemotherapy. Developing novel approaches to administer these derivatives by some alternative parenteral route would be valuable in overcoming their therapeutic limitations. Nanotechnology has the potential to improve the safety and efficacy of drug delivery and help the cause of malaria eradication. Many of the problems associated with conventional dosage forms and delivery systems such as poor bioavailability, non-specificity, rapid metabolism and excretion amongst others can be solved through pharmaceutical nanotechnology. This review highlights on the novel approaches such as lipid and polymeric nanoparticles in drug delivery for the treatment of malaria.

KEYWORDS: anti-malarial, nanotechnology, drug delivery, lipid nanoparticles, polymeric nanoparticles

INTRODUCTION:

Malaria is responsible for more than 1 million deaths annually and it mainly occurs in Sub-Saharan Africa [1-2]. Malaria is caused by five species of parasite that affect humans, and all of these species belong to the genus *Plasmodium*: *P.falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. *P.falciparum* is responsible for almost all malaria related deaths worldwide. Malaria parasites are transmitted by female *Anopheles* mosquitoes. The sporozoites are inoculated into the human host bloodstream, infect the liver cells and mature into merozoites (exoerythrocytic schizogony), which, after rupture of the liver cell, continue their cycle in red blood cells (erythrocytic phase). The erythrocytic phase is responsible for the clinical manifestations [3].

The increasing resistance of malaria parasites to available drugs increases the burden of disease and the need to develop new and effective anti-malarial agents [4]. To enhance the therapeutic efficacy of antimalarial agents which are already in use, new strategies which will be able to deliver high concentration of drug in the parasitophorous vacuole where the parasite resides are urgently required [5-6]. Hence, the

best strategy that could be adopted to tackle the aforementioned crisis associated with antimalarial therapeutics is by developing nanocarrier systems [7]. Nanotechnology is an emerging field with wide biomedical applications which has potential to cure deadly diseases like malaria.

A compound in order to be used as a drug should have favorable absorption, distribution, metabolism, excretion and toxicity (ADMET) characteristics. Among the most popular drug delivery systems, lipid nanoparticles have emerged in the latest years as promising approaches for poorly soluble drugs. Polymeric nanoparticles are among the drug delivery systems which have been studied in comparison to liposomes for many applications [8-10]. This review mainly focuses on lipid and polymeric nanoparticles as novel drug delivery systems for the treatment of malaria.

APPLICATIONS OF NANOPARTICLES IN ANTI-MALARIAL DRUG DELIVERY:

A. Lipid Nanoparticles:

Lipid nanoparticles have been reported as useful tools in developing injectable dosage forms for poorly soluble drugs.

1. Solid-Lipid Nanoparticles :

Solid lipid nanoparticles are lipid based submicron colloidal carriers consisting of physiological and biocompatible/biodegradable lipids such as highly purified triglycerides, complex glyceride mixtures, waxes and para-acyl-calix(4)arenes which are solid at room and body temperatures and which can be stabilized by using surfactants [11-13].

Artemether Loaded Lipid Nanoparticles:

Artemether, a derivative of artemisinin, contains sesquiterpene lactone rings with an endoperoxide bridge that is cleaved by an iron-dependent mechanism. Artemether plays a crucial role in the treatment of multi-resistant malaria parasite and has been found to inhibit haemozoin formation as well as haemoglobin degradation, due to the presence of the haem group that is a potent inhibitor of cysteine protease [14]. Since, there are some limitations in using this drug some researchers worldwide are involved in developing advanced drug delivery systems to enhance the anti-malarial activity of artemether [15-17]. A promising strategy to tackle novel approaches is developing lipid nanoparticles (LNP) consisting of a solid matrix (melting point > 40 ◦C) prepared by blending solid lipid (>70%, w/w) with increasing ratio of liquid lipid (i.e., oil up to 30%, w/w) [18]. One study has reported the production of artemether-loaded lipid nanoparticles (ARM-LNP) composed of 5% (w/v) lipid mass by a modified thin-film hydration method [19] using glyceryl trimyristate (solid lipid) and soybean oil (as liquid lipid in a concentration ranging from 0 to 45% (w/v) with respect to the total lipid mass). The particles were loaded with 10% of the anti-malarial ARM and surface-tailored with a combination of non-ionic, cationic or anionic surfactants. ARM-LNP were further characterized for their mean particle size, zeta potential and encapsulation efficiency, reporting optimized values below 120nm (PI < 0.250), −38mV and 97% (w/w), respectively. ARM-LNP composed of 45% soybean oil depicted a spherical-like shape by transmission electron microscopy and a biphasic release profile in phosphate buffer. Haemolytic activity was within the acceptable range (7%) revealing low toxicity risk of LNP for parenteral delivery of ARM. Histopathological analysis showed no significant histological changes in liver and kidney tissues in adult Swiss Albino mice treated with the selected formulations. *In vivo* anti-malarial activity of ARM was enhanced when formulated as LNP, in comparison to a conventional plain drug solution and to a marketed formulation which are currently in use to treat malaria patients. Loading of lipid nanoparticles with artemether will overcome

some important shortcomings such as the avoidance of fast drug metabolism and production of the toxic metabolite dihydroartemisinin (attributed to the sustained release of artemether from lipid particles). Furthermore, this approach will allow parenteral administration of this drug aiming to treat cerebral malaria, overcoming the artemether short half-life in the body, and its current use in combination with other anti-malarial drugs [20].

Curcuminoids Loaded Lipid Nanoparticles:

Curcuminoids are isolated from the rhizome of *Curcuma longa Linn* and consist primarily of three phenolic compounds: curcumin, demethoxy-curcumin and bisdemethoxycurcumin [21-22]. Curcuminoids are reported to have a promient antimalarial activity both *in vitro* (chloroquine resistance and sensitive *Plasmodium falciparum* lab strains) and *in vivo* (*Plasmodium berghei*) studies [23-26], but they have poor water solubility and high sensitivity to light and moisture [27]. To overcome these shortcomings, one study has reported a novel approach for the delivery of curcuminoids when formulated in lipid nanoparticles suitable for parenteral administration. In this work, curcuminoids-loaded lipid nanoparticles were successfully prepared by a nanoemulsion technique employing high-speed homogenizer and ultrasonic probe. For the production of nanoparticles, trimyristin, tristerin and glyceryl monostearate were selected as solid lipids and medium chain triglyceride (MCT) as liquid lipid. Scanning electron microscopy (SEM) revealed the spherical nature of the particles with sizes ranging between 120 and 250nm measured by photon correlation spectroscopy (PCS). The *in vivo* pharmacodynamic activity revealed 2-fold increase in antimalarial activity of curcuminoids entrapped in lipid nanoparticles when compared to free curcuminoids at the tested dosage level. Advantages of using this approach include the easy manufacturing process with mild preparation conditions, use of biocompatible lipids, production in aqueous media avoiding organic solvents, and high encapsulation parameters. The drug release characteristics exhibited controlled delivery of curcuminoids for longer periods, which may improve bioavailability of the drug in the active, native form [28].

2. Liposomes :

Liposomes are microscopic structures consisting of one or more concentric spheres of lipid bilayers, enclosing aqueous compartments **[29]** and are used extensively for controlled delivery drug formulations.

Artemisinin and Artimisinin plus Curcumin Liposomal Formulations:

Artemisinin is derived from the wormwood plant (*Artemisia annua*) that is found in parts of Asia. Artemisinin and its derivatives are considered the keystones of the treatment for *Plasmodium falciparum* malaria due to their high potency and rapid action [30]. Since, artemisinin is not very stable and it easily decomposes by the opening of the lactone ring [31], its encapsulation into conventional and PEGylated liposomes prolongs its circulating time in blood plasma and enhance its half-life [32]. Furthermore, curcumin is relatively abundant and cost-effective and is an attractive partner drug for artemisinin, due to its short half-life (1–2 h), closely matching that of artemisinin. But, curcumin has a similar drawback to artemisinin: a poor bioavailability [33-34]. One study has reported the therapeutic efficacies of novel liposomal delivery systems based on artemisinin or artemisinin-based combination therapy with curcumin. The developed liposomal formulations have proper characteristics as drug carriers for parental administration in terms of particle size, polydispersity, encapsulation efficacy and ζ-potential. The mean diameters of all the

artemisinin-based vesicles was ≤200 nm and resulted suitable for the intraperitoneal administration. The *in vivo* antimalarial activity of artemisinin-based liposomal formulations was tested in *Plasmodium berghei* NK-65 infected mice. Artemisinin, alone or in combination with curcumin, was encapsulated in conventional and PEGylated liposomes according to the film hydration method and its *in vivo* performance was assessed by comparison with the free drug. Mice were treated with artemisinin at the dosage of 50 mg/kg/days alone or plus curcumin as partner drug, administered at the dosage of 100 mg/kg/days. Artemisinin alone began to decrease parasitaemia levels only 7 days after the start of the treatment and it appeared to have a fluctuant trend in blood concentration which is reflected in the antimalarial effectiveness. By contrast, treatments with artemisinin-loaded conventional liposomes (A-CL), artemisinin-curcumin-loaded conventional liposomes (AC-CL), artemisinin-loaded PEGylated liposomes (A-PL), artemisinin-curcumin-loaded PEGylated liposomes (AC-PL) appeared to have an immediate antimalarial effect. Both nanoencapsulated artemisinin and artemisinin plus curcumin formulations cured all malaria-infected mice within the same post-inoculation period of time. Additionally, all formulations showed less variability in artemisinin plasma concentrations which suggested that A-CL, AC-CL, A-PL and AC-PL give a modified release of drug(s) and, as a consequence, a constant antimalarial effect during time. In particular, A-PL seems to give the most pronounced and statistically significant therapeutic effect in this murine model of malaria. The enhanced permanency in blood of A-PL suggests the use of these nanosystems as suitable passive targeted carriers for parasitic infections [35].

Chloroquine Liposomal Formulations:

Liposomes bearing cell-specific recognition ligands on their surfaces have been widely considered as drug carriers in therapy [36-37], and liposome encapsulation has been assayed for the targeted delivery of compounds against murine malaria [38-40]. Despite these promising results, liposomal-based targeted delivery has not progressed due of the lack of sufficiently specific markers for *Plasmodium*-infected cells. RBCs have very poor endocytic processes, and for this reason liposomes docked by specific antibodies to RBCs can be an efficient system to deliver cargo into the cell by a simple membrane fusion process [41–43]. Studies with encapsulation of chloroquine in liposomes bearing antipRBC antibody on their surfaces has markedly increased its efficacy against *Plasmodium berghei* infections in mice [44]. In one study liposomes composed of phosphatidylcholine (PC); phosphatidylethanolamine (PE); CHOL and 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) were fabricated using thin film hydration method. Fluorescent probe pyranine, carboxyl quantum dots or chloroquine were encapsulated within the liposomes and it was attached with specific half-antibodies against *P. falciparum* late form infected pRBCs for selective targeting. 200-nm liposomes loaded with quantum dots were covalently functionalized with oriented, specific half-antibodies against *P. falciparum* late form-infected pRBCs. In less than 90 min, liposomes dock to pRBC plasma membranes and release their cargo to the cell. 100.0% of late form-containing pRBCs and 0.0% of noninfected RBCs in *P. falciparum* cultures were recognized and permeated by the content of targeted immunoliposomes. Liposomes not functionalized with antibodies were also specifically directed to pRBCs, although with less affinity than immunoliposomes. In preliminary assays, the antimalarial drug chloroquine at a concentration of 2 nM, ≥10 times below its IC50 in solution, cleared 26.7±1.8% of pRBCs when delivered inside targeted immunoliposomes. This study with *Plasmodium falciparum* show that if the

specific targeting molecules are available sufficiently, drugs carried by liposomes can be delivered to pRBCs with complete specificity relative to non-infected RBCs. But the liposomes docked to pRBCs are not internalized as a whole. Since, quantum dots were delivered inside liposomes and liposome bound targeting antibody and quantum dots always colocalize in pRBCs, it proves that liposomes fuse and deliver their contents inside pRBCs [45].

B. Polymeric Nanoparticles :

Polymeric nanoparticles are made up of biodegradable and/or biocompatible synthetic polymers like poly(D,L-lactide-co-glycolide) (PLGA), polyalkylcyanoacrylates (PACA), curdlan, chitosan, albumin and gelatin [46] for which therapeutic and targeting moiety can be either adsorbed, entrapped or covalently attached [47].

Cryptolepine Hydrochloride Loaded Gelatin Nanoparticles:

Cryptolepine hydrochloride (5-methyl, 10H-indolo [3,2-b] quinoline hydrochloride) , an alkaloid derived from *Cryptolepis sanguinolenta* (Lindl), has shown antimalarial activity [48-49]. The compound acts within the acidic food vacuole of the parasite where it interferes with β-haematin activity [50-51], and this interference inhibits the conversion of the toxic by-product of haemoglobin digestion into the harmless pigment hemozoin, resulting in cell lyses and death. Since, cryptolepine has been reported to be potentially cytotoxic [51-53], one study has developed and characterised cryptolepine hydrochloride-loaded gelatine nanoparticles as a means of exploring formulation techniques to improve the pharmaceutic profile of the compound. Gelatine is a natural polymer which has already been approved for use in parenteral products [54]. It is nontoxic, biodegradable and biocompatible and possesses basic nitrogenous groups which make it attractive as a carrier for the quinoline compounds. Cryptolepine hydrochloride-loaded gelatine-type (A) nanoparticles was formulated according to the double desolvation approach [55]. The study was conducted at two different formulation pH values (2.5 and 11.0) and by two different approaches to drug loading. Three cryoprotectants—sucrose, glucose and mannitol—were investigated for possible use for the preparation of freeze-dried samples. Nanoparticles with desired size mostly less than 350 nm and zeta potential above ±20 were obtained when formulation pH was between 2.5 and 5 and above 9. Entrapment efficiency was higher at pH 11.0 than pH 2.5 and for products formulated when drug was loaded during the second desolvation stage compared to when drug was loaded onto pre-formed nanoparticles. Further investigation of pH effect showed a new isoelectric point of 6.23–6.27 at which the zeta potential of nanoparticles was zero. Sucrose and glucose were effective in low concentrations as cryoprotectants. The best formulation produced an EC50 value of 227.4 μM as a haemolytic agent compared to 51.61 μM by the free compound which is an indication of reduction in haemolytic side effect. There was sustained released of the compound from all formulation types over a period of 192 h. Thus, cryptolepine hydrochloride-loaded gelatine nanoparticles exhibited reduced haemolytic effect compared to the pure compound and can be developed further for parenteral delivery [56].

Curcumin Bound Chitosan Nanoparticles:

Chitosan, a copolymer of D-glucosamine and N-acetyl- D-glucosamine, is a deacetylated derivative of chitin and is a naturally occurring polysaccharide found abundantly in marine crustaceans, insects and fungi. Due to its unique polymeric cationic character it has been extensively examined for the development of drug delivery systems in the pharmaceutical industry [57-58]. Since, it has free amino groups available, it carries a positive charge and reacts

with many negatively charged surfaces such as the cell membrane, mucus lining (due to negatively charged sialic acid residues), and also with other anionic polymers [59]. In one study, to improve the bioavailability and chemical stability of curcumin, curcumin was bound to chitosan nanoparticles. Chitosan nanoparticles were prepared by the procedure based upon ionic gelation between positively charged chitosan and negatively charged penta-sodium triphosphate [57]. Dynamic light scattering showed that the mean diameter of the particles was 178 nm with zeta potential +78±7.6 mV. It was found that curcumin bound to chitosan nanoparticles did not degrade that rapidly in comparison to free curcumin when such particles were incubated in mouse plasma *in vitro* at room temperature. The uptake of bound curcumin from chitosan nanoparticles by mouse RBC was much better than from free curcumin. Curcumin loaded chitosan nanoparticles when delivered orally improved the bioavailability of curcumin in the plasma and RBC. While mice infected with a lethal strain of *Plasmodium yoelii* (N-67) died between 8 and 9 days post infection, feeding of chitosan nanoparticles alone made them to survive for five more days. Curcumin inhibited parasite induced β hematin synthesis in vitro in a dose dependent manner and showed a lower IC50 value (122 µM±2.7) than chloroquin (198 µM±3.7). It was found that binding is pH dependent and maximum binding occurred at pH 4. The improved bioavailability of curcumin resulted in prevention of hemozoin synthesis leading to the death of the parasite and cure of the animals [60].

Nanoencapsulation of Qunine:

Quinine (QN) acts in the erythrocytic phase against all types of *Plasmodium*. Although QN treatment is generally effective against chloroquine-resistant *falciparum* malaria, its use is limited by its narrow therapeutic index, cardiotoxicity and the development of cinchonism syndrome characterised by neurological, cardiovascular and gastrointestinal toxicity as well as hypoglycaemia and hypersensitivity reactions [61-62]. To overcome these shortcomings, one study has developed QN-loaded nanocapsules, to evaluate their efficacy *in vivo* and to determine their pharmacokinetics and erythrocyte partition coefficient. Nanocapsules are a specific type of nanoparticle composed by an oil core surrounded by a polymeric membrane, stabilised by surfactants. The nanocapsules containing QN were prepared with poly(_-caprolactone) and Polysorbate 80. *Plasmodium berghei* infected Wistar rats were used to evaluate the efficacy of QN-loaded nanocapsules using different dosing regimens. QN-loaded nanocapsules presented an adequate particle size (176 nm), narrow particle distribution (0.19), negative zeta potential (−18mV) and high drug content and encapsulation efficiency. Intravenous administration of QN-loaded nanocapsules at 75 mg/kg/day to infected rats resulted in 100% survival, representing an almost 30% reduction compared with the free QN effective dose (105 mg/kg/day). The QN partition coefficient into infected erythrocytes doubled (6.25±0.25) when the drug was nanoencapsulated compared with the free drug (3.03±0.07). Nanoencapsulation may be an interesting approach to improve the efficacy of drugs such as QN, used to treat malaria, owing to the increase in penetration of the drug's target, the red blood cells [63].

CONCLUSIONS:

The treatment with drugs that targets malaria parasite *Plasmodium* has become increasingly a challenge, since parasites turn to acquire resistance against the existing drugs. These results pointed out the optimization of existing drugs efficacy by applying innovative formulation strategies. Nanotechnology is emerging as a rapidly growing field with its application in science and technology and has the

potential to reduce side effects of antimalarial drugs and improve their efficacy. This review gives a better understanding of the novel approaches such as lipid and polymeric nanoparticles as delivery systems for malaria treatment. Advantages of lipid nanoparticles include their lipid composition from biodegradable and biocompatible materials, possibility of controlled release, protection of drugs from chemical degradation and enhanced drug solubility [12,64-65]. In recent years, biodegradable and biocompatible polymeric nanoparticles have attracted a considerable attention as potential carriers for the controlled and site-specific delivery of drugs [66]. The nanodrug delivery systems seem to be a promising and viable approach for improving malaria treatment [67]. There is an urgent need to develop and explore new delivery systems, if the goal of malaria eradication has to be achieved.

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