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DRUG-RESISTANT MALARIA IN SOUTH ASIAN COUNTRIES: A REVIEW OF EVIDENCE AND FUTURE PROSPECTS OF NANOMEDICINE BASED STRATEGIES FOR PROPHYLAXIS AND TREATMENT

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ABSTRACT

International experts raised the alarm over the spread of drug-resistant malaria in several Southeast Asian countries, saying it endangers major global gains in fighting the mosquito-borne disease that kills more than 600,000 people each year. The availability of therapies using the drug artemisinin has helped cut global malaria deaths by a quarter in the past decade. But over the same period, resistance to the drug emerged on Thailand's borders with Myanmar and Cambodia and has spread tremendously. It has been detected in southern Vietnam and probably exists in southern Laos. Once it reaches a higher level of resistance where the drugs don't work, we are technically stuffed, Scientists have been working for decades to develop a malaria vaccine, but none is yet available. To counteract this trend, research has been done in nanotechnology and nanomedicine, for the development of new biocompatible systems capable of incorporating drugs, lowering the resistance progress, contributing for diagnosis, control and treatment of malaria by target delivery. In this review, we discussed the main problems associated with the spread of malaria and the most recent developments in nanomedicine for anti-malarial drug delivery.

KEYWORDS: Malaria, Parasite, Treatment, Nanoemulsions.

1.0 INTRODUCTION

Malaria is caused by protozoan parasites belonging to the genus Plasmodium. There are more than 120 species of Plasmodia out of which human malaria is caused by four Plasmodium species – *Plasmodium malariae, Plasmodium ovale, Plasmodium vivax and Plasmodium*

falciparum. P. falciparum and P. vivax are the two major species involved in global malaria. Out of the four species, only P. malariae may infect other primates in addition to humans. Malaria is transmitted from host to host by the bite of an infected Anopheles mosquito. It has been shown that one bite can introduce several genetically different parasites into the host.[1] Even though Anopheline mosquito is a vector, only 60 out of 380 species of Anopheline mosquito can transmit malaria. Clinically, the paroxysms may occur daily (quotidian), on alternate days (tertian) or with an interval of three days between chills (quartan). Despite broad control efforts, the occurrence of the disease is not declining in most malaria prevalent areas of the world and in some it is clearly going up. In recent years, due to the interest received from several public and private organizations e.g. Bill and Melinda Gates Foundation, Medicines for Malaria Venture, Drugs for Neglected Diseases initiative (DNDi) and the Institute for One World Health (IOWH), the situation improved to some extent. Several new potential therapeutic candidates has been discovered or developed to treat these neglected diseases.[2] However, new drugs are still mandatory and their discovery is a time consuming and expensive (Fig. 1).[3]

To overcome the problem of inadequate participation and long duration for new drug discovery and development, various approaches have been explored. One among them is the development of new delivery system which can enhance the therapeutic potency of existing drugs by improving their adsorption, distribution, metabolism and excretion (ADME), reducing over drug toxicity and targeted delivery to the target organism or organ. In the present review, attempts have been made to summarize the various general aspects of malaria and application of novel drug delivery systems to improve the therapeutic aspects of existing antimalarial drugs using above mentioned approach.

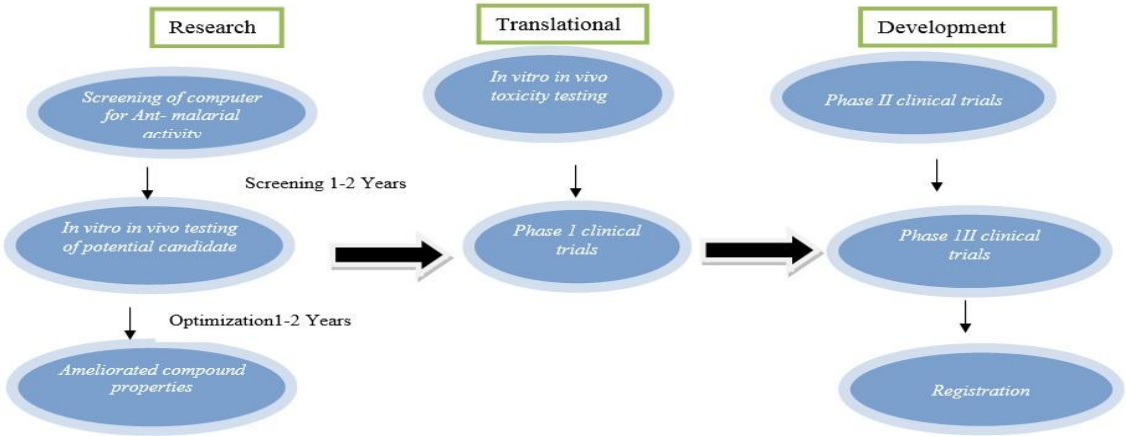

Figure 1: Sequential steps involved in the development of anti-malarial drugs

1.1 Life cycle of malarial parasite

The Plasmodium genus of protozoan parasites has a life cycle that is divided between a vertebrate host and an insect vector. The Plasmodium species, with the exception of P.malariae (which may affect the higher primates) are exclusively parasites of man. Malarial parasites in the form of sporozoites in the salivary gland of mosquitoes gain access to liver through the blood stream during a mosquito bite. The merozoites released from the liver enter the red blood cell (RBC) and an asexual cycle consisting of rings, trophozoites, schizonts and again merozoites leads to parasite multiplication and in turn they would be ready to infect fresh RBC. In this process, a few gametes are also formed which gain access to the mosquito midgut during the bite. The sexual cycle in the mosquito eventually gives rise to sporozoites in the salivary glands.

Therefore, the parasite has a sexual cycle in the mosquito and an asexual cycle in human, later being divided into the exo-erythrocytic cycle in liver and intra-erythrocytic cycle in RBC. The intra-erythrocytic phase elicits the clinical manifestations of malaria.[4,5] The basic life cycle of the parasite is shown in Fig. 2.

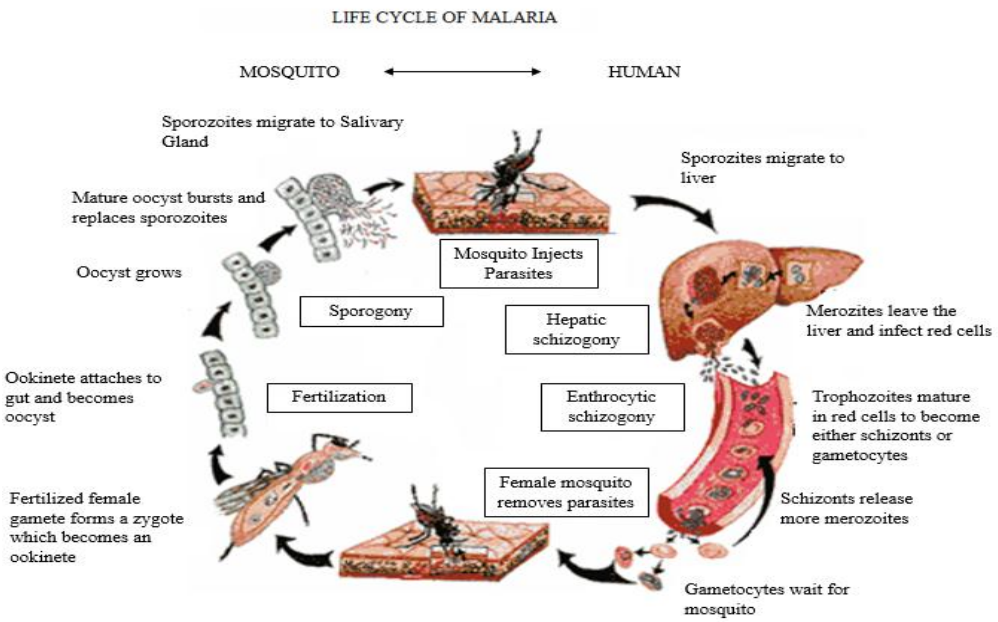

Fig 2: Life cycle of Malaria

Current Trends in treatment of Malaria

In 1967, the government of the People's Republic of China embarked on a systematic examination of native plants used in old-fashioned remedies as sources of drugs. One such plant, a pervasive weed with a long history of use is known as qing hao (Artemisia annua L.,

sweet wormwood, annual wormwood).[6,7] Artemisinin (ART) is proven to be a sesquiterpene, a natural product composed of fifteen carbon atoms based on three isoprene molecules usually joined head-to-tail, with additional oxygen atom functionality. The really striking feature of ART was the peroxide bridge spanning one of the molecules rings, which was shown to be its active ingredient, responsible for its activity against plasmodium.[8] The artemisinins including artesunate (AS), artemether (ARM), arteether (AE) and dihydroartemisinin (DHA) are the most effective anti-malarial drugs known today. They retain an extraordinarily wide therapeutic index. They have the capability to quickly kill a broad range of asexual parasite stages at safe concentrations that are consistently achievable through standard dosing regimens.[9] ART and its derivatives are considered the keystones of the treatment of P. falciparum malaria due to their high potency and efficacious ACT formulations to combat this deadly parasitic disease.

Table 1 Current antimalarial drugs and associated limitations.

Drug	Associated problems	References
Artemisinin (Quinghasu)	Recrudescence; neurotoxicity; cost	[10] [11]
Atovaquone (Mepron)	Limited experience; rapid development of resistance in monotherapy	[12]
Azithromycin	Limited use; efficacy to be defined	[13,14]
Chloroquine (Aralen)	Drug resistance worldwide	[15–20]
Doxycycline	Phototoxicity; not used in pregnant women children; gastrointestinal intolerance; skin and nail disorders	[21–26]
Fansidar	Drug resistance worldwide; severe allergic reactions	[27,28]
Halofantrine (Halfan)	Severe allergic reactions; resistance worldwide cardiotoxicity, poor absorption, sporadic	[29]
Mefloquine (Larium)	Gastrointestinal and neurological disturbances; drug resistance in Indochina and Africa	[30–34]
Primaquine	Photochemically unstable; in vitro phototoxic; narrow therapeutic index; development of drug resistance; not used in G6PD-deficient patients: hemolytic anemia as side effect. Administration of high doses can cause: nausea, headache, disturbance of visual accommodation, prurits and abdominal cramps.	[35–39]
Proguanil (Paludrine)	Mouth ulcers; resistance worldwide	[40,41]
Quinine sulfate	Tinnitus; resistance in Brazil and Indochina	[42,44]
Quinidine gluconate	Limited availability; requires cardiac monitoring	[45–47]

FORMULATION BASED APPROACHES

Nanocrystals

Drug nanocrystals are particles made from 100% drug; typically, they are stabilized by surfactants or polymeric steric stabilizers.[48,49] Hence, these particles possess a 100% drug

loading in contrast to matrix nanoparticles consisting e.g. of a polymeric matrix (polymeric nanoparticles[50] or a lipidic matrix (nanoemulsions[51,52], liposomes[53,54] and lipid nanoparticles[55] (Fig. 1). The high loading makes them very efficient in transporting drug to or into cells, reaching a sufficiently high therapeutic concentration for the pharmacological effect. The nanocrystals are typically produced in a liquid dispersion medium, i.e. the nanocrystals are suspended in the liquid (Nanosuspensions).

According to National Science Foundation of USA, nanotechnology deals with controlling or restructuring of the material dimension more or less between 1 and 100 nm. This top down or bottom up processes makes these materials different from their original ones either in surface related properties or quantum properties.[56] Though magic bullet concept of Paul Ehrlich "specifically targeting the causative agent" is the main focus in nanomedicine research, executing this concept pose a major challenge to malaria drug discovery and delivery scientists due to intracellular and strewn location of parasite. Here nanosized systems can be used for treatment, imaging, vector control and diagnosis of malaria. Understanding the pathophysiology of *Plasmodium* and the concept of Nanoparticulates delivery systems results in overcoming the pharmacokinetic mismatch associated with therapeutic molecules and it will help for the precise and early detection of the disease.

Pheroids: Pheroids are used to encapsulate, transport and deliver the therapeutics to a specific organ or part of the body. They are biocompatible and biodegradable and hence they are safe to use in medicine, as they are made up of naturally occurring compounds.

Recently, this technology was used to deliver the anti-malarial drugs artemisone and artemiside. These two are derivatives of artemisinin with good antimalarial activity but poor and erratic absorption upon oral administration. The pheroid formulation was constituted using vitamin F ethyl ester, Cremaphor® EL, D-α-tocopherol and butylated hydroxyanisole as oil phase and Nitrous oxide water as water phase. This water phase was added to oil phase to get the final pheroid formulations. The fabricated pheroids were 3.81 μm in size.

Aetemiside exhibit marginal improvement in pharmacokinetic properties after encapsulating in Pheroids.[57] Also, pheroids have been used to improve the stability of another antimalarial drug mefloquine and its efficacy was compared with mefloquine loaded liposomes. Pheroids were composed of vitamin-F, Cremophor®, RH40 and DL-α-tocopherol and mefloquine.Water was then added to this nitrous oxide saturated. Liposomes were produced

using CHOL and L-α-phosphatidylcholine., significant increase in size was observed in liposome formulations (~5 to 9 μm) with larger aggregates in 3 months stability whereas in pheroids it was negligible. EE was ~69% which remained stable even after accelerated stability test of 3 months. No variation was observed in structure or size on the micrographs.[58]

Solid Lipid Nanoparticles

Solid lipid nanoparticles has been regarded as an alternative to liposomes and nanoemulsions due to various advantages such as ease of manufacture, particulate nature ability to sustain the release of the drug. Additionally, the ability of Solid lipid nanoparticles to sustain the delivery of therapeutic agents could be useful in combating the recrudescence which **i**s commonly observed with the Artemether (ARM) monotherapy . Nisha Raina et al, formulated Solid lipid nanoparticles of ARM by using biocompatible excipients for passive targeting to liver cells and was evaluated for its potential in improving the antimalarial efficacy of ARM in comparison to the conventional IM oily formulation by means of a suitable in vivo model. The poor aqueous solubility of ARM significantly hampers its therapeutic efficacy. The oral bioavailability of ARM is low (~40%), due to its poor aqueous solubility and degradation in stomach acids. Whereas the current oily intramuscular (IM) injection suffers from disadvantages such as severe pain due to oily nature of the formulation and erratic absorption on intramuscular administration. The oily injection is not very appropriate when rapid extinction of the malarial infections is required. It has been demonstrated that intra venous delivery of ARM results in the highest availability to body as compared to all other routes and can lead to rapid eradication of the malarial infection. However, currently, no ARM product is available that enables IV delivery of ARM. So, it is necessary to have IV formulation of ARM that enables its quick availability to the body with concomitant reduction in the pain on injection.

Hemolysis is an important parameter and needs to be examined since the drug showed its optimum therapeutic activity at RBC stage when hemolytic study was performed to evaluate cytotoxicity of the formulation. It was inferred that the assembling of aforementioned components in solid lipid nanoparticles structure changes their mode and degree of interaction with the erythrocytes and hence the combination of all these components does not show any additive effect on the hemolysis of erythrocytes. Similar observations have been reported for the lipid emulsions which corroborate this observation. This result further

confirmed the biocompatible nature of lipid and surfactant selected for formulation. It was also found that Percentage antimalarial activity observed 70% and 50% for preventive and curative stage respectively indicated artemether based solid lipid nanoparticles was more effective in preventive stage than curative stage.[59]

Liposomal formulations

Liposomes, first described in 1976, were the first type of particulate drug delivery system applied in disease therapy.[60,61] These are self-assembling spherical, closed colloidal structures composed of phospholipid bilayers that surround a central aqueous space. These amphiphilic phospholipid molecules form a closed bilayer sphere, shielding the hydrophobic groups from the aqueous environment, while maintaining contact with the aqueous phase through the hydrophilic head groups.[62,61] Anti-malarial drugs such as Chloroquine (CQ), Quinine (QN), Primaquine (PQ), Artesunate (AS), Artemether (ARM), Arteether (AE) and very recently, a combination of Artemisinin (ART) and curcumin have been encapsulated in neutral conventional or long circulating liposomes using different preparation techniques.[63- 70] From the findings of these studies, the pH gradient technique seems to be the best for enhancing the encapsulation efficiency of anti-malarial. Investigations into the possibility of encapsulating drugs like QN and CQ in neutral large unilamellar vesicles (LUVs) applying the pH gradient method have been concluded and reported.[63,64] In the study, uptake of 148 and 104 nmol/ømol after 15 min, and 81 and 88 nmol/ømol were also reported after 2 h were reported for QN and CQ respectively. This finding showed that drugs such as QN and CQ were able to accumulate within these LUVs which exhibit a proton gradient. Bayomi et al, encapsulated AE for oral administration in neutral multilamellar liposomes.[69] In their study, multilamellar liposomes prepared with dibehenoylphosphatidylcholine (DBPC), cholesterol (CHOL) and AE at a ratio of 1:1:2 presented a mean size of 3.20 μ± 1.03 μm and entrapment efficiency (EE) of 82.3 %. The daily release rate of AE from liposomes prepared with mixtures of DBPC and dipalmitoylphosphatidylcholine (DPPC) at a ratio of 1:1 was 0.818 %/day while it was 0.783 %/day when CHOL was added to DBPC at a ratio of 1:1, and 0.616 % when CHOL was used at a ratio of 1:2. These findings showed that the increase in the length of acyl chain of phospholipids as well as the addition of CHOL led to a decrease in the release rate of AE. This might be due to the ability of CHOL to induce drug/phospholipid interactions in the bilayer, leading to a decrease in drug release. Its in vivo evaluation compared with that of an oral aqueous suspension showed that orally administered liposomes of AE gave a relative

bioavailability of 97.91 % while its oral aqueous suspension gave 31.83 %. Gabriels and Plaizier-Vercammen have reported the encapsulation of AS in neutral liposomes using a pH 5 buffer solution as aqueous phase to prevent the aqueous instability of AS.[67] The EE of the AS-loaded liposomes was approximately 100 % and the liposomes remained stable for 10 days at 25 ÀC. They reported that the release of the drug from the liposomes was influenced by the lipid content as the release rate decreased with increase in lipid concentration.

Furthermore, Chimanuka et al. reported the encapsulation of β- artemether (βAM) in neutral liposomes, and also evaluated its therapeutic efficacy in mice infected with Plasmodium chabaudi.[68] They reported an EE of about 100 % for their formulations, which also retained their stability for 3 months at 4 ÀC. When administered to the infected mice, a 100 % cure was observed after 22 days of infection. Benedetta et al. reported the encapsulation of ART and a combination of ART and curcumin in conventional and PEGylated liposomes using the film hydrationmethod.[70] In the study, ART conventional liposomes (A-CL) were formulated using Phospholipon® 90G (P90G), CHOL and ART. ART-loaded PEGylated liposomes (A-PL) were formulated using polyethyleneglycol-2000-distearoylphosphatidylethanolamine (PEG2000-DSPE), P90G, CHOL and ART. ART-Curcumin-loaded conventional liposomes (AC-CL) were formulated using P90G, CHOL, ART and curcumin while ART-Curcumin-loaded PEGylated liposomes (AC-PL) were formulated using PEG2000-DSPE, P90G, CHOL, ART and Curcumin. They reported that the mean diameter of all the ART-based vesicles was [a] 200 nm and suitable for intraperitoneal administration. HPLC analysis of the vesicles gave EE of 78 % and 68 % for conventional liposomes and PEGylated liposomes respectively. The EE of curcumin showed that the EE for AC-PL was smaller than that of AC-CL. This could be due to the smaller size of PEGylated liposomes which decreases the bilayer capacity of solubilizing lipophilic drugs. All the ART-loaded vesicles remained stable for a period of 1 month. When the ART-loaded vesicles were administered in mice infected with Plasmodium berghei, parasitaemia was reduced faster with AC-PL to about 60% after only 3 days. In all liposomal treatments, parasitaemia was reduced more than 95 % from day 5. Interestingly, the infection was almost totally reverted in mice treated with A-CL after 7 days and with AC-CL, A-PL and AC-PL after 5 days. This is as shown in table 2. On the basis of the above studies, liposomes have been extensively used as an effective carrier of anti-malarial drugs in the treatment of experimental malaria. This could be explained from their ability to reduce the toxicity profile of these chemical entities, show improved

experimental therapeutic efficacy against the Plasmodium strains, modify the bioavailability of these drugs as well as ensure prolonged in vivo release.

Table 2 Some particulate drug formulations available in the market

Brand Name	Drug	Formulation Type	Route of Administration	Application	Company
Rapamune®	Rapamycin	Nanoparticles	Oral	Immunosuppressant	Wyeth Pharma, USA
Intelectol®	Vinpocetine	Liposomes	Oral	Cerebrovascular disorders	Menory Secret Inc., USA
Nurofen®	Ibuprofen	Nanocapsules	Oral	NSAIDs	Abbott AG, USA
Lipofen®	Fenofibrate	Liposomes	Oral	Hypercholesterolemia	Kowa Pharma Inc., USA
Ambisome®	Amphotericin B	Liposome	Intravenous Infusion	Fungal Infections	Astellas Pharma Inc., USA
Mevacor®	Lovastatin	SLNs	Oral	Hyperlipidaemia	Merck and Co. Inc., USA
Procardia®	Nifedipine	Nanosuspension	Oral	Hypertension	Pfizer Labs Inc., USA
Cesol®	Praziquantel	SLNs	Oral	Antihelmintic	Merck KGaA, Germany
Abraxane®	Paclitaxel	Nanoparticles	Intravenous Injection	Metastatic breast cancer	American Biosciences, USA
Efudex®	N3-o-toluyl-Fluorouracil	Liposomes	Oral	Tumour Inhibition	Valeant Pharma. Intl, USA

Micro/nanoemulsions

Nanoemulsions are the thermodynamically steady isotropic structure in which two immiscible liquids (water and oil) are assorted to appear in a single phase by means of a suitable surfactants or its mix with a droplet of approximately 0.5–100 μm in diameter. Since it is a stable isotropic system, careful balance of the three phases is essential for the system to achieve thermodynamically balanced state. The proportion of the three phases is determined using three phase diagram which denotes the ratio of constituents that exist in balance to form nanoemulsion.[71]

Primaquine has primarily been used against gametocytes and hypnozoites (liver reservoirs) responsible for the relapsing forms of P. vivax and P. ovale. Despite its good oral absorption, this molecule has a short half-life and needs to be administered daily. To overcome this problem, a delivery system was fabricated using the chylomicrons nanoemulsions to target the primaquine to gametocytes and hypnozoites which hibernate in liver. The fabricated chylomicrons encapsulated with primaquine were able to deliver the drug specifically to liver

in comparison to free primaquine parenteral administration. In addition to this enhanced therapeutic efficiency, the primaquine entrapped in nanoemulsions was more stable than free primaquine in Serum and shows controlled release from the carrier.[72]

In another effort, primaquine loaded nanoemulsions were fabricated and tested on P. berghei infected mice. Primaquine loaded nanoemulsions with 100–200 nm size was able to reduce the therapeutic dose by 25%when administered orally which was evident from reduced parasite progression and increased survivality period. Pharmacokinetic studies have revealed more promaquine accumulation in liver in comparison to plain drug solution.[73]

Microemulsion of artemether was produced using the pseudoternary phase diagram (NanOsorb). The microemulsion preconcentrate after dilution yielded particles of ~180 nm, with a narrow polydispersity index (0.498). In vivo antimalarial activity was tested in P. berghei infected mice and compared with marketed formulation (Larither®). In addition to this, the toxicity was tested as prescribed by Organization for Economic Cooperation and Development (OECD) guidelines. Both the drug loaded and empty NanOsorb showed better antimalarial activity and reduced toxicity than marketed formulation and artemether oily solution.[74]

Dendrimers: A dendrimer is described as a macromolecule with high degree of surface functionality and versatility characterized by its highly branched 3D structure. Dendrimers are also referred to as "Polymers of the 21st century". An initiator core, Interior layers (generations) composed of repeating units radically attached to the interior core and an exterior core (terminal functionality) attached to the outermost interior generations constitutes the dendrimers.[75,76]

Artemether, a fat soluble derivative of artemisin was tried to entrap in the PEG-lysine type dendrimer and chondroitin sulfate A was coated to provide the controlled delivery of artemether from the dendrimer after parenteral administration. The produced dendrimers were able to encapsulate ~18 molecules of artemisinin. The amount of drug loading and controlled release property enhanced as both generation (4G to 5G) and concentration of chondroitin sulfate increased. In addition to this, coating of dendrimers with chondroitin sulfate A increased its biocompatibility as evident from hemolytic assay and size. Antimalarial activity increased 2-fold in uncoated and 4-fold in chondroitin sulfate coated dendrimers.[77] Similar type of PEGlysine type dendrimer and chondroitin sulfate coated

dendrimers were produced for the encapsulation of chloroquine. Enhanced in vitro and in vivo antimalarial activities were observed after encapsulating the chloroquine in dendrimers in comparison to free chloroquine.

Solution.[78] Bhadra et al. encapsulated liver schizonticide primaquine in polypropyleneimine (PPI) dendrimers-coated peripherally with galactose. The aim of the study was to direct the dendrimers carrying primaquine to liver using galactose as targeting moiety. PPI dendrimers were synthesized by consecutive Michael double addition reaction (using ethyelenediamine as core) followed by hydrogenation reaction. Ring opening reactions were carried out for conjugating galactose followed by Schiff's reaction and reduction to secondary amine in sodium acetate buffer (pH 4.0). Synthesis of uncoated and coated dendrimers was confirmed using MASS spectroscopy, infrared spectroscopy and nuclear magnetic resonance. The galactose coating resulted in increase in encapsulation efficiency up to 15 times in comparison to non-coated dendrimers. Adding to this, galactose coating also resulted in controlled release of primaquine from the dendrimers. The biocompatibility of the dendrimers system was proved by absence of macrophage and white blood cell (WBC) stimulation and also RBC counts remained constant. The pharmacokinetic studies revealed the presence of primaquine in targeted organ for a longer period.[79]

Future trends in Anti-malarial activity

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.[80,81] Most of the antimalarials are associated with pharmacokinetic mismatch such as low solubility and therefore, reduced bioavailability, drug-resistance, stability, tolerability, toxicity and inability to reach the strewn location of the parasite.

The concentration of drug in the microenvironment of the intracellular parasite plays an important role rather than increasing the bioavailability of drug to whole body. Many promising antimalarial compounds are charged and therefore require transportation into the parasite. The route of administration is also an important aspect to be considered in malaria. The oral route should be the first choice for clinically uncomplicated malaria. However, parenteral therapy is advocated in severe or complicated malaria. Hence, the best strategy that could be adopted to tackle the aforementioned crisis associated with antimalarial therapeutics is by developing nanocarrier systems.

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