Review: Anti-Malarial Drugs and Oral Lipid-Based Drug Delivery System

Nilesh Jaiswal [1], Prof, (Dr.) Mehta Parulben [2]

[1] Research Scholar, School of Pharmacy, LNCT University, Bhopal, Madhya Pradesh, India.

[2] Professor, School of Pharmacy, LNCT University, Bhopal, Madhya Pradesh, India.

Email: [1] nileshjaiswal2@gmail.com, [2] parulmehta1@rediffmail.com

Abstract

Lipids have been well known for decades for contributing to the greater bioavailability of their carrier drug molecules. Especially those drug candidates that have a greater problem of either struggling in their solubility in polar solvents and/or facing first-pass metabolism often become the ideal candidates for lipid-based drug delivery systems. Anti-malarial drugs are among those candidates that too face such challenges, and hence this research review is conducted to touch base on the main aim of formulations to enhance their oral bioavailability, especially where there is a history of first-pass effect or enzymatic drug degradation before the drug reaches the site of absorption or therapeutic action. The application of lipids as one of the prime formulation approaches is believed to be a highly promising concept. Lipoidal-based drug delivery systems are one of the best and most reliable formulation approaches to counter the challenges of properties such as aqueous solubility and bioavailability of lipophilic drug molecules. Lipoidal and lipid-based formulations can be engineered to meet a complete range of product needs, especially by route of administration, disease indications, toxicity, cost considerations, and product stability. When we investigate anti-malarial drugs, they too have poor aqueous solubility and a positive postprandial food effect, making them ideal candidates for lipid-based drug delivery in order to attain increased oral bioavailability. This review covers the various stages of how a drug-micelle conjugate gets emulsified, digested, and absorbed when ingested and explains how it is a very useful tool these days and an ideal delivery system for, especially, drugs of the malaria class. The review also captures the various factors affecting the physical properties of the drug-micelle structures.

Keywords: drug-micelle conjugate, lipoidal formulations, digestion, emulsification, lipolysis.

1. Introduction

Malaria, caused by Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax, and/or Plasmodium knowlesi species, is a deadly disease that commonly affects humans in many countries in tropical and subtropical regions worldwide[1]. Malaria continues to be the leading cause of morbidity and mortality worldwide. In fact, malaria has caused between 21 and 260 million cases and nearly 400,000 deaths per year over the past decade.

In 2020, about 94% of malaria cases were recorded in sub-Saharan Africa. Nigeria (about 27%), the Republic of the Congo (about 12%), Uganda (about 5%), Mozambique (about 4%), and Niger (about 3%) are responsible for approximately 51% of all malaria cases worldwide.

Pregnant women and children under 5 years of age in these regions are responsible for approximately 85% of deaths[1].

Factors contributing to this disadvantage include poor quality of antimalarial drugs, particularly lack of bioavailability (poor water solubility, permeability, and/or un-stability towards GUT enzymes), and serious side effects that render patients ineffective. To solve all these problems, nanotechnology-based drug delivery systems have emerged as important therapeutic tools in malaria control. In fact, the benefits of nanotechnology as a drug delivery system include increasing efficacy, reducing drug toxicity, improving patient compliance, and overcoming the development of drug resistance. In addition, nano-drug delivery systems can provide cell adhesion sources and materials to bind certain ligands on their surfaces, leading to poor transport and/or selective drug delivery at target or other sites.

Nanodrug delivery systems include nanocarriers, which are dispersions or colloidal particles from 1 to 1000 nm in size. These nanoparticle structures consist of polymers and, in a few cases, lipids and/or inorganic materials with which the active ingredients can be dissolved, encapsulated, absorbed, and/or chemically bonded.

2. Malaria: The Protozoal Life Cycle

Generally, the malaria incubation period lasts from ~7 to ~10 days[2]. However, in the case of Plasmodium vivax and P. ovale infection cases, some schizonts convert into hypnozoites, which is a dormant stage that, if untreated, can persist in the liver for months or even years. Afterward, the hypnozoites can re-activate into schizonts, leading to re-lapses, by entering the bloodstream in the absence of an infectious mosquito bite.

In 2017, the P. vivax parasite was primarily responsible for approximately 7.4 million cases of malaria throughout the world, of which 82% were recorded in India, Afghanistan, Ethiopia, and Pakistan. Mature merozoites that invade erythrocytes evolve into early trophozoites (ring stage). Haemoglobin and plasma nutrients are fed by the parasites, which grow in mature trophozoites (trophozoite stage). These trophozoites replicate their DNA to convert into intra-erythrocytic schizonts, which consist of numerous daughter merozoites. After nearly eight divisions of cycles, the schizonts rupture and release the merozoites in the bloodstream. The latter, once entering other erythrocytes to perpetuate the blood-stage cycle, exponentially increase in number.

The approximate time for one replication cycle is ~48 h for P. falciparum, P. vivax, and P. ovale, whereas P. malariae and P. knowlesi display a 72-h and a 24-h asexual life cycle, respectively. In erythrocytes, the Plasmodium parasites induce physical and chemical stress that results in programmed erythrocytic (RBC) death, named "eryptosis" (erythrocyte apoptosis). After several such cycles, many of the merozoites differentiate into female or male gametocytes (which are also called the "sexual erythrocytic stage"). When the Anopheles mosquitoes feed on blood meal, the gametocytes reach their guts, where they develop into new sporozoites, passing through the zygote, ookinete, and oocyst stages (named the "sexual mosquito stage").

Moreover, in the early 1980s, Gregoria, one of the prominent researchers, studied that liposomes coated with cell-specific ligands may prove highly useful as carriers for target site-specific drug delivery in the bio-phase. The target site-specific drug delivery potential makes them a useful delivery system for targeting infected hepatocytes and RBCs, viz., passive and/or active targeting. On the contrary, active pharmaceutical drug targeting was realized by

the surface activation of the liposomes through the inclusion of surface-binding ligands (e.g., glycolipids, carbohydrates, proteins) binding to the RBC's that were already infected.

Most recently, in 2022, Beavogui et al. mentioned in their research study that artesunate-pyronaridine as a fixed-dose combination therapy was included in the WHO list of prequalified medicines for the management of malaria.[3]

The incubation period for malaria usually lasts 7 to 10 days. In the case of P. vivax and P. ovale, however, some schizonts turn into hypnozoites (named "dormant stage") that can remain in the liver for months or years if not corrected. Then, in the absence of infected mosquito bites, hypnozoites can enter the bloodstream and multiply as schizonts, causing proliferation. Plasmodium vivax is one of the main causes of approximately 7.4 million malaria cases worldwide, of which about 82% occur in India, Afghanistan, Ethiopia, and Pakistan. Mature merozoites affecting erythrocytes transform into early trophozoites (ring stage). Haemoglobin and plasma nutrients are fed by protozoa growing in mature trophozoites (named the "trophoblast stage"). The trophozoites copy their DNA and develop into intra-erythrocytic schizonts containing many daughter merozoites. After about eight cycles of divisions, the schizont ruptures and releases merozoites into the bloodstream. Second, they increase exponentially when they enter other erythrocytes to maintain their blood levels.

The estimated duration of the reproductive cycle of Plasmodium falciparum and Plasmodium vivax is approximately 48 hours. Strains like P. malariae and P. knowlesi display 72 and 24 hours of life, respectively. The Plasmodium protozoa induces physical and chemical stress on red blood cells, resulting in red blood cell (RBC) death called "erythrocytic apoptosis." After several cycles, many merozoites differentiate into female or male gametocytes (also known as the "sexual erythroid stage"). When Anopheles mosquitoes feed on blood, the gametocytes reach their intestines, where they pass through the zygote, ookinete, and oocyst stages and develop into new forms (nomenclated as the "sexual mosquito stage").

In the mid-1990s, researchers proved that liposomes coated with specific cell ligands are useful as carriers for drug release in the "biological phase." The ability to target specific drug delivery sites makes them useful for targeting hepatocytes and erythrocytes. Instead, active drug targeting is achieved by encapsulating ligands (such as liposomes, glycolipids, carbohydrates, and proteins) that bind to red blood cells.

Beavogui et al., in 2022, extensively studied the usage of artesunate-pyronaridine as a fixed-dose combination therapy in malarial patients. This was later also included in the WHO's first list of drugs for the treatment of malaria.[3]

In another study, halofantrine and lumefantrine, which are anti-malarial drugs belonging to the aryl-amino alcohol pharmacophore type like quinine, also acted as schizonticides. However, the effectiveness and usefulness of halofantrine as an anti-malarial drug are currently limited these days because of its cardiotoxicity. On the other hand, lumefantrine is only administered against uncomplicated plasmodium falciparum and plasmodium vivax malaria in combination therapy with Artemether[4].

Similarly, chloroquine, a potent schizonticide with gametocytocidal properties, has also been the best in the class for the prophylactic treatment of malaria in the past many years, due to its affordability and/or efficacy. Another very prominent combination anti-malarial therapy like sulfadoxine and pyrimethamine has also been used for many years to treat uncomplicated

and chloroquine-resistant *Plasmodium falciparum* malaria. This combination therapy is currently recommended as one of the most intermittent prevention treatments for pregnant women (including infants) in most African countries where malaria is endemic.

In 2013, Memvenga et al., in their research study, explored the use of curcumin-loaded lipid-based systems combined with arteether as an antimalarial agent. In this study, two lipid-based drug delivery formulations comprising a very high concentration of curcumin (i.e., as high as 30 mg/g) were prepared. In a gastrointestinal medium, curcumin conjugated with lipids was prepared, which exhibited self-emulsifying properties to form nano-sized particles. During the *in vitro* lipolysis process, nearly ~5–10% w/w of curcumin precipitated from the lipid-based drug delivery system in amorphous solid form with a very high rate of re-dispersibility in fasted simulated intestinal fluids. Due to the increased curcumin solubility, lipid-based drug delivery systems increased the transport of curcumin across Caco-2 cell lines in comparison with the transport of free drugs.

3. Lipid-Based Drug Delivery Systems in Anti-Malarial Therapy

The disadvantages of intravenous administration can be avoided by oral administration, making it the preferred method of administration. However, oral administration is limited by other problems like the physicochemical characteristics, such as limited aqueous solubility, lack of intestinal permeability, lack of chemical stability, and rapid metabolism, all of which reduce oral bioavailability.

However, thanks to drug development and process development, many molecules with therapeutic potential have been created, minimizing most of the above challenges. Most of these new pharmaceuticals have a high molecular weight and fall under the category of Biopharmaceutical Classification System (BCS)-II (i.e., have very low water solubility and are mostly membrane permeable).

All such challenges owing to oral route have been overcome by researchers by the selection of a lipid drug delivery approach (Liposomes: about 30 nm in diameter) as a new drug/delivery platform objectively for the prevention and treatment of malaria. Similarly, lipoidal spherical vesicles are also another kind of new drug delivery approach wherein one or more phospholipid bilayers are surrounded by a water core. Since the core is water-soluble, hydrophilic drugs can be formulated in such a way that the same gets entrapped in the vicinity and surrounded by bilayer phospholipids. Such hydrophilic molecules will be resistant to enzymatic and/or chemical degradation within GUT (if any). Such vesicles, based on their molecular size and the number of bilayers, exhibit the potential to load hydrophilic drugs or act as carriers. Similarly, medium-sized vesicles are called MLVs (medium-sized liposome vesicles), exhibiting more than 500 nm in diameter. Oligolamellar vesicles (nomenclated here as OLVs) exhibit a diameter of about 100–500 nm; giant unilamellar vesicles (UV's) bear a diameter of about 1000 nm; large unilamellar vesicles (LUVs) have a diameter of about 100–1000 nm; and small uni-lamellar vesicles (SULV's) are in the range of 10–100 nm. The physical properties of such structures, like structural stiffness, integrity, permeability, and surface charge, will govern absorption, digestion, and transport through intestinal epithelial enterocytes. Such vesicular structures also comprise synthetic and/or natural phospholipids in a concentration range of about 50–55% w/w and cholesterol—nearly about 30-45% w/w. In addition, hydrophilic and/or lipophilic surfactants are also integral parts of the 3-dimensional structure. Cholesterol is specially added to liposomes to increase

the strength, integrity, elasticity, and permeability of the bilayer structure, resulting in better stability. The molar concentration of cholesterol mainly depends on the desired physicochemical properties. Due to their dual hydrophilicity and lipophilicity, biodegradability, biocompatibility, and low toxicity, liposomes represent a potentially interesting platform for the participation of lipophilic antimalarial drugs. In contrast, those pharmaceutical drugs that are known for their active transport absorption mechanism (the drug molecules are interacted with surface ligands, just like peptides, proteins, glycolipids, carbohydrates, and/or antibodies), which bind to red blood cells (RBCs).[7]

4. Types of Lipid-Based Drug Delivery Systems

In the year 2000, the lipid formulation classification system (LFC) was started, and later in the year 2006, an extra "type" of formulation was added. In the past few years, LFCs have been primarily discussed within the pharmaceutical industry to gather a consensus that can be accepted widely as a framework for distinguishing the performance of lipid-based formulations among themselves, including their applications per se. The main objective of these LFCs was to enable the *in vivo* studies to be interpreted more readily and subsequently to enable the identification of the most fit formulations for specific drug design, that is, with reference to their physiochemical characteristics, as mentioned in Fig. 1.

Formulation type	**Material**	**Characteristics**
Classification type-I (Highly lipophilic)	Long and medium chain triglyceride oils (they do not contain any surfactants or cosolvents)	They are non-dispersible and require lipolysis/digestion
Classification type-II (Lipophilic)	Contain combination of MCT Oils and/or lipophilic surfactants	Self-emulsifying drug delivery prepared by using no hydrophilic soluble surfactants
Classification type-III (Hydrophilic)	Contains oils, surfactants, and/or cosolvents (Both lipophilic and hydrophilic surfactants are used)	Self-emulsifying/emulsifying drug delivery prepared by using hydrophilic soluble surfactants
Classification type-IV (Highly Hydrophilic)	Contain only hydrophilic surfactants and co-solvents	Such lipoidal formulations readily disperses in polar solvents and form micelles readily.

Fig. No. 1: Classification of lipid formulation classification system

5. Lipid Formulation and Distribution Through the Lymphatic System

When we discuss lipids, it is important to understand "micelles," especially in terms of how they are absorbed and digested. These micelles play an important role in transporting hydrophobic molecules through the intestinal epithelial cells, which are involved in the transport process. Micelles are lipid structures that rearrange themselves in the solvent in a spherical (mostly) structure. They are amphiphilic in nature because of the fatty acids, i.e., they have both hydrophilic domains (due to polar head-groups) and lipophilic domains (due to long hydrophobic chains). This structural arrangement allows them to be converted into a spherical shape to reduce the steric hindrance. Glycolipids and phospholipids also contain fatty acids with two lipophilic chains but are too large to fit into spherical micelles; thus,

glycolipids and phospholipids form "lipid bilayers." As mentioned above, micelles have a special aggregation property in water, and this special arrangement is due to the amphiphilic nature of the molecules. The rate-limiting step in the coupling process is the lipophilic interactions that the molecules undergo. The preferred lipid structure in aqueous solutions is in the form of lipid bilayers, sheets rather than spherical micelles. This is because the two fatty acid chains are large enough to fit in micelles and long enough to be bulkier. Therefore, the micelles have one hydrocarbon chain instead of two. Lipid bilayers form rapidly in aqueous media and are highly stable through lipophilic, van der Waals, and electrostatic interactions. The main function of the lipid bilayer is to form a barrier between the two sides of the membrane. Because lipid bilayers are composed of lipophilic fatty acid chains, charged ions, and multipolar molecules, they face problems in transport across the intestinal epithelial layers. For a lipophilic molecule to be transported through the lipid bilayer, it must move from a hydrophilic aqueous medium to a lipophilic medium and back into the aqueous medium. Therefore, the intrinsic permeability of small lipophilic molecules is related to the solubility of the molecule in lipophilic vehicles or solvents and the solubility of the molecule in polar media. Lipophilic drugs are the best candidates for lipid-based delivery systems (in addition to exhibiting a positive postprandial "food effect") and have the advantage of increasing absorption through the intestinal wall when compared to those formulated without lipid-based delivery. As depicted in Fig. No. 2

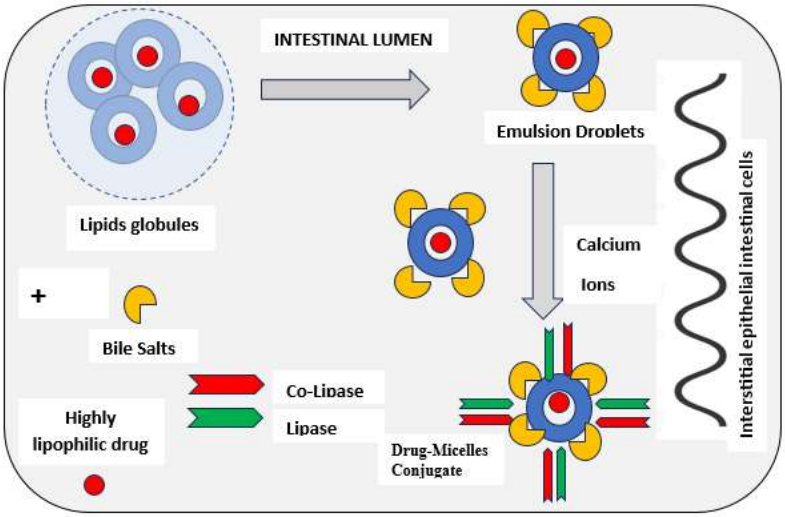

Fig. No. 2: In-situ emulsification process of lipids/oil

Drug-loaded micelles, when entering the intestinal lumen through the oral route and reaching the duodenum region of the GUT, meet pancreatic lipase and colipase to form a compound structure in the presence of calcium ions called "*emulsified oil globules conjugated with drug."* Bile salts, such as sodium taurocholate (as depicted in Fig no. 3), are ionized in nature and selectively bind to the micelle surface, thereby reducing its surface tension, so that the structure can readily undergo the emulsification process. The stability of such emulsified micelles depends on many factors, but is not limited to :-

i. Stochiometric ratio of drugs to lipids
ii. nature and HLB value of the lipids chosen.
iii. Dielectric constant of the lipids chosen
iv. Solubility of a lipophilic drug in lipids

v. temperature at which drug-micelle conjugate is formed
vi. Size and mol wt. of the lipids
vii. Physiological state of the patient as bile concentration may alter from person to person and vary in different GUT disorder conditions (if any).

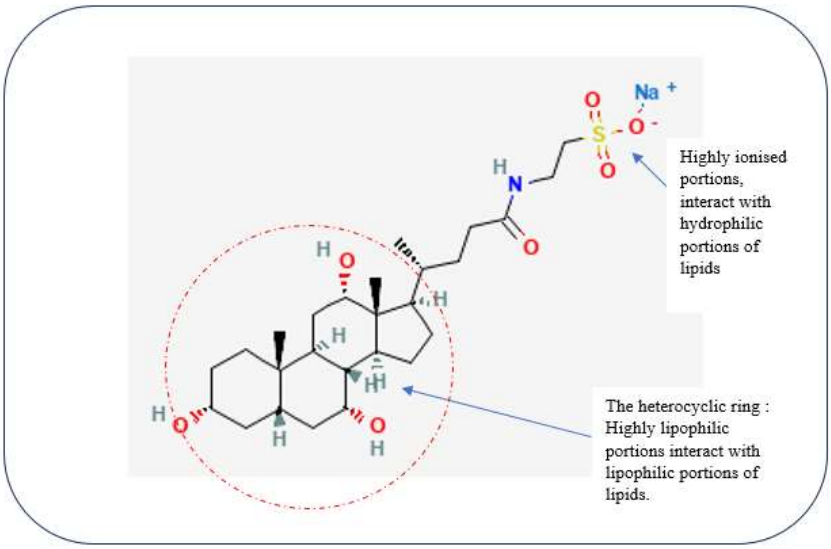

Fig No. 3 : Structure of sodium taurocholate.

All the above factors would determine the in-situ stability of the drug-loaded micelle during the digestion process and would also determine which pathway of internalization favors it most. Among the different internalization processes depicted in Fig. 4.

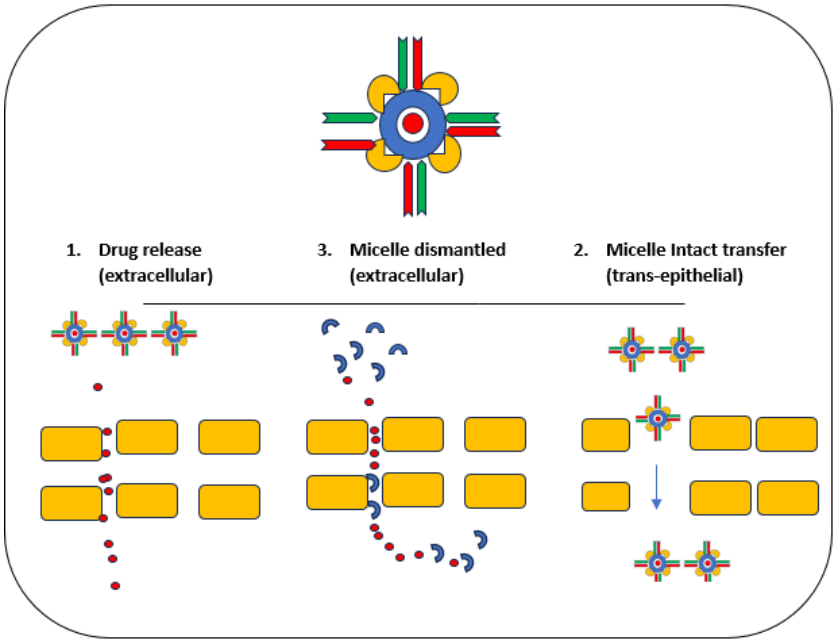

Fig No. 4 : Diagramatic represntation of the Insitu micelle internalisation process

The main pathway for micelle internalization (i.e., the process of movement of intact drug-loaded micelles into the intestinal epithelial cells, which is called "endocytosis") is based on micelle interactions with the intestinal epithelial layer, followed by the uptake in cells and the transport into endosomes. This pathway has high potential for target-specific drug delivery applications nowadays. We will now discuss the different internalization processes.

6. Drug Release at the Surface of the Epithelial Layer (Apical Side)

The intestinal epithelial cells, which look like "brush borders," also contain a thick mucus layer. This mucous layer is created by the exudate of goblet and mucous cells present in the epithelial lining of the intestine at the apical side of the membrane. The purpose of the mucous layer is to not only act as a defensive mechanism but also aid the immunity of the body by not allowing any pathogenic bacteria or viruses to infect the wall of the intestine. The outermost layer of mucous is thin, and as one moves inside towards the apical epithelial surface of cells, the mucous becomes thicker. The viscosity of the mucous layer is because of the mucin protein, which forms the structured network (as per Fig. 5).

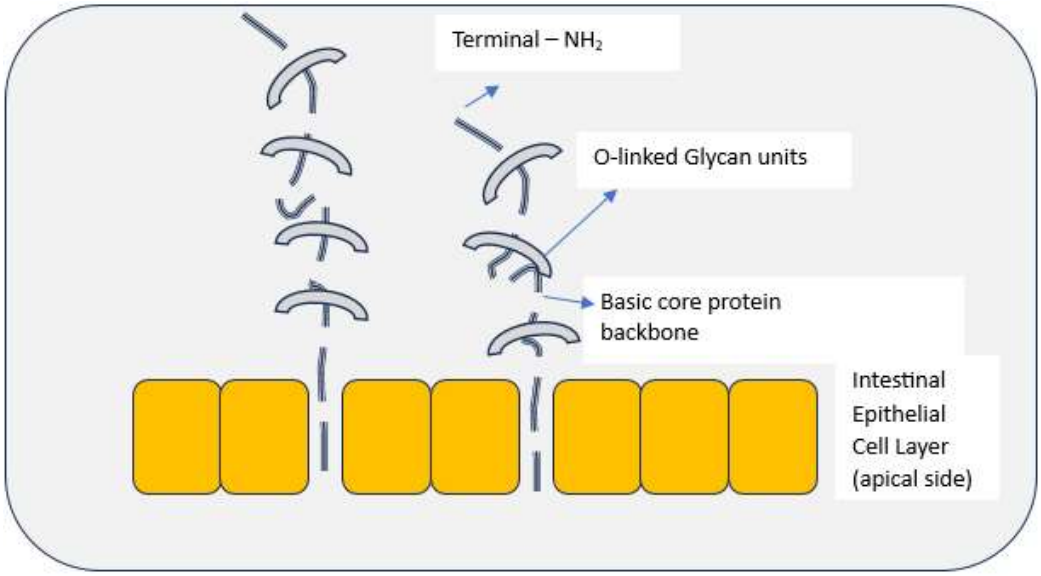

Fig No. 5: Pictorial representation of mucin protein

In the internalization process, the affinity or bond between drug lipids are not strong enough to withhold the lipid digestion process, which occurs naturally within the intestinal lumen. The bile salts induce a reduction of surface tension over the surface of the emulsified oil globules, and lipase and colipase induce lipolysis at the surface of the epithelial cell layer, owing to which the drug particles enter through the epithelial layer. Lipid formulations of the classification type-1 category, which are basically oil-miscible in nature and do not contain any lipid surfactant in the formulation, may undergo lipolysis and lead to the precipitation of drug particles at the surface of the epithelial layer of the intestinal lumen. And drugs that are available right at the transcellular junction get transported across and enter the cytoplasm. Lipid formulations of the classification type-2 category, wherein there is an exclusively lipophilic surfactant, may have a relatively stronger integrity as the entire micelle has more lipophilic portions due to the use of water-insoluble surfactants, which need more time to get lipolyzed or digested. Lipid formulations of the classification type-3 category, wherein there is the presence of both hydrophilic and lipophilic surfactants used, have a more balanced structure of hydrophilic and lipophilic portions, and hence may not need very high concentrations of bile salts to wet and hence may undergo lipolysis or digestion relatively easier than classification type-2 and classification type 1. Lipid formulations of the classification type-4 category, wherein cosolvents and exclusively hydrophilic surfactants are used, may not also need a very high concentration of bile salts to wet and may escape the intercellular space of the intestinal epithelial cells and pass through intactly without being digested or lipolyzed in the lumen. Such drug-loaded micelle formulations usually disperse to

form micelles. Hence, the type-4 category of LFCS is ideal for target-specific drug delivery to specific desired sites of the body. In addition, those drug molecules that are prone to pre-systematic metabolism in the GUT lumen are the best candidates for a lipid-based drug delivery system, as the formation of micelles in situ would prevent the first-pass effect. In addition, such drug-loaded micelles have a higher affinity for the lacteal (i.e., lymphatic vessels in the intestine) and hence partition to absorb through the lymphatic circulatory system. In this way, drugs that are lipophilic, have less aqueous solubility, and exhibit a first-pass effect are ideal candidates to formulate using a lipid-based drug delivery system if the problem of poor oral bioavailability is intended to be solved.

Such drug-loaded micelles, which enter the cytosol of the enterocytes, get transported to the Golgi apparatus via the endoplasmic reticulum. From the Golgi apparatus, the intact drug-micelles partition between the cytosol of the enterocytes and the lacteal capillaries of the lymphatic circulatory system. In general, the lymphatic system is more lipophilic when compared to the systematic circulatory system; hence, micelles are more affinized to the lymphatic system and get absorbed directly into it. Pouton, in 2008, mentioned the same in his publication[8,9].

In cases where there is a tumor (metastasis stage) drained into the lymph nodes, conventional anti-cancer drugs fail to reach the target site in abundance as they lack lymphatic absorption in the absence of lipids in the formulation. However, when anti-cancer drugs are formulated using water-soluble surfactants and cosolvents (i.e., especially classification type-4 category LFCS), the in-situ "anti-cancer-loaded micelles" formed get directly absorbed through the lymphatic system to reach the lymph nodes to counter the cancerous cells. A similar study was done in March 2023 by Xuan He et al., wherein paclitaxel was formulated using suitable lipids and administered to cancerous patients suffering from cancerous lymphatic metastasis[10].

7. The Future of Lipids in the Target Drug Delivery System

In 2008, Liu et al.[11] mentioned colloidal drug delivery systems, wherein different natural and synthetic polymers were used as carriers to conjugate with anti-cancer drugs to achieve less opsonization by the reticulo-endothelial system. Similarly, in 1997, Sharma and Sharma[12] mentioned liposomal formulations (spherical lipid vesicles), which are basically known today as the first generation of lipid-based nanoparticle drug carriers.

References

[1] World Malaria reports of WHO. World Health Organization ; 2020 ; Nov. p 221.

[2] Ménard R, Tavares J, Cockburn I, Markus M, Zavala F, Amino R. Looking under the skin: the first steps in malarial infection and immunity. Nat Rev Microbiol. 2013 Oct;11(10):701-12.

[3] Beavogui AH, Camara A, Delamou A, Diallo MS, Doumbouya A, Kourouma K, Bouedouno P, Guilavogui T, Dos Santos Souza S, Kelley J, Talundzic E, Fofana A, Plucinski MM. Efficacy and safety of artesunate-amodiaquine and artemether-lumefantrine and prevalence of molecular markers associated with resistance, Guinea: an open-label two-arm randomised controlled trial. Malar J. 2020 Jun 24;19(1) : 223.

[4] World Health Organization. Guidelines for treatment of malaria ; 2015 ; Nov. p. 313.

[5] Ecker A, Lehane AM, Clain J, Fidock DA. PfCRT and its role in antimalarial drug resistance. Trends Parasitol. 2012 Nov;28(11):504-14.

[6] Peters PJ, Thigpen MC, Parise ME, Newman RD. Safety and toxicity of sulfadoxine/pyrimethamine: implications for malaria prevention in pregnancy using intermittent preventive treatment. Drug Saf. 2007;30(6):481-501

[7] Santos-Magalhães NS, Mosqueira VC. Nanotechnology applied to the treatment of malaria. Adv Drug Deliv Rev. 2010 Mar 18;62(4-5):560-75.

[8] Pouton CW. Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. Eur J Pharm Sci. 2006 Nov;29(3-4):278-87.

[9] Pouton CW, Porter CJ. Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. Adv Drug Deliv Rev. 2008 Mar 17;60(6):625-37.

[10] He X, Deng T, Li J, Guo R, Wang Y, Li T, Zang S, Li J, Zhang L, Li M, He Q. A core-satellite micellar system against primary tumors and their lymphatic metastasis through modulation of fatty acid metabolism blockade and tumor-associated macrophages. Nanoscale. 2023 May 11;15(18):8320-8336.

[11] Liu Z, Jiao Y, Wang Y, Zhou C, Zhang Z. Polysaccharides-based nanoparticles as drug delivery systems. Adv Drug Deliv Rev. 2008 Dec 14;60(15):1650-62.

[12] Sharma A, Sharma US. Liposomes in drug delivery: Progress and limitations. International Journal of Pharmaceutics. 1997 Aug 26 ;154(2):123–40.