LIPID NANOPARTICLES: SITE SPECIFIC TARGETING AND TOXICOLOGICAL IMPLICATIONS

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ABSTRACT

Lipid based nanoparticulate drug delivery system can be explored as potential vehicle for site specific drug delivery to the various organs/tissues/systems such as lymphatic, brain, lung and skin. Recently, lipid nanoparticles are used for targeting the drugs to the oral mucosa for local therapeutic effects. Lipid nanoparticles (lnps) can help in enhancing the efficacy and lowering the toxicities of anticancer drugs to treat the tumors particularly in lymph after metastases of tumors. Cellular uptake of drugs can also be enhanced using lnps and therefore, lnps are the ideal carrier for treating intracellular infections. Lnps are more effective to treat parasitic infection particularly in the brain. However, the same unique physical and chemical properties that make nanoparticle so attractive may be associated with their potentially calamitous effects on cells and tissues. Thus, in the present review article possible toxic implications of lipids at nanoscale is also discussed in addition to its applications in site specific targeting.

Keywords : *Lipid nanoparticles, targeting, metastases tumors, toxicological implications*

Introduction

The foremost objectives for developing novel drug delivery system are to target the delivery of drug at the site of action, prolong the release of drug and improve the therapeutic benefit-risk ratio. One of the promising and exciting drug delivery system which attracts the many researchers is nanoparticulate based drug delivery systems. Nanoparticles used in the drug delivery system can be further classified as metallic nanoparticles, polymeric nanoparticles and lipid nanoparticles. The major concern with the metallic and polymeric nanoparticles is the toxic effects of metals and polymers used in preparation of nanoparticles. The lipids used in the preparation of lipid nanoparticles (LNPs) are included in the category of GRAS (Generally Recognized as Safe) substances (1). Moreover, preparation of LNPs at large scale can be possible by using high pressure homogenization method (2-3). Some other features of LNPs include good tolerability, controlled drug release, stability (stabilized by surfactants), protection of drugs liable to degradation and site specificity (1, 4-5). These advantages of LNPs enticed the researchers to investigate them as site specific drug delivery system. LNP are transported to the systemic pool via intestinal lymphatics and thus avoid the hepatic metabolism of drugs and enhances the bioavailability of drugs (6-7).

LNPs are intensively studied for targeting drug molecules to skin strata (8-10). Corticosteroids loaded solid lipid nanoparticles (SLN) is accumulated in the upper layer of skin and avoids the side effects of corticosteroids (11). Thus, LNPs can be useful to treat many diseases such as microbial and viral infections associated with epidermis and dermis of skin (9).

LNPs have potential to enhance the internalization of drug into cell due to membrane affinity of lipid material and their submicron size (12). Cellular internalization of LNPs ascribe to the weakness of lipid–lipid interactions, leading to a mosaic of dynamic, reversible lipid domains in the bilayer of biological membranes (13).Thus, LNPs facilitated the accumulation and sustained the release of drug in brain tissues and also in cytoplasm. Accumulation of LNPs in cytoplasm is useful for drugs having targets in cytoplasm such as antiviral agents which act by inhibition of viral reverse transcriptase in cytoplasm (14). Further, surface of lipid nanoparticles can be modified and employed to increase the specificity towards cells or tissues of brain to improve the bioavailability of drugs in brain (15-16). Brain targeting potential of SLN can be very useful for the treatment of CNS stage of parasitic infections (17).

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Several antitumour agents act on nucleus. LNPs can be accumulated near to the nucleus and also functionalized for active targeting to tumor cells (18-19). Further, metastases of tumors to lymphatic system can be effectively treated by lipid nanoparticles (20).

Therefore, the focus of the present article is to review the diverse applications of lipid based nanoparticulate drug delivery system to the specific sites or tissue or cells. However, the same unique physical and chemical properties that make nanoparticle so attractive may be associated with their potentially calamitous effects on cells and tissues. Thus, possible toxicological implications of lipids at nanoscale are also discussed.

Lymphatic targeting

Lymphatic targeting has attracted lot of attention for providing a preferential anticancer chemotherapy, improving bioavailability of drugs that undergoes hepatic first pass metabolism and achieving mucosal immunity. Many particulate systems such as emulsions, lipid solution, micro emulsions, micellar solutions, liposomes, polymeric nanoparticles and lipid nanoparticles are investigated for targeting of drugs to the lymphatic system (7, 21-25). Solid lipid nanoparticles composed of particularly triglycerides resembles to the chylomicrons and may alter the absorption behaviour of drugs including avoidance of first pass metabolism (6). LNPs, composed of nonpolar core and polar heads oriented to the aqueous phase, promote the absorption of lipophilic drugs into intestinal lymphatic system and organs. Although, lipid emulsions also resembles to the chylomicrons but are not able to sustain the release of drug as solid lipid nanoparticles. Liposomes are also comparable to the solid lipid nanoparticles in lymphatic targeting but degradation of liposomes by intestinal lipases prohibits their use via oral route (26).

Bargoni et al., (1998) reported that LNPs were found to be stable at gastric physiological pH, even after 180 minutes of administration, and suggested them as a suitable carrier system for lymphatic drug delivery. Thus, solid lipid nanoparticles can be utilized as an ideal carrier system to provide prolonged release and target the drugs to lymphatic system which is the site of many diseases such as metastatial tuberculosis, cancer and filiariasis (27).

The size and composition of LNPs can play an important role in uptake pathway. Size is important for achieving high efficiency in targeting as it is established that carrier particles less than 100nm in size are preferable for targeting to regional lymph nodes (28).

Lymphatic targeting can also provide an effective anticancer chemotherapy to prevent the metastasis of tumor cells by accumulating the drug in the lymph nodes. 50-60% of the breast cancer patients are found to be suffered from lymph node metastases in armpit. Thus, lymph node metastasis is a serious problem in case of breast cancer and difficult to treat (29). Mitoxantrone, an anticancer agent used in breast cancer, loaded solid lipid nanoparticles having size less than 100 nm achieved high efficiency in the targeting of the drug in lymph nodes (20). In addition to targeting, LNPs can also change the pharmacokinetic profile of anticancer drugs which helps in reduction of toxicities of drug e.g. solid lipid nanoparticles reduce the cardiac and nephric toxicity of temozolomide (30).

Many times chemotherapy fails to treat tumor because of development of resistance in tumor cells due to expression of an important transporter protein, known as P-glycoprotein (P-gp). P-gp reduced the overall concentration of drug in the tumor cells. Dong et al., (2009) evaluated the ability of Doxorubicin and Paclitaxel loaded lipid nanoparticles to overcome multidrug resistance by inhibiting P-gp (31). They proposed the two following reasons for enhanced cytotoxicity of anticancer loaded lipid NPs in P-gp mediated resistance cell

1. Increased uptake of drug (endocytosis of NPs)
2. Decreased efflux rate of drug through inhibition of P-gp function and ATP depletion caused by Brij (surfactant), a component of nanoparticles.

Recently, Goutayer et al., (2010) made lipid nanoparticles functionalized for active targeting to tumors. Tumor targeting ligand such as cRGD (cyclic Arg-Gly-Asp) peptide is able to bind with $\alpha v\beta 3$ integrins (receptors over-expressed on the angiogenic vessels grown during tumor development) and improve the accumulation of

functionalized LNPs in tumor than that of non functionalized LNPs (19). Teskac and Kristl, (2010) established that LNPs could also provide benefits by delivering anticancer drugs like resveratol locally near the nuclear target site (18).

Brain targeting

Drug delivery to the brain is limited due to presence of Blood Brain Barrier (BBB) which restricts the delivery of wide variety of drugs to the brain. BBB consist of endothelial cells with tight junctions that lines cerebral capillaries. Thus, tight junctions in epithelium of brain endothelial eliminate a paracellular pathway of solute across the BBB. In addition to tight junctions, many efflux transport pathways like P-glycoprotein and active organic acid are present in brain endothelial cells to remove unwanted substances (32). LNPs have higher affinity towards brain endothelial cells and can also overcome the problem of efflux transport by inhibiting the P-gp glycoprotein.

The brain targeting of LNPs can be very useful for the treatment of CNS diseases such as brain tumors, AIDS, CNS stage of many parasitic diseases, other neurological and psychiatric disorders (33-34). CNS stage of trypanosomiasis and even cerebral malaria can be targeted easily and treated effectively by using lipid nanoparticles (15-16). LNPs not only help the drug moiety to pass the blood brain barrier but also enable to enhance the intracellular uptake of drug which results in the eradication of intracellular parasitic infections (35). LNPs of β-artemether were fabricated in order to hasten its uptake and prolong its release. These LNPs were dispersed in aqueous phase which eliminate the problem of severe pain associated with oily injection (for intramuscular delivery) of Artemether (36-37).

Active targeting has emerged as a major breakthrough in CNS and Neuro-oncology drug delivery. Active targeting of lipid nanoparticles towards the brain can be achieved by modifying the surface of LNPs with target specific recognition moieties such as thiamine, transferrin and other ligands capable of recognizing brain capillary endothelial cells (15, 38-39).

Skin targeting

LNPs draw lot of attention for developing topical products for medicinal as well as cosmetic purposes. Dermatologists have five different targets such as surface of skin, stratum corneum, skin appendages, dermis and systemic circulation for treating disorders associated with skin. LNPs provide edge for delivering the drugs to the first four targets and avoid the systemic circulation.

Mechanism of enhancing penetration of drug into the skin from lipid nanoparticles involved occlusion effect and film formation of lipid nanoparticles on the skin. The occlusion effect of lipid nanoparticle can greatly influenced by its particle size and crystallinity of the particles (40). LNPs showed 15 times higher occlusion effect than that of microparticles. Nanoparticles provide larger surface area and contact points to adhere the skin layer than that of microparticles. LNPs can provide benefits of accumulation of drug in the skin strata to treat various skin diseases such as acne and eczema as reviewed by Korting et al., (41).

Drugs required on the surface of skin include UV protectant (sunscreen), insect repellants and antimicrobials. Lipid nanoparticles can also act as UV sunscreen system (42). Placebo LNPs showed greater UV-blocking efficacy than emulsions containing tocopherol acetate as the molecular sunscreen. Incorporation of tocopherol acetate into SLN leads to an over additive UV-blocking effect (43). Inorganic sunscreen can also encapsulate or bonded to lipid nanoparticles which enhance the UV protection properties of both. The skin targeting potential of lipid nanoparticles was investigated for many drugs belong to different classes such as podophyllotoxin, indomethacin, isotreonin, retinol, miconazole, prednicarbate and betamethasone 17- valerate (9-10, 44-47). Lipid nanoparticles favor drug penetration into skin and provide sustained release to avoid systemic absorption (48).

LNPs also reduce the irritation of the drugs. Encapsulation of tretinoin in LNPs resulted in remarkably less erythematic episodes as compared to currently marketed tretinoin cream. Thus, LNPs based tretinoin gel can offer improved topical delivery of tretinoin as compared to existing conventional tretinoin formulations in terms of skin tolerability by patients and would be a viable alternative for them (49). In past years, many topical products based on lipid nanoparticles were launched in market (Table 1).

Table 1 Lipid Nanoparticulate based topical products in market

Product Name	Producer/Distributor	Main active Ingredients
Nano Lipid Restore	Chemisches Laboratorium Dr. Kurt Richter, CLR Berlin	Coenzyme Q-10 and omega unsaturated fatty acids
NLC Deep effect	Beate Johnen	Cocunut oil, tamanu tree extract,
Intensive Serum Nanorepair Q-10	Dr. Rimpler Gmbh	Q-10, Polypeptide, Mafane extract (Anti-wrinkle effect)

Pulmonary targeting

Pulmonary drug delivery system is the most suitable route of drug administration to treat respiratory diseases and noninvasive mean to provide local lung effects. Pulmonary drug delivery system also provides high systemic bioavailability of drugs undergoes first pass metabolism due to avoidance of metabolism and high surface area for absorption. Many colloidal drug delivery systems such as microspheres, polymeric nanoparticles, emulsion and liposomes are investigated for delivery of drugs targeting to the lungs. Microspheres have been extensively investigated for their lung targeting effects but safety of lung targeting microspheres is uncertain as microsphere with diameter more than 5 micron may block the blood capillaries and causes chronic obstructive emphysema (50). Recently, attention has been focused on solid lipid nanoparticles (SLNs) (1, 51), which potentially have the advantages of both liposomes and microspheres while avoiding some of their disadvantages (52).

Tuberculosis is caused by *Mycobacterium* organisms which posses cell walls impermeable to most of the antibacterial and make these diseases chronic and necessitate prolonged treatment. Moreover, its incidence is increasing due to the AIDS epidemic (53). Mycobacteria grow slowly and may be dormant in the host for long periods; thus, they are relatively resistant to the effects of antibiotics. Further, a portion of mycobacteria can reside inside macrophages, adding another permeability barrier that effective agents must cross. Intracellular trafficking of solid lipid nanoparticles is allowing an efficient cytosolic delivery system (51, 54). This characteristic is important for the range of therapeutic targets, which could be located in the cytosol or at a deeper level within intracellular organelles (such as causative agents of tuberculosis reside in macrophages). Thus, solid lipid nanoparticles can act as a novel carrier to enhance the efficacy of antibiotics against resistant bacteria of tuberculosis and also achieved sustained release of drugs at the site of action (26).

Solid lipid nanoparticles can also assist in targeting of drugs to the lymph nodes present in lungs as observed by deposition and clearance of SLNs after inhalation of aerosolized insoluble particles using gamma-scintigraphy imaging analysis (55, 56). Thus, inhalation can be an effective route to deliver drug-containing LNPs to the lymphatic systems and used as potential drug carriers for lung cancer therapy, as well as for vaccine delivery.

Oral mucosal targeting

Most of the oral mucosal delivery systems are based on aqueous gel as delivery vehicles. Other delivery system such as oral rinses, local injections and mucoadhesive patches are also frequently used in oral mucosal delivery of the drugs. However, formulation of poorly water soluble compounds and unstable compounds in aqueous based topical bases are most challenging. These challenges can be overcome by LNPs based formulations. Holpuch et al., (2010) demonstrated, for first time, that monolayer human oral squamous cell carcinoma (OSCC) cells internalize LNPs and these nanoparticles penetrate to the basal and superficial underlying connective tissue layers of intact normal oral epithelium (57). This finding suggests that LNPs can be used as novel carrier to target therapeutics to treat diseases associated with oral mucosa.

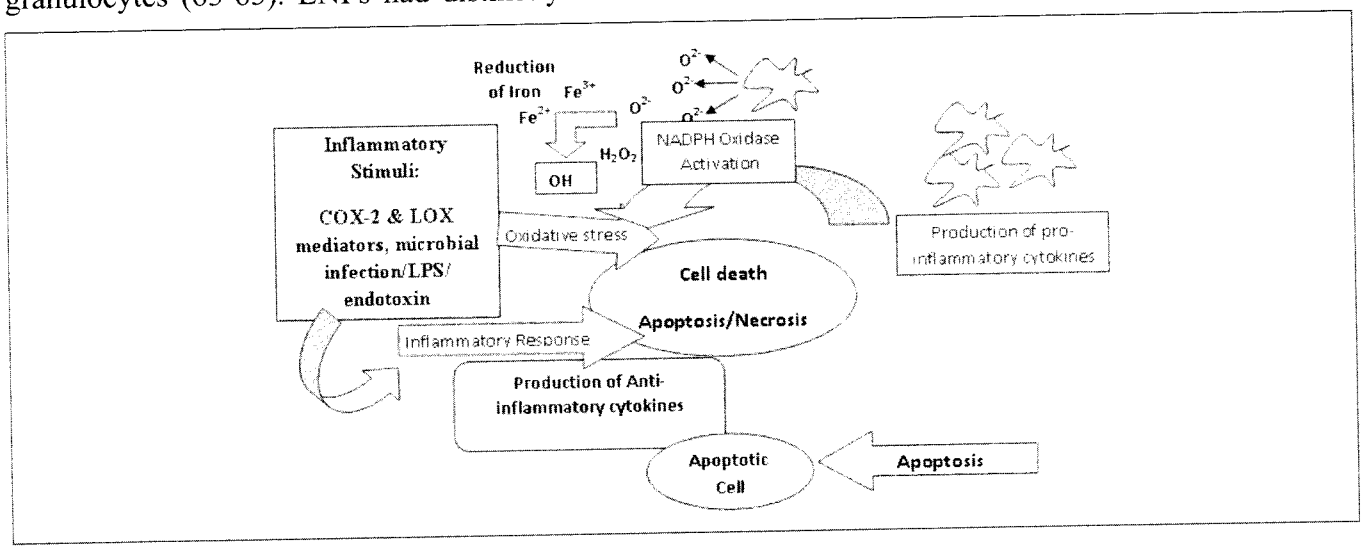
Toxicological implications

As the use of nanomaterials increases worldwide, concerns for worker and user safety are mounting. Immune system recognizes nanoparticles as foreign body and tries to destroy them. Thus, nanoparticles can activate the immune system and induce inflammation, cause allergy and other immune responses. Therefore, studies are needed in order to know the potential deleterious or beneficial effects of nanoparticles in the immune system.

Nanoparticle's initial target for cytotoxic effects is type I epithelial cells whose necrotic death stimulates a proinflammatory response and recruitment of inflammatory cells (58). The major pathways involved in this process are explained in Fig.1. LNPs show comparatively less inflammatory responses in terms of cytokine production. Few reports on interaction of Dynasan 114 SLN with peritoneal mouse macrophages support the above finding (59-61).

Olbrich et al. reported that LNPs formulations were well tolerated with no significant tissue toxicity (62). There are other reports which studied the interaction of LNPs and their respective cytotoxicities with different granulocytes (63-65). LNPs had distinctly lower uptake by phagocytosis resulting in prolonged circulation time in blood. Additionally, LNPs shows 10-fold low cytotoxicity in comparison to polymeric nanoparticles (63). Thus, LNPs can be used as intravenous carriers because of their prolonged circulation time and high toxicological acceptance.

Scholer et al. showed that the cytotoxicity of LNPs is dependent on concentration of LNPs as IL-6 production is based on the concentration of the lipid (64). However, the cytotoxicity was independent of the size of LNPs. The result of histopathological in vivo study suggests that the toxicity is dependent on the lipid matrix as well as the dose administered. Toxicity of LNPs made of two different lipids (compritol and cetyl palmitate) was evaluated by *in vivo* experiments (66). Two types of LNPs, one made from Compritol (GRAS approved) and the other made from Cetyl palmitate (less physiologic) were injected at very high dose, six times within a period of 20 days. Results of this study show that high dose compritol-containing formulation led to the accumulation of lipid in liver and spleen of mice and results in subsequent pathological alterations. But, no untoward results were obtained with cetyl palmitate LNPs. The alterations with compritol-containing formulation



were reversible within six weeks after intravenous administration. This difference is attributed to faster degradation of cetyl palmitate than that of compritol in in-vivo study. The further reduction in the compritol dose to 0.25 g (still high dose) all these observed alterations did not occur any more. It was further reported in another study that the cell toxicity was found to be dependent on the composition of LNPs and the method of purification used (67).

Conclusion and future perspectives

In current scenario, emphasis is given on the development of carrier for site specific drug delivery. Lipid nanoparticles possess many advantages along with its ability to deliver the drugs to specific sites. Lipid based nanoparticles could be able to deliver the drugs to the resistant tumors which develop due to expression of P-gp in tumor cells. Metastases of cancer in the lymph nodes could also be treated by the lymphatic targeting of lipid based nanoparticles, which otherwise is very difficult to treat. Thus, lipid based nanoparticles could be explored to treat the diseases for which conventional dosage form is not suitable or has lower therapeutic effect. Targeting capability of LNPs to the oral mucosa opens the possibility to treat the diseases associated with oral cavity in efficient manner. Active targeting of a drug is achieved by conjugating a nanocarrier system (drug loaded) to a tissue- or cell-specific targeting ligand. Active targeting has raised the importance of nanomedicine and this can now be achieved by a number of specific interactions, such as ligand-receptor binding. These specific interactions result in preferential accumulation of nanomedicine into molecular targets. However, the biocompatibility of lipid nanoparticles with blood components and other tissues is essential for the successful regulatory clearance of lipid nanoparticles. This necessitates the need for studying the toxicological implications of lipid nanoparticles.

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