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Review Article

Theme: Lipid-Based Drug Delivery Strategies for Oral Drug Delivery
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Potential of Lipid Nanoparticles (SLNs and NLCs) in Enhancing Oral Bioavailability of Drugs with Poor Intestinal Permeability

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Abstract. Lipid-based drug delivery systems has become a popular choice for oral delivery of lipophilic drugs with dissolution rate limited oral absorption. Lipids are known to enhance oral bioavailability of poorly water-soluble drugs in multiple ways like facilitating dissolution as micellar solution, enhancing the lymphatic uptake and acting as inhibitors of efflux transporters. Lipid nanoparticles are matrix type lipid-based carrier systems which can effectively encapsulate both lipophilic and hydrophilic drugs. Lipid nanoparticles namely solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are versatile drug delivery system and can be used for multiple routes of delivery like parenteral, topical, ocular, transdermal, and oral. Lipid nanoparticles are particularly attractive vehicles for peroral delivery of drugs with oral bioavailability problems as they are composed of lipid excipients which are cheap, easily available, and non-toxic; manufacturing technique is simple and readily scalable for large-scale production; the formulations provide controlled release of active components and have no stability issue. A large number of drugs have been incorporated into lipid nanoparticles with the objective of overcoming their poor oral bioavailability. This review tries to assess the potential of lipid nanoparticles for enhancing the oral bioavailability of drugs with permeability limited oral absorption such as drugs belonging to class IV of Biopharmaceutic Classification System (BCS) and protein and peptide drugs and also discusses the mechanism behind the bioavailability enhancement and safety issues related to such delivery systems.

KEY WORDS: lipid; nanoparticles; SLN; NLC; oral bioavailability; permeability; toxicity.

INTRODUCTION

The peroral route of drug delivery has remained the most desirable route of drug delivery despite of recent advances in alternate routes of drug delivery as it is the most convenient, easiest, and cheapest way of non-invasive administration. Delivery by this route for a number of drugs still poses a great challenge to the formulation scientists. According to the Biopharmaceutic Classification System (BCS) put forward by Amidon et al. (1), for any drug, its solubility in the gastrointestinal fluid and permeability across the biological membrane are the key parameters affecting oral bioavailability of drugs. Potential drug candidates developed with the help of high-throughput screening methods generally have higher molecular weights and tend to be lipophilic in nature (2). Other factors contributing to low oral bioavailability of drugs are low stability in the gastrointestinal environment and poor membrane permeability. Many drugs are substrate to intestinal efflux transporters like p-glycoprotein resulting in poor oral bioavailability (3). In the past few decades, lipid-based drug delivery techniques have emerged as a leading strategy to overcome the solubility and permeability issues associated with drugs having oral bioavailability problems. The effect of dietary lipids on oral bioavailability of lipophilic drugs has been well documented for lipophilic drugs like cyclosporine, griseofulvin, and halofantrine (4). High-fat meal increases gastrointestinal residence time, stimulates secretion of bile and pancreatic enzymes, stimulates lymphatic transport, increases permeability of intestinal wall, decreases presystemic metabolism, and alters blood flow rate to the mesentery and liver, leading to an improvement in oral bioavailability of drug (5,6). The different novel lipid-based formulations that have been developed include micro and nanoemulsions, self-emulsifying formulations, liposomes, lipid nanoparticles, and lipid-drug conjugates. Among these, nanoparticulate lipid formulations have generated

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considerable interest as they incorporate the interesting properties of nanoparticles with non-toxic and low-cost lipid excipients. Solid lipid nanoparticles (SLNs) were described for the first time in 1991 and the technology is currently owned by SkyePharma (7). The nanostructured lipid carriers (NLCs), which are considered to be the next generation of lipid nanoparticles, were developed in the early twenty-first century (8). The main differentiating feature of the SLN and NLCs lies in the physical properties of the lipids used in their composition. SLNs generally contain a single lipid or a mixture of lipids which do not melt at room temperature and at physiological temperature. On the other hand, NLCs are composed of solid lipid mixed with a liquid lipid (oil). The resulting nanoparticles prepared by this blend remain solid at temperature up to about 40°C (7). The advantages associated with lipid nanoparticles are high drug loading capacity (9,10), feasibility of entrapping both hydrophilic and hydrophobic drugs (11), long shelf-life (12–14), possibility of extended drug release, and ease of scaling-up for large-scale manufacturing (15). The lipid nanoparticles are versatile drug delivery systems and can be utilized for topical, transdermal, and parenteral routes besides oral administration. The potential of SLNs in enhancing oral bioavailability of drugs has been reviewed earlier (16,17). The application of NLCs in oral delivery of different drugs has also been reviewed (18,19). The main focus of this review article is to analyze the potential of SLNs and NLCs in oral bioavailability enhancement of drugs with poor intestinal permeability, *i.e.*, BCS class III and class IV drugs. The various mechanisms by which SLNs and NLCs may modulate the oral absorption of poorly permeable drugs; formulation aspects affecting bioavailability of such drugs and the safety aspects of orally administered lipid nanoparticles are also summarized here.

EXCIPIENTS USED

The main three components used in the production of SLNs are solid lipid/s, emulsifying agent/s and water. Classes of lipids that are generally used include triglycerides (*e.g.*, tripalmitin, tristearin, trilaurin), partial glycerides (e.g., Witepsol 85E, Imwitor, Compritol® 888 ATO), fatty acids (*e.g.*, palmitic acid, stearic acid), hard waxes (*e.g.*, gleceryl mono stearate, glyceryl behenate), sterols (*e.g.*, cholesterol). Emulsifiers that can be used in SLNs intended for oral delivery should be generally regarded as safe (GRAS) and can include phospholipids and nonionic surfactants. A detailed account of excipients used in the formulation of SLNs and NLCs and corresponding production techniques have been reviewed in detail elsewhere (19,20).

The main disadvantage associated with SLNs which necessitated the advent of NLCs was the fact that drug entrapment capacity of SLNs is affected by the polymorphic form of the solid lipid. If the SLNs are consisting of single lipid of high purity, a perfectly crystalline lattice may form during storage, which may result in decreased solubility of the drug in the lipid matrix and ultimately expulsion of the encapsulated drug. When SLNs are formed, lipids crystallize in disordered α and β′ crystalline structures. During storage, the lipid molecules gradually order themselves in a more stable ordered structure resulting in generation of the βi and β crystalline forms from which the entrapped drug may be expelled (21).

NLCs were designed to overcome this limitation by having a controlled nanostructure of the lipid particle matrix where the matrix structure would be as imperfect as possible. This may be achieved by using a blend of solid lipid and an oil which are spatially very different. Three distinct classes of NLCs can be formed according to the nature of lipids used. The type I NLC consists of a highly disordered, imperfect lipid matrix structure which can accommodate dissolved as well as dispersed drug molecules. This type of structure forms due to the difference in structure of solid lipids and liquid lipids and conditions during the crystallization process (22). The type II NLCs consist of a high concentration of liquid lipids (oils). During the cooling stage of the production process, a miscibility gap is created between the solid lipid and the oil which leads to phase separation and precipitation of nano sized oil globules in the solid lipid matrix. High drug loading capacity can be achieved with type II NLCs as drug solubility is commonly more in oils than solid lipids. The type III NLCs are composed of an amorphous solid lipid matrix. The formation of the amorphous matrix is aided by use of lipids such as hydroxyoctacosanylhydroxystearate, isopropylmyristate, dibutyl adipate *etc.* (19).

Commonly used oils in NLCs are vegetable oils and oils comprising mixtures of mono-, di-, and triglycerides containing fatty acids of varying chain lengths and degree of unsaturation (23,24). Many of the commonly used oils are known bioavailability enhancers. It has been reported that medium-chain triglyceride (MCT) oils are more effective than long-chain triglycerides (LCT) in terms of absorption enhancement (25).

PRODUCTION TECHNIQUES

Numerous methods have been reported for preparation of SLNs and NLCs. Among them, high-pressure homogenization (HPH) and microemulsion techniques have emerged as the most preferred methods owing to the strong possibility of large-scale adaptation of these processes (26,27). In this method, the lipid or the lipid oil mix is first melted at approximately 5–10°C above the melting point of the lipid/blend and the drug is dissolved or dispersed in the molten lipid blend. The emulsifier is dissolved in water separately and this solution, preheated at the same temperature, is then added to the drug–lipid melt and mixed using a high speed stirring device to form the pre-emulsion. This hot pre-emulsion is then subjected to high-pressure homogenization at the same temperature repeatedly until a nanoemulsion is formed. On cooling the lipid, droplets of the nanoemulsion solidify and form lipid nanoparticles with solid matrix. In another variation of the HPH method known as cold HPH, the drug–lipid melt is rapidly cooled down by means of liquid nitrogen or dry ice and subsequently milled to microparticles by suitable milling technique like ball mill. The resulting microparticles are then suspended in cold water containing a surfactant and homogenized at or below room temperature forming lipid nanoparticles. Cold HPH method is better suited for hydrophilic and/or thermosensitive drugs (20).

In the microemulsion method for the preparation of SLNs/NLCs (28–30) similarly, the drug is first dissolved or

dispersed in the molten lipid mixture followed by addition of the hot aqueous solution containing the surfactant and the co-surfactant. The mixture is mildly agitated to aid in the formation of transparent microemulsion which is then dispersed in large volume of cold water of temperature 2–10°C with mild agitation. This sudden dilution results in conversion of the microemulsion into nanoemulsion, droplets of which immediately crystallize to form SLNs/NLCs. This method uses a large volume of water (25–50 times the volume of hot microemulsion) for dilution of the hot microemulsion which can be removed by lyophilization. One of the major disadvantages of this method is the use of high concentrations of surfactant and co-surfactant which may not be desirable from the safety point of view.

Other techniques that can be used for production of lipid nanoparticles include emulsification solvent evaporation (31,32), solvent diffusion (33,34), and solvent injection (35) methods. All of these methods suffer from the drawback that organic solvents are used. A microwave-assisted one pot and one step method has been reported recently (36).

ORAL BIOAVAILABILITY ENHANCEMENT OF DRUGS WITH POOR INTESTINAL PERMEABILITY

A number of research articles have been published in the past decade which deals with improvement of oral bioavailablity of drugs belonging to Class IV of Biopharmaceutics Classification System (BCS) or are peptides/proteins. The findings of some of those studies are discussed in the subsequent section.

Cyclosporine A (CyA): CyA is highly lipophilic, has polar surface area and high molecular weight (37–39), and belongs to BCS class IV. The absorption of CyA from the gastrointestinal tract is impeded by a variety of factors including its narrow absorption window in the upper part of GI tract, P-glycoprotein-mediated efflux from enterocytes, and extensive pre-systemic metabolism in the gut wall and liver (40,41). Wang et al. compared the oral bioavailability of cyclosporine A (CyA) from different formulations, namely poly(lactic-co-glycolic acid) (PLGA) nanoparticles, NLCs, and self-microemulsifying drug-delivery systems (SMEDDS) in beagle dogs (42). The NLCs were prepared by melting emulsification method using Precirol ATO 5 as the solid lipid, Captex100 as the liquid lipid, and Tween 80 as the surfactant. SMEDDS was composed of Labrafil M 1944 CS, Cremophor EL, and Transcutol P. The relative bioavailability of the formulations was determined in beagle dogs compared to commercial Sandimmun Neoral®. NLCs exhibited maximum relative oral bioavailability of 111.8% followed by SMEDDS which had a relative bioavailability of 73.6%. PLGA NPs exhibited the smallest relative bioavailability of about 22.7%. This study demonstrated the superiority of lipid nanoparticles over polymeric nanoparticles in enhancing oral bioavailability of BCS Class IV drug CyA.

In an earlier study, Muller et al. (43) compared the oral bioavailability of CyA from SLN formulations with that from CyA nanosuspensions. The SLN formulation consisted of Imwitor®900 as the lipid and was prepared by HPH method. Oral bioavailability studies were performed for the SLN and the nanosuspension in young pigs and compared with oral bioavailability of Sandimmun Neoral®. The SLN formulation generated a mean plasma profile which had high degree of similarity to that of the control but was devoid of the initial peak concentration of more than 1000 ng/ml present in the plasma profile generated by the control, while the nanocrystals of cyA generated a mean plasma profile with very low concentrations of drug. The authors concluded that the lipids present in the SLN system play a significant role in promoting the absorption of the drug and opined that SLN has the potential to be considered as an alternative delivery system for drugs where nanosuspension fails to elicit the desired result.

Amphotericin B: Amphotericin B (AmB), an amphiphilic polyene antifungal antibiotic is considered as a model BCS class IV drug which apart from being practically insoluble in water, is unstable at gastric pH and is a substrate of p-glycoprotein (44,45). Chaudhari et al. (46) reported the preparation and evaluation of AmB loaded SLN using glyceryl dilaurate as the lipid. The SLNs were prepared by probe sonication-assisted nanoprecipitation technique. The developed formulation was found to be stable in simulated gastric and intestinal fluids. AmB was found to be in the non-toxic superaggregated form in the SLN formulation. The relative oral bioavailability of AmB-loaded SLN formulation was compared to that of Fungizone® given by intravenous route in rats and it was found to be 1.05. The findings suggested that SLNs can be a viable option for oral bioavailability enhancement of drugs with permeation limited oral absorption. Nephrotoxicity of the SLN formulations was also found to be significantly less compared to Fungizone®.

Curcumin: Curcumin is a poly-phenolic compound isolated from *Curcuma longa* which has proven to possess a myriad of pharmacologic activities like anti-inflammatory, antioxidant, anticancer, antiviral, and neurotrophic activity. Extremely poor oral bioavailability of curcumin, however, has been an impediment in its development as a therapeutic molecule. Curcumin can be classified as a BCS Class IV molecule as it is poorly soluble in water and has low intestinal permeability (47). In the Caco-2 cell lines, it was demonstrated that curcumin undergoes intestinal first-pass metabolism and gets accumulated intracellularly. Fang et al. reported preparation of curcumin NLCs using the ethanol dripping method (48). The prepared NLCs had an entrapment efficiency of more than 95% and exhibited a sustained release of curcumin *in vitro* in pH 6.8 phosphate buffer. Bioavailability of the prepared NLCs was measured in rats in comparison with a curcumin suspension. Curcumin NLCs demonstrated significant increase in both rate and extent of oral absorption compared with curcumin suspension.

Curcumin SLNs with TPGS and Brij78 were prepared, and intestinal permeability and oral bioavailability studies were performed (49). The prepared SLNs exhibited sustained release, and *in vivo* pharmacokinetic study in rats showed that the area under the curve (AUC) of curcumin from the SLNs was about 12 times higher than curcumin suspension and the relative bioavailability of SLNs was 942.53%. Results of the *in situ* intestinal absorption study revealed that the effective permeability co-efficient value of curcumin for SLNs was significantly higher compared to curcumin solution.

Baek et al. (50) reported preparation and evaluation of N-carboxymethyl chitosan (NCC)-coated curcumin SLNs. The aim of the surface coating was to suppress the fast

release of curcumin in gastric environment and increase the bioavailability. The NCC-coated SLN was found to inhibit the burst release in simulated gastric fluid whereas released curcumin in a sustained release pattern in simulated intestinal fluid. The oral bioavailability and lymphatic uptake of the NCC-modified SLNs were found to be significantly greater than that of curcumin solution leading to the conclusion that this formulation could be a superior vehicle for enhancing oral bioavailability of curcumin.

Decitabine (DCB): Decitabine, a cytidine analog, is an antineoplastic agent having poor oral bioavailability mainly attributable to its poor aqueous solubility and low intestinal permeability, as well as its rapid deamination by cytidine deaminase in the intestine (51). Decitabine-loaded NLCs were prepared by cold homogenization technique using Precirol ATO5 as a solid lipid and Transcutol HP as a liquid lipid and were optimized by the Box–Behnken experimental design (52). The entrapment efficiency of optimized NLC was found to be 84% with about 8.5% drug loading. The optimized NLCs exhibited sustained *in vitro* drug release possibly by Fickian diffusion as suggested by the release kinetics studies. Permeation of drug as obtained by *ex vivo* gut permeation study was found to increase by four times compared to the drug solution. γ-Scintigraphy imaging and MTT assay results indicated that DCB-loaded NLC had excellent cytotoxic activity against cancer cells implying that the NLCs can be potentially utilized for oral delivery of decitabine.

Tacrolimus (TL): Various factors have been reported for poor and variable oral bioavailability of tacrolimus, a macrolide lactone immunosuppressive agent, including low solubility, site-dependent permeability, extensive pre systemic metabolism in gut and liver, and Pgp-mediated drug efflux (53). Khan et al. reported preparation and *in vivo* pharmacokinetic studies of TL-loaded NLCs (54). The NLCs were prepared by modified solvent emulsification evaporation-probe sonication and modified high pressure homogenization. The NLCs consisted of a MCT and LCT based binary lipid matrix. *In vitro* lipolysis studies revealed that significantly high amount of drug was solubilized from the NLCs in aqueous phase compared to TL suspension. The *in vivo*, lymphatic, and organ distribution studies were performed in albino wistar rats and the results revealed that relative bioavailability of TL from NLCs was 7.2 times higher in comparison to TL suspension. Lymphatic transport of TL was found to be greatly enhanced from the NLCs thereby avoiding the pre-systemic metabolism.

Etoposide: Etoposide, a widely used anticancer drug, exhibits low and variable oral bioavailability. The low bioavailability is mainly attributable to the drug being a substrate for the efflux transporter, P-glycoprotein (55). Zhang et al. prepared and evaluated etoposide NLCs (56). Plain NLC containing monostearin and soyabean oil as well as surface-modified NLCs coated with polyethylene glycol (PEG) and distearoylphosphoethanolamine PEG (DSPE-PEG) were manufactured by an emulsification and low-temperature solidification method. The absorption of the NLCs in the intestine was evaluated by the diffusion chamber method and it was found that drug transport across the mucosal side to the serosal side was more for NLCs with lower particle size. *In vivo* oral bioavailability study in rats revealed that all the three types of NLCs had significantly higher oral bioavailability compared to the suspension. The DSPE-PEG-coated NLCs had the highest bioavailability and demonstrated highest cytotoxic activity against carcinoma cell lines used in the study.

Saquinavir: Beloqui et al. used saquinavir (SQV) to study NLC transport mechanisms across the intestinal barrier (57). Saquinavir is a BCS class IV drug and P-gp substrate. Three different NLC formulations consisting of Poloxamer 188, Precirol ATO® 5, and Mygliol in varying concentrations were evaluated. Their findings suggested that SQV transport across Caco-2 monolayers was 3.5 times higher from NLCs than that from SQV suspension and transport of SQV NLCs was not influenced by the M cells. Intestinal permeability, the transcytosis pathway, and the efflux of SQV by P-gp were found to be influenced by the size and concentration of surfactant in the NLCs. The same research group also assessed the effect of dextran–protamine (Dex–Prot) coating on NLCs on SQV permeability enhancement (58). Their findings suggested that Dex–Prot complex coating can enhance permeability of SQV across biological membrane to a large extent.

Lopinavir: Alex et al. reported successful encapsulation of lopinavir in glyceryl behenate-based solid lipid nanoparticles (59). SLNs which were produced by hot homogenization process followed by ultrasonication showed a slow *in vitro* release profile in both gastric and intestinal pH. Intestinal lymphatic transport study performed in rats indicated that the lymphatic uptake of lopinavir was about five times higher from SLNs compared to the drug suspension. *In vivo* bioavailability studies performed in rats suggested that oral bioavailability of lopinavir from SLNs was twice the oral bioavailability from suspension.

Docetaxel: Docetaxel (DTX) is a semisynthetic anticancer drug which is structurally similar to paclitaxel. Oral bioavailability of DTX is less than 3% as it is degraded at gastric pH, is substrate to P-gp mediated efflux in the apical membrane of intestinal epithelial cells, and undergoes cytochrome-P450 (CYP 450)-mediated pre-systemic metabolism in the liver or intestinal tract (60). Fang et al. prepared NLCs loaded with DTX by emulsification-ultrasonication using Precifac ATO 5 as solid lipid and MCT as the oil (61). The NLCs were found to be able to prevent DTX degradation in simulated gastric and intestinal fluids and to provide prolonged drug release for 48 h. An *in vivo* pharmacokinetic study demonstrated that the extent of bioavailability was about four times higher than that of DTX solution. It was also found that lymphatic transport pathway has a major influence in the absorption of DTX from NLCs.

Solid lipid nanoparticles surface engineered by Tween 80 or D-alpha-tocopheryl poly(ethylene glycol 1000) succinate (TPGS 1000) were prepared and evaluated as potential oral delivery vehicle for docetaxel by Cho et al. (62). The SLNs were tristearin-based whereas Tween 80 and TPGS 1000 were used as emulsifiers. The SLN formulations significantly improved intestinal permeation, lymphatic uptake, and oral bioavailability of docetaxel compared to marketed formulation in rats. TPGS 1000 emulsified SLNs showed much better intestinal permeation and oral bioavailability compared to Tween 80 emulsified SLNs,

presumably because of greater inhibiting effect of TPGS 1000 on DTX efflux and higher lymphatic uptake.

Paclitaxel: Paclitaxel (PTX), a potent naturally occurring anticancer agent, is a non-ionizable, lipophilic, molecule with poor aqueous solubility and is a P-glycoprotein (P-gp) substrate (63). Pandita et al. prepared SLNs containing PTX by modified solvent injection method using stearylamine as lipid and poloxamer 188 and lecithin as emulsifying agents (64). *In vitro* release study from the SLNs showed sustained release following Higuchi kinetics. *In vivo* pharmacokinetic studies conducted in mice revealed that the SLNs after oral administration produced significantly greater drug concentrations in plasma and tissues compared to the free PTX solution and both the rate and extent of absorption was much greater from the SLNs compared to the solution.

In another study, Pooja et al. developed wheat germ agglutinin (WGA) conjugated, SLNs to improve the oral delivery of paclitaxel (65). Results of biodistribution studies performed in rats indicated that the WGA-conjugated SLNs significantly increased the oral bioavailability and lung targeting of PTX which can be explained by the bioadhesive property of the SLNs and targeting specificity of the conjugated ligand.

ORAL DELIVERY OF PROTEIN AND PEPTIDES USING LIPID NANOPARTICLES

Oral delivery of peptide and protein drugs still remains a challenge due to their large molecular size, poor lipid solubility, and instability in the gastrointestinal fluid due to presence of proteolytic enzymes and poor permeability through the gastrointestinal epithelial membrane (66). Among the many novel formulation techniques utilized, lipid nanoparticles like SLNs and NLCs seem to be very much promising (67). The benefits of SLNs/NLCs for the oral delivery of peptides and proteins could be the stabilization of peptides by the lipid matrix and possible permeation enhancing effect of the lipid (68). Dumont et al. have reviewed the production techniques for encapsulating proteins and peptides in lipid nanoparticles and evaluation techniques for measuring bioavailability enhancement (69). Oral bioavailability enhancement of insulin and salmon calcitonin (sCT) through utilization of lipid nanoparticles has been reported in the literature and is summarized below.

Chen et al. reported preparation of four sCT encapsulated SLNs by micelle–double emulsion technique (70). The lipid used was either solely stearic acid or a blend of stearic acid with triglycerides. It was found that the stearic acid and tripalmitin blend was most effective in improving the stability of the resulting SLNs and enhancing the drug stability in the simulated intestinal fluids, as well as the internalization of sCT. The mechanism of cellular uptake was studied and was found to be clathrin and caveolae-dependent endocytosis. The SLNs formulated with stearic acid and tripalmitin combination resulted in superior hypocalcemic activity after intraduodenal administration in rats and also produced six times higher oral bioavailability compared to the sCT solution.

Ansari et al. reported preparation of insulin-loaded SLNs by double emulsion solvent evaporation method, employing glyceryltrimyristate (Dynasan 114) as solid lipid and soy lecithin and polyvinyl alcohol as the emulsifiers (71). The optimized formulation had high entrapment of insulin (56.5%) and afforded better protection from gastrointestinal environment compared to the insulin solution. *In vivo* pharmacokinetic studies performed in rats indicated that the SLN formulation resulted in approximately five times greater relative oral bioavailability of insulin compared to the insulin solution.

ORAL DELIVERY OF NUCLEIC ACIDS

Lipid-based delivery systems have been recognized as non-viral vectors with great potential for gene transfection. Within the family of lipid-based systems, lipid nanoparticles have shown promising efficacy as gene and RNAi delivery systems *in vitro* and *in vivo* (72). Although a number of studies have reported the potential of SLNs as a gene delivery system based on *in vitro* cell lines studies and *in vivo* studies after parenteral or ocular delivery, very few studies on oral delivery of nucleic acids *via* lipid nanoparticulate systems are reported in literature. Ball et al. (73) studied delivery of siRNA *via* lipidoid (amphiphilic lipid like molecules) SLNs under simulated stomach and intestinal conditions *in vitro*. It was found that lipid nanoparticles were able to protect the entrapped nucleic acid in simulated gastric conditions. Effect of different concentrations of pepsin and bile salts were studied on the stability of the siRNA-loaded SLNs and it was observed that exposure to the concentration corresponding to the fed state had a greater effect on the stability of the nucleic acid than the fasted state concentration. Potency of the SLNs was found to be reduced when mucin was present on Caco-2 cells, which could be countered by increasing the concentration of PEG in the SLNs. Biodistribution studies performed in mice revealed that siRNA-loaded SLNs were retained in the GI tract for a minimum period of 8 h and the nanoparticles were able to enter the epithelial cell lining of the colon and small intestine. This study shows that lipid nanoparticles can be potentially used for delivery of siRNA to intestinal epithelial cells.

MECHANISM OF ORAL ABSORPTION ENHANCEMENT

Different mechanisms have been suggested for the oral bioavailability enhancing property of nanoparticulate drug carriers. One of these mechanisms is the general adhesiveness of nanoparticles to the gastrointestinal mucosa and release of the drug at the exact place of absorption (74). This property of bioadhesion is not specific for lipid nanoparticles but is a general behavior of all nanoparticles. Nanoparticles also by virtue of their small size and thereby resulting large surface area are able to increase the dissolution of poorly soluble drugs.

The second mechanism suggested is applicable for any lipid-based drug delivery system including emulsions, self-emulsifying formulations, and lipid nanoparticulate matrix systems. The fact that lipids can promote the absorption of lipid soluble drugs like vitamins A, D, E, and K (75) is well known. The absorption enhancing effect of lipid has been explained by the studies performed by Charman and co-workers (4,76–78). The lipids after oral administration are

broken down by lipolytic enzymes pancreatic lipase and its cofactor co-lipase to diglyceride, monoglyceride, and fatty acid. The presence of lipids in small intestine stimulates the gall bladder to secrete bile salt, phospholipid, and cholesterol. The monoglyceride, fatty acid, and lysophospholipid produced by the lipolysis are thereafter solubilized with the help of the bile salts by incorporation into multilamellar and unilamellar vesicles, mixed micelles and micelles. The lipid digestion products and drugs dissolved in the lipids are solubilized in these micelle and mixed micelle leading to a significant increase in dissolution and absorption of the solubilized species. An unstirred water layer separates the brush border (apical) membrane of enterocytes from the bulk fluid phase of the small intestinal lumen. This layer is separate from the luminal bulk phase and forms an acidic microclimate adjoining the enterocytes together with the mucus layer (79). This unstirred layer poses a major barrier to the diffusion of lipids and lipophilic molecules owing to their negligible solubility in water. Micellar solubilization of fatty acid, monoglycerides, and lipophilic molecules greatly improves their solubility in the unstirred water layer and aids in their transport across the stagnant layer (80). Micelles cannot be absorbed as such across the epithelial membrane. The acidic pH of the unstirred water layer microclimate might facilitate micellar dissociation (80,81) and solutes should first leave the mixed micellar phase before it can partition into the epithelial cells. The free fatty acid, monoglyceride, and lipid soluble molecules can partition across the brush border membrane by simple passive diffusion or by active transport or might be subjected to efflux transport by efflux transporter. The absorption enhancing effect of the lipid depends on the chain length of the lipid used and generally LCTs exert a more profound effect than MCTs (82).

Another possible mechanism by which lipidic excipients enhances oral bioavailability is the lymphatic transport. The absorption profile of drugs *via* the lymphatic route is affected by the acid chain length of the triglyceride, degree of saturation, and volume of the lipid administered. Short and medium chain fatty acids are majorly absorbed into the systemic circulation, whereas long chain fatty acids and monoglycerides are converted back to tryglycerides and entrapped within the chylomicrons which are then secreted into the lymph vessels by exocytosis (83). Thereby the bioavailability of drug compounds which are subject to hepatic first pass metabolism also may increase when administered as a lipid-based formulation. Another entry point for the lymphatic system is the gut-associated lymphoid tissue (GALT) especially the M cells of the Peyer's patches which can take up particulate systems (84–86).

The mechanisms discussed above can satisfactorily explain the increase in oral bioavailability of lipophilic drugs with high membrane permeability belonging to the Class II of the BCS. The oral bioavailability enhancement of BCS class IV drugs and of protein and peptides from lipid-based delivery systems need to be explained on the basis of not only enhancement of dissolution rate but also a possible mechanism by which the permeability barrier can be overcome. An important factor contributing to the poor or variable oral absorption of many drugs is the drug efflux mediated by xenobiotic transporters with broad specificity which are present in the epithelial cell membranes (87). P-glycoprotein (P-gp) is a membrane bound transporter that is responsible for active transport efflux of a wide variety of drugs and other xenobiotics out of the cells. This protein is expressed by liver, blood-brain barrier, kidney, and placenta as well as by the apical membranes of the enterocytes throughout the length of the gastrointestinal tract. P-gp driven efflux reduces the intracellular concentration of xenobiotics resulting in low oral bioavailability of the substrate drugs. Commonly used pharmaceutical excipients like lipids, surfactants, and polymers can act as nonspecific inhibitors of P-gp (88,89).

Some researchers have specifically tried to pinpoint the exact mechanism of oral bioavailability enhancement of poorly permeable drugs when formulated as lipid nanoparticles. Beloqui et al. investigated the mechanism of transport of SQV-loaded NLCs across intestinal barrier (57). They studied transport of SQV-loaded NLC across Caco-2 cell monolayers in presence of different inhibitors of endocytosis such as chlorpromazine and nystatin. Effect of P-gp-mediated efflux was studied in presence of P-gp inhibitor verapamil. Intracellular uptake of the NLCs by Caco-2 cells was studied by flow cytometry using nanoparticles loaded with Coumarin-6. Permeability of SQV across Caco-2 monolayer and follicle-associated epithelium (FAE) monolayers was also evaluated. The results of the study showed that NLC increased the permeability of SQV more than three times compared to the suspension. The cellular uptake of the NLCs was influenced by both the size and surfactant concentration of the particles. SQV transcytosis was found to be both caveolae and clathrin mediated depending on the formulation. One NLC formulation was found to circumvent P-gp-mediated efflux as it was able to be transported using clathrin-mediated endocytosis. Processing parameters also found to affect the transport mechanism as same composition when processed without homogenization and therefore having greater particle size was not able to circumvent the P-gp-mediated efflux.

The mechanism of transport of docetaxel NLCs was studied by Fang et al. (61). They utilized endocytosis inhibitors to understand the mechanisms of transport by using rat everted intestinal sacs. The data obtained from their experiment suggested that endocytosis was the prevalent pathway in the absorption of the NLCs and clathrin, caveolae, and macropinocytosis all contribute in the uptake process. They also studied intestinal lymphatic drug transport in rats using cycloheximide which is a known inhibitor of lymphatic transport. The results indicated that the lymphatic pathway also has a contribution to the transport of docetaxel into the systemic circulation. The authors concluded that probably several mechanisms combinedly contribute to the enhanced oral absorption of docetaxel by NLCs. Firstly, the small size and increased stability of NLCs in GI fluids enhance the passive diffusion of the solubilized DTX across the enterocytes. Secondly, direct internalization of the NLCs by the enterocytes bypasses the P-gp-mediated efflux. Lastly, uptake by the intestinal lymphatic pathway helps to avoid first-pass metabolism.

Chen et al. studied the uptake mechanism of sCT-loaded SLNs in Caco-2 cells by labeling the sCT SLNs with fluorescein isothiocyanate (FITC) and studying the transport process in the presence of different endocytosis inhibitors (70). The results indicated that the intracellular uptake

mechanism of sCT SLNs was mainly active transport *via* both clathrin- and caveolae-dependent endocytosis.

Shangguan et al. examined the *in vivo* bioavailability of SLNs and NLCs in comparison with their lipolysates and fast-release formulations using silymarin as model drug (90). Pharmacokinetics analysis in beagle dogs showed that the intact SLNs and NLCs had superior bioavailability compared to their products of their lipolysis. The relative bioavailability of lipolysates was about 59% and 75% compared to the intact SLN and intact NLC, respectively. Their findings suggested that the intact nanoparticles were only marginally better to their lipolysate counterparts in terms of bioavailability enhancement. The authors concluded that the major absorption mechanism of silymarin from lipid formulations is lipolysis and the contribution of the whole lipid nanoparticles is minimal.

Abuasal et al. compared the oral bioavailability of γ-tocotrienol (γ-T3) from SLNs and from mixed micelles (MM) (91). γ-T3 is a lipophilic compound with low oral bioavailability and low intestinal permeability. The SLNs demonstrated tenfold higher permeability, three times higher oral bioavailability and two times higher cellular uptake compared to MM. Study of absorption mechanism *in vitro* showed that enhancement of passive diffusion was the primary mechanism for increased bioavailability from SLN, while endocytosis plays a minor and formulation independent role.

EFFECT OF SURFACE MODIFICATION ON ORAL BIOAVAILABILITY

The lipid nanoparticles can be further surface coated by different polymeric and non-polymeric materials to further aid in improving oral bioavailability. Different strategies like (i) utilizing mucoadhesive polymer coating, (ii) enhancing mucopenetrating properties by coating with neutral or hydrophilic polymers, and (iii) coating with cell penetrating peptides which facilitate active transport have been used for this purpose. Surface modification with mucoadhesive polymers like chitosan can result into increased mucoadhesiveness of the lipid nanoparticles resulting in increased gastrointestinal residence time and better contact with the absorbing surface leading to increased absorption by passive diffusion. Surface coating with neutral or hydrophilic polymers like polyethylene glycol (PEG) results in increased mucopenetrating properties overcoming the barrier properties of mucus. Coating with cell-penetrating peptides (CPPs), which are short cationic or zwitterionic peptides with the ability to transport micromolecules and macromolecules, increases the chances of absorption *via* active transport.

Coating with positively charged polymers, such as chitosan, increases the mucoadhesiveness of the nanoparticles as it results in electrostatic attraction with negatively charged mucin (92). Thiomers are thiolated polymers which can form covalent disulfide bonds between the sulfhydryl groups of thiomers and the cysteine-rich portions of the mucus layer (93) and as a result increase mucoadhesion and prolong gastrointestinal residence time. Thiomers are also known to have affinity towards P-gp and thereby can enhance the absorption of drugs which are substrate to this efflux pump (94). Fang et al. reported that cysteine conjugation onto the surface of docetaxel-loaded NLCs resulted in significantly enhanced mucoadhesion with mucin *in vitro* in comparison to unconjugated NLCs (95). Results of *in situ* intestinal perfusion study indicated that permeability coefficient of docetaxel was significantly higher from the conjugated NLCs in comparison to drug solution and plain NLCs. The results of the *in vivo* pharmacokinetic study indicated that the AUC of cysteine-coated NLCs was increased 12.3-fold and 1.64-fold compared with docetaxel solution and unconjugated NLCs, respectively.

Fonte et al. prepared and characterized chitosan-modified SLNs containing insulin using Witepsol 85E as the lipid (96). Insulin permeation was found to be significantly improved in Caco-2 cell monolayer model as well as Caco-2/HT29 monolayer model. Oral administration of insulin-loaded SLN to diabetic rats resulted in a considerable hypoglycemic effect for 24 h, and the effect was significantly higher with the chitosan-coated SLN compared to the uncoated SLN. Relative bioavailabilities were found to be 8% and 17% for uncoated and chitosan-coated SLN, respectively. Only with the chitosan-coated SLN, labeled insulin could be found on the intestinal walls and inside the epithelial cells after oral administration. Prolonged retention of the labeled insulin on the intestinal surface demonstrated that the formulation was sufficiently mucoadhesive which can explain the increased absorption of insulin from the chitosan-coated SLNs.

Sarmento et al. used murine macrophage cell line to study macrophage uptake of chitosan-coated SLNs containing insulin (97). Their findings suggested that chitosan-coated Witepsol 85E-based SLN was not taken up by the macrophage cells whereas uncoated SLN and polystyrene latex nanoparticles used as positive control were completely internalized. These results show that chitosan coating can impart stealth properties to the nanoparticles and can avoid phagocytic uptake by macrophages present in lymph nodes, liver, spleen, and bone marrow. Pooja et al. reported that WGA conjugated, SLNs of paclitaxel significantly increased the oral bioavailability and lung targeting of PTX (65). The increase in oral bioavailability from the WGA conjugated SLN was attributed to the enhanced bioadhesive property of the nanocarrier system.

Beloqui et al. evaluated the uptake mechanism of NLCs coated with Dex-Prot complexes containing SQV in the presence of mucus (58). SQV permeability from the NLCs was found to be nine times more from the coated NLCs compared to the uncoated ones. SQV permeability in enterocyte like model and a mucus model was found to be dependent on the surface charge and NLCs with surface charge close to neutral resulted in maximum permeability compared to positively charged NLCs and uncoated NLCs indicating that a neutral surface charge results in better mucopenetration.

Cell-penetrating peptides (CPPs) are generally short chain length peptides consisting mainly of basic amino acids and are polycationic or amphipathic in nature. CPPs can transport both small and large molecules, including nanoparticles, across cell membranes (98). Major absorption mechanisms of CPPs are presumed to be endocytosis and translocation (99). These peptides can bind efficiently *via* electrostatic or covalent linkage to macromolecules such as proteins, oligonucleotides, SLNs, and liposomes with minimal

toxicity and aid in their cellular uptake (100–104). Zhang et al. prepared SLNs loaded with CPP and studied their potential for oral delivery of insulin (105). Octaarginine (R8) was used as the CPP. SLNs loaded with insulin and R8 showed initial rapid release followed by extended release *in vitro*. The relative pharmacological bioavailability of the SLN was found to be significantly greater than the insulin solution.

Fan et al. reported preparation of two kinds of peptide ligand-modified SLNs loaded with sCT (106). Compared with unmodified SLNs, the peptide ligand-modified SLNs showed better protection of the drug in gastrointestinal fluid, better internalization of drug on Caco-2/HT29-MTX co-cultured cells, and better permeation in excised rat duodenum mucosa. The internalization mechanism of the peptide ligand-modified SLNs was found to be mainly active transport *via* both clathrin- and caveolae-dependent endocytosis. The absolute bioavailability of modified SLNs was significantly higher than the unmodified SLNs.

In another study, PTX-loaded SLN and PTX-loaded SLNs surface modified with hydroxypropyl-β-cyclodextrin were prepared and characterized (107). The surface-modified SLNs showed higher cytotoxicity compared to that PTX solution presumably because of higher cellular uptake. The results of *in vivo* bioavailability studies indicated that surface-modified SLNs resulted in significantly increased extent of absorption of PTX. The lymphatic uptake of PTX was also maximum from the surface-modified SLNs. The authors surmised that HPCD surface modification results in reduction of the particle size of the PTX nanoparticle and leads to increase in its solubility and dissolution**.**

Taurocholic acid (TCA) has been used as a ligand for bile-acid transporter-mediated uptake of NLCs for improvement of oral bioavailability of curcumin (108). TCA-modified curcumin NLCs exhibited improved absorption rate and permeability coefficient during *in situ* intestinal perfusion studies. And also displayed a significant increase in oral bioavailability of curcumin in rats compared to unmodified NLCs. The same research group prepared and utilized N-acetyl-L-cysteine-polyethylene glycol (100)-monostearate (NAPG) as a novel conjugate for enhancing mucoadhesion and mucus penetration of curcumin NLCs (109). The results of *in vivo* pharmacokinetic studies suggested that the oral bioavailability of curcumin in rats was proportional to the degree of functionalization of NLCs with NAPG. Extent of bioavailability of NLCs surface modified with NAPG modified was more than 500 times and 117 times than that of curcumin solution and unmodified Cur-NLC, respectively.

The conclusion that can be drawn from the research work carried out by different researchers as discussed above regarding the mechanism of bioavailability enhancement is that a combination of different mechanisms are at play resulting in the overall enhancement of bioavailability from orally administered lipid nanoparticles. The findings by different research groups are summarized in Table I. Lipolysis and resulting higher solubility and dissolution play a major role for poorly water soluble drugs. Lymphatic transport through chylomicron formation and uptake by Peyers patches is also an important factor. For poorly permeable molecules like proteins and peptides, bioadhesion and endocytosis may be the predominant mechanisms. Surface coating of lipid nanoparticles leads to further increase in bioavailability presumably by facilitating bioadhesion/mucopenetration and endocytosis. The suggested mechanisms of increased uptake of poorly permeable drugs from intestinal lumen are depicted in Fig. 1.

SAFETY OF ORALLY ADMINISTERED SLN/NLC

In comparison to polymeric nanoparticles, SLN and NLC are expected to pose much less challenges related to safety as they are generally composed of lipids which are GRAS listed and can be metabolized in the body by the normal physiological metabolic pathways. Surfactants used as emulsifiers may have some toxic effect which needs to be evaluated. Numerous studies have been reported regarding the evaluation of *in vitro* and *in vivo* toxicity of lipid nanoparticles using a variety of cell lines. Table II summarizes the methods used for evaluation of toxicity and findings of some of these studies. Muller et al. assessed the *in vitro* cytotoxicity of solid lipid nanoparticles (SLNs) with respect to the lipid and surfactant used in the formulation (122). They assessed the viability of HL60 cells and human granulocytes after incubation with SLNs made of different lipids (Dynasan 114, Compritol ATO 888) and stabilizing surfactants (poloxamers, Tween 80, soya lecithin, and sodium dodecyl sulfate). Cellular uptake of the SLNs was quantitatively evaluated by chemiluminescence measurements. The authors concluded that the viability of the cells was not affected by the nature of the lipids but the nature of the surfactants had a significant effect. It was found that cytotoxic effect of the surfactants decreased significantly with binding to the SLN surface. The cytotoxicity of SLNs was found to be lower compared to polyalkylcyanoacrylate and polylactic/glycolic acid (PLA/GA) nanoparticles.

How et al. evaluated the cytotoxicity of various solid lipids namely trilaurin, docosanoid acid tripalmitin, and hydrogenated palm oil (HPO) and surfactants (Polysorbate 20, 80, and 85) on BALB/c 3T3 cells (123). The HPO and Polysorbate 80 were found to be least cytotoxic when utilized along with olive oil in the NLC formulation. This study also revealed that toxicity of NLC was less to BALB/c 3T3 cells compared to Polysorbate 80 alone implying that association with SLN/NLC reduces the cytotoxic effect of surfactants. Cationic surfactants, however, are known to be cytotoxic and need proper evaluation. Tabatt and co-workers (124) reported formulation of SLN by using two different matrix lipids and six different positively charged surfactants and evaluating the *in vitro* cytotoxicity of these formulations using COS-1 cells. Their findings revealed that cytotoxicity depends on the nature of the cationic lipid used and SLN made from one-tailed cationic surfactants were highly cytotoxic whereas the two-tailed cationic lipids were much safer. Saedi et al. investigated the effect of liquid lipid types on different features of NLC (125). They used four types of oils such as fish oil, coconut oil, linseed oil, and black seed oil to prepare curcumin-loaded NLCs. MCF-7 cell lines were used to study the cell viability and the results indicated that the blank NLCs had a mild inhibitory effect on the viability of the cells. The blank NLCs composed of linseed oil had the lowest IC_{50} value whereas coconut oil NLCs showed the least cytotoxicity.

Table I. Mechanism of Bioavailability Enhancement and Study Method Used for Evaluation of the Same

Formulation type	Main components	Entrapped drug	Method of study	Model/cell line/s used	Mechanism of uptake	Ref.
NLC	Poloxamer 188 Precirol ATO® 5, Mygliol	Saquinavir	Uptake study in presence of endocytosis inhibitors	Caco-2 FAE	Clathrin and caveolae mediated endocytosis, inhibition of P-gp mediated effux	(57)
NLC	Poloxamer 188, Precirol ATO® 5, Mygliol, Dextran-protamine surface coating	Saquinavir	Comparative permeation study	Caco-2 (enterocyte-like model) Caco-2/HT29-MTX (mucus model)	Mucopenetration, surface charge close to neutral results in more efficient permeability	(58)
NLC	Precifac ATO 5, MCT	Docetaxel	Uptake study in presence of endocytosis inhibitors Inhibition of lymphatic uptake by cycloheximide	Rat everted intestinal sacs, Rats	Clathrin and caveolae mediated endocytosis, macropinocytosis, Lymphatic transport	(61)
SLN	Stearic acid, tripalmitin, trimyristin or trilaurin	Salmon calcitonin	Uptake study in presence of endocytosis inhibitors	Caco-2	Clathrin and caveolae mediated endocytosis	(70)
SLN	Witepsol 85E Chitosan (surface coating)	Insulin	Comparative permeation study Flow cytometry	Caco-2 Caco-2/HT29	Mucoadhesion	(94)
SLN	Witepsol 85E Chitosan (surface coating)	Insulin	Flow cytometry	Murine macrophage RAW 264.7	Inhibition of phagocytosis (stealth property)	(95)
SLN	polyoxyethylene (40) stearate, CPP IRQ and	Salmon calcitonin	Uptake study in presence of endocytosis inhibitors	Caco-2/HT29-MTX, excised rat duodenum mucosa	clathrin- and caveolae-dependent endocytosis	(105)
SLN, NLC, Corresponding lipolystaes	Precirol ATO 5 Oleic acid	Silymarin	*In vivo* pharmacokinetics	Beagle dogs	Lipolysis plays a major role	(90)
SLN, mixed micelle	Lutrol, Compritol ATO	γ - Tocotrienol	Uptake study in presence of endocytosis inhibitors	HepG2 cells	Endocytosis plays a minor role, passive diffusion major mechanism of uptake from SLNs	(91)

Lipid nanoparticles intended to be delivered by oral routes should be also non-toxic to the epithelial cell lining of the gastrointestinal tract. Some studies have reported the effect of SLN/NLC formulations on integrity and survival of gastrointestinal cells. Fang et al. performed biocompatibility studies on rat intestine after oral administration of blank NLC formulations to rats by performing histopathologic examination of the isolated intestines (61). The mucosal erosions and disruption of the intestinal epithelium cells were not observed after administration of blank NLCs in increasingly higher concentrations, indicating that the amount of blank NLCs used did not generate any toxic effect to the epithelial cells. Similar findings have been reported for docetaxel-loaded SLNs by Cho and co-workers (62). They evaluated the toxicity of docetaxel-loaded SLNs in rat intestinal mucosa by histological staining and could not find any sign of damage to the intestinal cells after 8 h of oral administration of the SLNs.

Acute toxicity studies of zerumbone (ZER)-loaded NLC (ZER-NLC) as well as the blank NLC have been performed by Rahman et al. (126). The protocol of the study involved treating the BALB/c mice with a single oral dose of either water, olive oil, ZER, NLC, or ZER-NLC for 14 days. The animals were observed for symptoms of toxicity, behavioral changes, and abnormalities in feeding and gross appearance. Histological examination for different organs and tissues, total hemogram, bone marrow examination in terms of cellular morphology, and serum biochemical parameters were also determined. At oral doses of 100 and 200 mg/kg, neither ZER-NLC nor the blank NLC resulted in any significant sign of toxicity or mortality.

Caco-2 cell lines have been also used to study the toxicity of lipid nanoparticles intended for oral delivery. Chen et al. evaluated intestinal toxicity of NLCs containing tripterine coated with CPP by performing MTT assay using Caco-2 cells (127). The findings of the study revealed that cytotoxicity was dose dependent and both the coated and uncoated NLCs had significantly less cytotoxicity than tripterine alone. The results led to the conclusion that the NLC formulations can be used to reduce the gastrointestinal side effects of tripterine.

Ball et al. determined influence of lipid nanoparticles on the integrity of tight junctions using cultured Caco-2 cell monolayers (70). Integrity of the tight junctions was evaluated by transepithelial electrical resistance (TEER) after

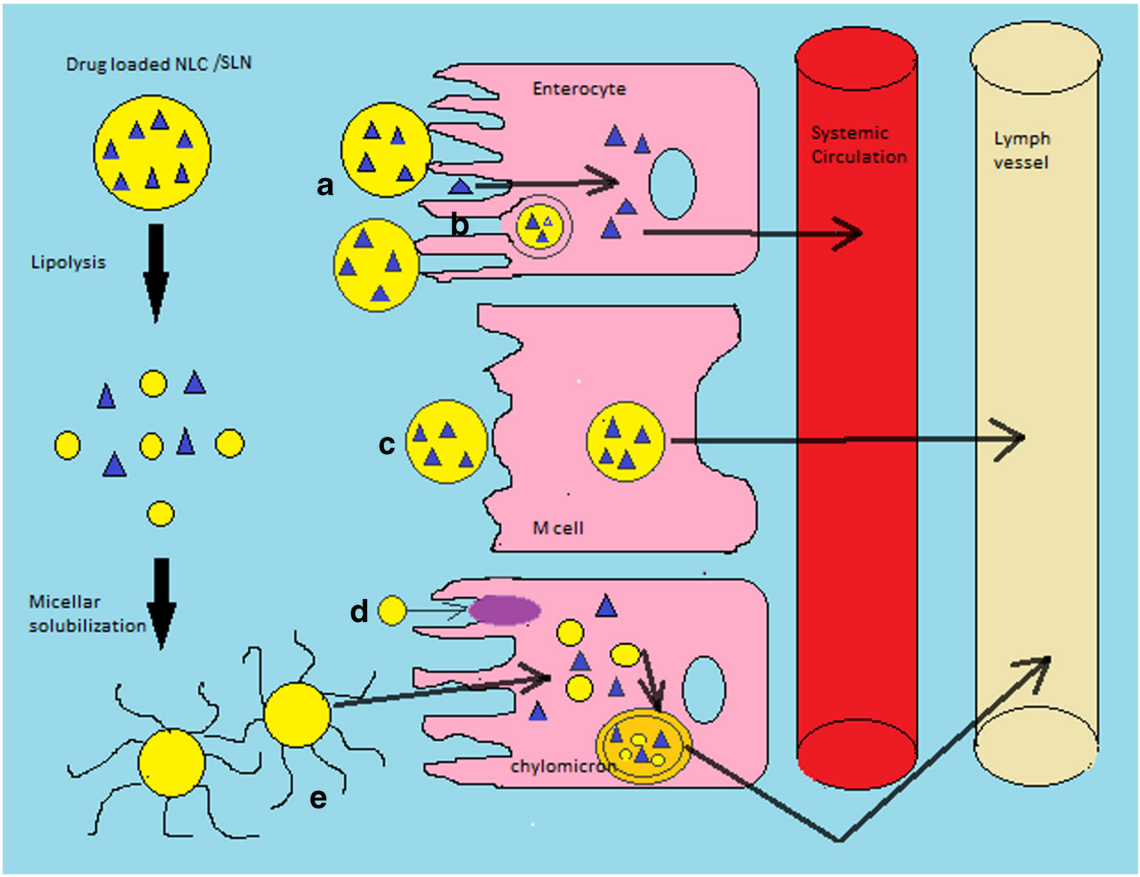

Fig. 1. Mechanism of permeation enhancement of poorly permeable drugs from lipid nanoparticles. **a** Bioadhesion leading to delivery of drug at the absorption window. **b** Clathrin/caveollae mediated endocytosis. **c** Uptake through Peyer's patches. **d** Inhibition of efflux transporters by excipients. **e** Micellar solubilization followed by passive diffusion into enterocytes and chylomicron formation

administration of LNPs loaded with siRNA to Caco-2 monolayers. TEER value did not change significantly even at very high concentrations indicating that the lipid nanoparticles did not affect barrier function. Visualization of the protein arrangement of the tight junctions after 3 h indicated that the protein arrangement was not affected by the presence of the lipid nanoparticles in the intestine.

Another approach used in studying toxicity of the SLN/NLC formulations intended for oral delivery is applicable when the drug loaded is itself cytotoxic. Comparison of cytotoxicity between blank nanoparticle formulation and drug-loaded nanoparticle formulation against various cell lines has helped researchers to conclude that the cytotoxicity exerted is due solely to the effect of the drug-loaded formulations and the blank NLC/SLN is innocuous. Zhang et al. studied the cytotoxicity of etoposide loaded NLCs on human epithelial like lung carcinoma cells (56). They observed that the IC_{50} value for the blank NLC formulations was more than 100 times large compared to the IC_{50} values for the drug-loaded NLCs. On the other hand, the drug-loaded NLCs had a much smaller IC_{50} value compared to the free drug, leading to the conclusion that the drug-loaded NLCs were cytotoxic whereas the blank NLCs were not.

In vivo cytotoxicity study based on survival of drosophila flies and their larvae has been reported by Fangueiro and co-workers (128). They used *Drosophila melanogaster* test to evaluate an insulin SLN formulation. This test evaluates the effect of the materials under study on the crossings between the flies and/or induced mutations on the developed larvae. The number of generated flies was considered normal between 300 and 500 and number of progeny below this range was considered to indicate toxicity risk. The findings of the study indicated that blank SLNs, insulin-loaded SLNs, as well as the lipid Softisan, used in the preparation of SLNs were non-toxic in the concentrations tested.

CONCLUSION

The SLN and its advanced version NLC hold a lot of promise for effective oral delivery of poorly soluble and poorly permeable drugs. Many lifesaving drugs like amphotericin B, cyclosporine A, paclitaxel, saquinavir, ritonavir *etc.* are poorly permeable through the intestinal epithelial barrier. The main reasons attributable to the poor permeability can be stability in the gastrointestinal tract, presence of narrow absorption window, and efflux by efflux transporters. SLN and NLC formulations have demonstrated significant effectiveness in overcoming these limitations by protecting entrapped drugs from degrading enzymes and harsh pH conditions, localizing the nanoparticles within the absorption window by virtue of their mucoadhesive nature and inhibiting the P-gp-mediated efflux. Lymphatic uptake of

Table II. Summary of *in vitro* and *in vivo* toxicity studies performed with lipid nanoparticles

Formulation type	Main components (inactive)	Entrapped drug	Method of toxicity study	Cell line/s used	Conclusion	Ref.
SLN	Precirol ATO® 5 or Compritol 888 ATO®	Alendronate	MTT, DAPI staining, DNA fragmentation	A549 (adenocarcinomic human alveolar basal epithelial cells)	No effect on cell viability, did not result in apoptosis/ necrosis and DNA fragmentation	(110)
Cationic SLN	Imwitor 900P, Compritol 888, ATO CTAB, Lutrol F68 Miranol C-32 Ultra	None	cell viability (alamar blue assay) and genotoxic potential (alkaline comet assay)	HepG2 and Caco-2	Among various formulations, Compritol based SLN was found to be most cytotoxic, and concentration dependent toxicity was observed. Among various cell lines HepG2 cells were found to be more affected	(111)
SLN	Softisan 154 (S154, Phospholipon 90G	None	Lactate dehydrogenase (LDH) and 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay	human alveolar epithelial cell line (A549) and murine precision-cut lung slices (PCLS)	Low toxicity was observed	(112)
SLN	Compritol 888 ATO, Soy phospholipid	Tetrandrine	Cell Counting Kit-8 (CCK-8) assay	Human lens epithelial cells (SRA 01/04)	Cationic SLN was found to be more toxic than non-cationic one, blank SLN and tetrandrine loaded SLN have showed low toxicity as compared to drug alone	(113)
SLN	Tristearin, trimyristin, cholesteryl myristate,polysorbate 80poloxamer 188, polyvinyl alcohol, soybean phospholipid, sodium glycocholate	None	MTT assay	L929 mouse fibroblasts	Crystalline nanoparticles were found to be more cytotoxic than the corresponding liquid or liquid crystalline particles. Cell viability were also affected by type of matrix lipid, stabilizer and the particle shape	(114)
SLN	Glycerol monostearate (Tristearin Lecithin Pluronic-F68	Rapaglinide	MTT assay	Rat macrophage cells	No cytotoxicity	(115)
SLN	Cetyl palmitate, myristyl myristate, and cetyl esters	None	MTT assay	Mouse 3T3 fibroblasts and human HaCaT keratinocytes	SLN less cytotoxic than polymeric nanoparticles	(116)
SLN	Softisan®100 Tween®80, Span®80 and Lipoid®S75	Insulin	Alamar blue assay	HEPG-2 and Caco-2	Non toxic to Caco-2 cells, low toxicity towards HEPG 2 cells	(117)
SLN	Tristearin, Solid white Vaseline USP, Vegetal lipids	None	cell viability assays, flow cytometry and ROS generation assessment	Fibroblasts: Vero and MDCK	Mild cytotoxicity with formulation containing sodium dodecyl sulphate	(118)
SLN, NLC, Nanoemulsion	Miglyol 812, Glyceryl monostearate, Lecithin, Polysorbate	None	MTT assay, ROS generation assessment, Hemolysis test, *in vivo* toxicity in mice	Monkey kidney epithelial cells (VERO) and acute lymphoblastic leukemia cells (L1210)	SLN, NLC more toxic than nanoemulsion, main mechanism of toxicity is the induction of oxidative stress in liver	(119)
NLC	Hydrogenated palm oil, lecithin, olive oil, polysorbate 80	Thymoquinone	*In vivo* acute and sub-acute toxicity studies	BALB/c mice	NLC at a dose of 10 mg/kg not toxic in mice	(120)
NLC	dialkyldimethyl ammonium bromide (DxDAB) of	None	Haemolysis	Human erythrocytes	Concentration dependent low haemolytic activity, no	(121)

Table II. (continued)

Formulation type	Main components (inactive)	Entrapped drug	Method of toxicity study	Cell line/s used	Conclusion	Ref.
	different alkyl chain length (x = 12, 14, 16, 18) as the solid lipid oleic acid (liquid lipid)				relation between chain length and haemolytic activity	

the lipid nanoparticles also plays a key role in increasing bioavailability of drugs which are subject to pre-systemic metabolism or are substrate to efflux transporters. Lymphatic uptake of lipid nanoparticles can be successfully utilized for targeted delivery of drugs to organs of the lymphatic system. Moreover, lipid nanoparticles have also been successfully exploited for oral delivery of peptide and protein drugs like insulin and calcitonin. Potential of SLNs and NLCs in oral delivery of nucleic acids is also being studied. Surface modification of lipid nanoparticles by coating with various polymers and peptides has been successfully performed and has shown to significantly increase the oral bioavailability. Lipid nanoparticles surface coated with cell penetrating peptides or hydrophilic polymers can be a potential strategy to successful oral delivery of proteins and peptides. Lipid nanoparticles are also shown to provide controlled release of the entrapped drug thereby reducing the toxic effects associated with high plasma concentrations of drugs like cyclosporine A and amphotericin B. *In vitro* toxicity studies on cell line and *in vivo* toxicity studies have indicated that oral delivery of lipid nanoparticles does not pose any significant risk of local or systemic toxicity and these are safer than polymeric nanoparticles. Lipid nanoparticles offer attractive characteristics like high entrapment efficiency, low cost production, easy scalability, better stability compared to liposomes and use of cheap, GRAS listed and easily available excipients make it a potential oral delivery system for drugs with problematic oral absorption. NLC technology is already adapted in the cosmetic market in the form of a number of cosmetic and cosmeceutical products worldwide (129). Lipid nanoparticles intended for oral delivery of poorly permeable drugs, however, will need further clinical studies before they can be approved for therapeutic use.

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