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Review Article

A REVIEW ON NOVEL LIPID BASED NANOCARRIERS

AJAY PATIDAR*, DEVENDRA SINGH THAKUR, PEEYUSH KUMAR, JHAGESHWAR VERMA

SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur 495009, Chattishgarh, India
Email : justmaildevendra@gmail.com

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ABSTRACT

The aims of review are the latest research development of the lipid based nanocarriers according to the recent relevant literatures**.** Each preparation of the lipid based nanocarriers has advantages and disadvantages. The SLN is an excellent drug delivery system and has broad prospects in the pharmaceutical field. This review discusses the recent developments in the fields of solid lipid nanoparticle (SLN), nanostructured lipid carriers (NLC) and lipid drug conjugates (LDC) nanoparticle pertinent to therapeutic agent delivery covering those systems tested and/or validated.

Keywords: SLN, NLC and LDC, Production technique, Pharmaceutical application.

INTRODUCTION

Rapid advances in the ability to produce nanoparticles of uniform size, shape, and composition have started a revolution in the sciences. The development of lipid based drug carriers has attracted increased attention over the last years. Solid lipid nanoparticle is the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicine and research, as well as in other varied sciences. Due to their unique size-dependent properties, lipid nanoparticles offers the possibility to develop new therapeutics. The ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery that could be used for secondary and tertiary level of drug targeting. Hence, solid lipid nanoparticle hold great promise for reaching the goal of controlled and site specific drug delivery and hence has attracted wide attention for researchers.

During the last 20 years there was only one novel carrier system which can be considered a major innovative contribution in the dermal area, the liposomes first introduced to the cosmetic market by Dior in 1986. After some years delay, liposomes appeared on the market in pharmaceutical products. Apart from technological benefits, the liposome as a novel carrier found broad attention among the public. There is quite a number of other formulation principles used during the last two decades, e.g. microemulsions, multiple emulsions and also solid particles (e. g. microsponge delivery system (MDS), thalaspheres). However, none of them found a broader application due to various reasons and none of them received comparable attention as the liposomes. Compared to liposomes and emulsions, solid particles possess some advantages, e.g. protection of incorporated active compounds against chemical degradation and more flexibilty in modulating the release of the compound. Advantages of liposomes and emulsions are that they are composed of well tolerated excipients and they can easily be produced on a large scale, the prerequisite for a carrier to be introduced to the market.

At the beginning of the 1990s, the solid polymeric nanoparticles comes which made from non-biodegradable and biodegradable polymers having size rang from10 to 1000 nm which are yet another innovative parenteral carrier system. Advantages of these particles are site-specific targeting and controlled release of the incorporated drugs[1]. However, the cytotoxicity of the polymers after internalization into cells is a crucial and often discussed aspect[2]. Also, large scale production of polymeric nanoparticles is problematic. Therefore, this carrier system has so far not been relevant for the pharmaceutic market.

In the middle of the 1990s, the attention of different research groups has focused on alternative nanoparticles made from solid lipids, the so-called solid lipid nanoparticles (SLN or lipospheres or nanospheres) [3–4]. The SLN combine the advantages of other innovative carrier systems (e.g. physical stability, protection of incorporated labile drugs from degradation, controlled release, excellent tolerability) while at the same time minimizing the associated problems. SLN formulations for various application routes (parenteral, oral, dermal, ocular, pulmonary, rectal) have been developed and thoroughly characterized in vitro and in vivo [5-6]. A first product has recently been introduced to the Polishmarket (Nanobase, Yamanouchi) as a topically applied moisturizer. At the turn of the millennium, modifications of SLN, the so-called nanostructured lipid carriers (NLC) and the lipid drug conjugate (LDC) nanoparticles have been introduced to the literature[7-8]. These carrier systems overcome observed limitations of conventional SLN. This paper intends to describe briefly the different lipid based carrier systems SLN, NLC and LDC, structure and associated features, stability, applied production methods, drug incorporation and drug release mechanisms. The bioactivity of SLN after parenteral application, i.e. tolerability, toxicology, cellular uptake, albumin adsorption, pharmacokinetics, tissue distribution and drug targeting is reviewed in detail.

NOVEL GENERATION OF LIPID NANOCARRIERS

Solid lipid nanoparticle (SLN)

Solid lipid nanoparticles (SLNTM) were developed at the midlines of the 1990s as an alternative carrier system to the existing traditional carriers, such as emulsions, liposomes and polymeric nanoparticles [9]. Solid lipid nanoparticles (SLN) prepared either with physiological lipids or lipid molecules with an history of safe use in human medicine, which attract increasing attention as colloidal drug carriers. Under optimized conditions they can be produced to incorporate lipophilic or hydrophilic drugs and seem to fulfill the requirements for an optimum particulate carrier system[10].

Advantages of SLN are the use of physiological lipids, the avoidance of organic solvents, a potential wide application spectrum (dermal, per os, intravenous) and the high pressure homogenization as an established production method. Additionally, improved bioavailability, protection of sensitive drug molecules from the outer environment (water, light) and even controlled release characteristics were claimed by incorporation of poorly water soluble drugs in the solid lipid matrix[10]. Common disadvantages of SLN are their particle growing, their unpredictable gelation tendency, their unexpected dynamics of polymorphic transitions and their inherent low incorporation rate due to the crystalline structure of the solid lipid[11].

Nanostructured lipid carriers (NLC)

A new generation of nanostructured lipid carriers (NLCs) consisting of a lipid matrix with a special nanostructure has been developed[12-13]. This nanostructure improves drug loading and firmly incorporates the drug during storage. These NLCs can be produced by high-

pressure homogenization and the process can be modified to yield lipid particle dispersions with solid contents from 30–80%.Carrier system. However, the NLC system minimizes or avoids some potential problems associated with SLN. The review by Mehnert and Mader[14] high lights these aspects:

1. Pay-load for a number of drugs too low
2. Drug expulsion during storage
3. High water content of SLN dispersions.

The new concept for the production of NLC, spacially very different lipid molecules are mixed, i.e. blending solid lipids with liquid lipids (oils). The resulting matrix of the lipid particles shows a melting point depression compared to the original solid lipid but the matrix is still solid at body temperature. Depending on the way of production and the composition of the lipid blend, different types of NLC are obtained. The basic idea is that by giving the lipid matrix a certain nanostructure, the pay-load for active compounds is increased and expulsion of the compound during storage is avoided.

Lipid drug conjugates (LDC) nanoparticle

A major problem of SLNs is the low capacity to load hydrophilic drugs due to partitioning effects during the production process. Only highly potent low dose hydrophilic drugs may be suitably incorporated in the solid lipid matrix[15]. In order to overcome this limitation, the so called LDC nanoparticles with drug loading capacities of up to 33% have been developed[10]. An insoluble drug-lipid conjugate bulk is first prepared either by salt formation (e.g. with a fatty acid) or by covalent linking (e.g. to ester or ethers). The obtained LDC is then processed with an aqueous surfactant solution (such as Tweens) to a nanoparticle formulation using high pressure homogenization (HPH). Such matrices may have potential application in brain targeting of hydrophilic drugs in serious protozoal infections.[9]

Advantages of lipid based nanocarriers

- Control and targeted drug release.
- Improve stability of pharmaceuticals.
- High and enhanced drug content (compared to other carriers).
- Feasibilities of carrying both lipophilic and hydrophilic drugs.
- Most lipids being biodegradable, SLNs have excellent biocompatibility.
- Water based technology (avoid organic solvents).
- Easy to scale-up and sterilize.
- More affordable (less expensive than polymeric/surfactant based carriers).
- Easier to validate and gain regulatory approval.

TECHNIQUES FOR SLN PRODUCTION

General ingredients and the emulsifiers

The matrixes of SLN are the natural or the synthetic lipids which can be degradated, including triglyceride (tri-stearic acid, tri-palmitic acid, tri-lauric acid etc. long- chain fatty acid), steroid (e. g. cholesterin) waxes (e. g., microcrystal paraffin wax, whale ester wax). The choice of the emulsifiers depends on the administration of the drug, to the parenteral system, there are limits to choose the emulsifiers16] including the phospholipids [e. g. , soybean phospholipids (LS 75, LS loo), yolk phospholipids (LE80) , lecithin(epikuron ~ooo)], nonionic wetting agent (e. g. , poloxamer 188, 182, 407, 9081, chleolate (e. g. , sodium cholate, sodium glycocholate sodium taurocholate , deoxy-sodium taurocholate 1 short-chain spirits (e. g. ,butanol, butanoic acid 1. Amphipathicity materials (e.g.,ionic and nonionic type) can stabilize the dispersion of SLN, on the surface of SLN, hydrophobic parts stretch to the core, hydrophilic parts stretch to the disperse medium, so drug with low water-solubility can be entrapped in the SLN to form the colloidal drug system.

High pressure homogenization

HPH is a suitable method for the preparation of SLN, NLC and LDC and can be performed at elevated temperature (hot HPH technique) or at or below room temperature (cold HPH technique) [15,9,16, 17-19]. The particle size is decreased by cavitation and turbulences. Briefly, for the hot HPH, the lipid and drug are melted (approximately 5 ^{o}C above the melting point of the lipid) and combined with an aqueous surfactant solution having the same temperature. A hot pre-emulsion is formed by high speed stirring. The hot pre-emulsion is then processed in a temperature controlled high pressure homogeniser, generally a maximum of three cycles at 500 bar are sufficient. The obtained nanoemulsion recrystallises upon cooling down to room temperature forming SLN, NLC or LDC. The cold HPH is a suitable technique for processing temperature labile drugs or hydrophilic drugs. Here, lipid and drug are melted together and then rapidly ground under liquid nitrogen forming solid lipid microparticles. A pre-suspension is formed by high speed stirring of the particles in a cold surfactant solution. This pre-suspension is then homogenised at or below room temperature forming SLN, NLC or LDC, the homogenising conditions are generally five cycles at 500 bar. The influence of homogeniser type, applied pressure, homogenisation cycles and temperature on particle size distribution has been studied extensively [15,16,20,21]. Both HPH techniques are suitable for processing lipid concentrations of up to 40% and generally yield very narrow particle size distributions [22, 23].

Film ultrasound dispersion

The term lipid and the drug were put into suitable organic solutions, decompression, rotation and evaporation the organic solutions, a lipid film is formed, then the aqueous solution which includes the emulsions was added. Using the ultrasound with the probe to diffuser at last, the SLN with the little and uniform particle size is formed. Wang et al use the soybean phospholipids as carrier and the film-ultrasound dispersion method to prepare the Oleanane solid lipid nanoparticles (OA-SLN).

SLN prepared by solvent emulsification/evaporation

For the production of nanoparticle dispersions by precipitation in o/w emulsions[24] the lipophilic material is dissolved in water-immiscible organic solvent (cyclohexane) that is emulsifed in an aqueous phase. Upon evaporation of the solvent nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. The mean diameter of the obtained particles was 25 nm with cholesterol acetate as model drug and lecithin/sodium glycocholate blend as emulsifer. The reproducibility of the result was confirmed by Siekmann and Westesen, who produced the cholesterol acetate nanoparticles of mean size 29 nm[25].

Production of SLN via microemulsions

The group of Gasco has developed and optimised a suitable method for the preparation of SLN via microemulsions which has been adapted and/or modified by different labs [26, 27, 28, 18, 29, 30]. Firstly,awarm microemulsion is prepared by stirring, containing typically c10% molten solid lipid, 15% surfactant and up to 10% cosurfactant. This warm microemulsion is then dispersed under stirring in excess cold water (typical ratio c1:50) using an especially developed thermostated syringe. The excess water is removed either by ultra-filtration or by lyophilisation in order to increase the particle concentration. Experimental factors such as microemulsion composition, dispersing device, temperature and lyophilisation on size and structure of the obtained SLN have been studied intensively. It has to be remarked critically, that the removal of excess water from the prepared SLN dispersion is a difficult task with regard to the particle size. Also, high concentrations of surfactants and cosurfactants (e.g. butanol) are necessary for formulating purposes, however less desirable with respect to regulatory purposes and application.

Lipid particles from supercritical fluid (SCF) technology

More recently, very attractive new techniques based on SCF technology have been studied as useful alternatives for drying pharmaceutical protein formulations, and to produce solvent-free particulate drug carriers. Carbon dioxide (CO2) has been used almost exclusively in SCF processing of pharmaceuticalsbecause of its low toxicity, its relatively low critical temperature and moderate critical pressure, and its low cost[31]. The main advantages of such techniques include mild processing conditions, possible sterilising

properties of supercritical CO2, ability of producing microparticles or nanoparticles in the form of dry powders and feasibility of scaling-up[32]. The SCF technology comprises several processes for micro/nanoparticle production such as rapid expansion of supercritical solution (RESS), particles from gas saturated solutions (PGSS), gas/supercritical antisolvent (GAS/SAS), aerosol solvent extraction system (ASES), solution enhanced dispersion by supercritical fluids (SEDS), which are selected according to the drug solubility in the SCF [33-35]. Several proteins have been processed by such SCF techniques, mainly by SAS and PGSS [33]. The extensive description of these methods being out of the scope of the present review, the reader is referred to recent reviews on particle formation using SCF [33,35]. With reference to solid lipids, the viability of using the PGSS process to obtain spherical hydrogenated palm oil-based solid lipid microparticles, forprolonged release of hydrophilic drugs such as theophylline was demonstrated by Rodrigues et al.[35, 36]. In addition, its application to protein molecules led to a modified SAS technique that combines the atomisation and the anti-solvent processes to prepare lysozyme spherical nanoparticles (100 to 400 nm) using water/ethanol solutions, while keeping enzyme integrity and stability throughout the process. Using a modified PGSS process to produce SLN, insulin protein was dissolved in dimethylsulfoxide (DMSO) and this solution was then incorporated into melted mixtures of tristearin, phosphatidylcoline and dioctyl sulfosuccinate[38]. The lipid nmass was mixed with compressed CO2. Atomisation of this mixture resulted in SLN of a particle size b500 nm presenting sustained release properties and preserving insulin biological activity. Unfortunately, the use of organic solvents even as mild as DMSO compromises the benign aspects of solvent-free SCF processing, especially when particles are intended for injection purposes. An original approach consists of coating protein crystals of a given particle size with solid lipids dissolved in supercritical CO2. As the drug is not dissolved and the process is carried out under mild conditions (e.g. 35 °C/200 bar or 45 °C/200 bar for 1 h) protein integrity is preserved. BSA crystals were coated using this process either with tripalmitin or Gelucire® 50-02, and the latter resulted in prolonged release lipid microcapsules (80% of intact BSA in 24 h)[39].

PHARMACEUTICAL APPLICATION

SLN as carriers for peptides and proteins drugs

Since their first description by Müller et al.[10], SLN have attracted increasing attention as an efficient and non-toxic alternative lipophilic colloidal drug carrier prepared either with physiological lipids or lipid molecules used as common pharmaceutical excipients. Two main production techniques were then established: the high-pressure homogenisation described by Müller and Lucks[38] and the microemulsion-based technique by Gasco[9]. Unlike most polymeric microsphere and nanoparticle systems, SLN production techniques do not need to employ potentially toxic organic solvents, which may also have deleterious effect on protein drugs. Furthermore, under optimised conditions they can be produced to incorporate lipophilic or hydrophilic drugs and seem to fulfil the requirements for an optimum particulate carrier system [10, 39]. Their colloidal dimensions and the controlled release behaviour enable drug protection and administration by parenteral and non-parenteral routes thus emphasising the versatility of this nanoparticulate carrier. Publications have described the use of lipid nanoparticles by parenteral routes including biodistribution and pharmacokinetic studies upon i.v. administration[40-44]. Higher amounts of drug were also found in the brain after i.v. injection, suggesting the potential use of SLN as a brain delivery of drugs such as doxorubicin, tobramycin, not capable of crossing the blood brain barrier[40-43].

The SLN production is based on solidified emulsion (dispersed phase) technologies. Therefore, due to their hydrophilic nature most proteins are expected to be poorly microencapsulated into the hydrophobic matrix of SLN, tending to partition in the water phase during the preparation process, which is further enhanced by the use of surfactants as emulsion stabilizers. In addition, SLN can present an insufficient loading capacity due to drug expulsion after polymorphic transition during storage, particularly if the lipid matrix consists of similar molecules. However, lipids are versatile molecules that may form differently structured solid matrices, such as the nanostructured lipid carriers (NLC) and the lipid drug conjugate nanoparticles (LDC), that have been created to improve drug loading capacity (reviewed by Wissing and Müller)[39]. Since the mid 1990's, authors have regularly published promising results concerning the incorporation of several peptides and proteins in solid lipid particulate carriers. Therapeutically relevant peptides (e.g. calcitonin, cyclosporine A, insulin, LHRH, somatostatin), protein antigens (e.g. hepatitis B and malaria antigens) and model protein drugs (e.g. bovine serum albumin and lysozyme) have been investigated for drug release kinetics, protein stability and in vivo performance.

Application of novel SLN-Gene vector formulations

Recent work of our group showed that pre-compaction of DNA with oligomers of the HIV-1 TAT peptide for the formulation of gene vector complexes led to an increase of up to two orders of magnitude in gene transfer efficiency[45]. The dimeric TAT peptide was found to be most efficient. This effect was related to the unique features of the HIV-1 TAT peptide which represents a protein transduction domain (PTD) [46, 47] and a nuclear localization sequence (NLS) [48]. The PTD could improve cellular uptake due to its cell penetrating properties. In addition, the NLS function could facilitate nuclear transport of the DNA due to interaction with the endogenous cytoplasmic nuclear transport machinery. In this study we attempted to apply this formulation technique (i.e., the formulation of ternary gene vector complexes consisting of DNA precompacted with a dimeric TAT peptide (TAT2) which were completed by the addition of a cationic gene carrier to SLN formulation. In order to further investigate whether the TAT2 peptide mediates a sequence-dependent effect, gene vectors were formulated by pre-compaction of DNA with either poly- L-arginine (pLa) or the dimeric peptide TAT2-M1 of the nuclear transport-deficient TAT-M1 mutant[48]. Transfec- tion experiments were performed on a bronchial epithelial cell line (16HBE14o-) in vitro. For in vivo application two methods were tested i) direct intratracheal instillation into the mouse lung or ii) whole body nebulization of the gene vectors.

SLN/LNC as a carrier in cosmetic and dermal preparation

At the beginning of SLN research, there were basically only three research groups working on this topic, apart from the groups of Muller and Gasco, the group of Westesen in Braunschweig[49]. The SLN system found more attention which was clearly documented in the increase of research groups working in this area and the number of published papers, a first review being published in 1995[10]. The increase in research groups working with SLN continued, which is documented in two major SLN reviews covering the last decade of SLN research in the last century[50, 51]. However, the research activities in SLN of this last decade focussed almost exclusively on pharmaceutical applications, and within these pharmaceutical applications mainly on non-dermal administration routes, i.e. oral administration and parenteral injection. However, during the last 4 years, SLN were used in topical formulations, not only for pharmaceutical but also for cosmetic products. Apart from the benefits of SLN for topical delivery of active compounds, another reason was the recognition that the time-to-market is very short for these products.

Lipid nanoparticle as a carrier for vaccine

For a long time particulate carriers have been sought as vehicles for protein antigens. An extensive work has been developed in the area of vaccine formulation using various biodegradable polymeric nanoparticles and microparticles, which release their payload of antigen in a controlled manner and possess adjuvant properties by parenteral or mucosal administration routes[52]. Taking into consideration that most peptide or protein antigens are ineffective for mucosal immunisation due to proteolytic degradation at mucosal sites, association with particulate carriers by microencapsulation or adsorption is an established strategy to improve vaccine efficacy. However, most of these polymers present problems associated with the costs and the potentially toxic organic solvents used for microsphere production. Among the biodegradable polymers used for antigen microencapsulation the PLA/PLGA are the most

commonly used. PLA/PLGA microspheres are very useful antigen delivery systems that are ingested by macrophages and dendritic cells, providing lasting immunity thanks to sustained release at relatively predictable times of the microencapsulated or adsorbed material[53, 54]. Concerning lipid-based systems, it has been established in several laboratories that liposomes can act as powerful immunological adjuvants inducing both cellular and humoral immunity for a variety of bacterial and viral antigens relevant to human disease. These seem to be effective when administered by different routes making an attractive vaccine delivery vehicle for the development of mucosal vaccines[55]. Protein antigens may be covalently linked to triacylglycerols using a controlled lipidation technique. Lipidated antigens have the ability to self assemble in water, mimicking viral particles that enhance both humoral and cellular immune responses at systemic and mucosal levels[56]. It was observed that lipid microparticles can trigger in vitro the internalisation of BSA by antigen-presenting cells[57]. However, apart from the already mentioned experiments performed with model protein antigens[57,58,59,60] the incorporation of protein antigens into lipid particles for immunization purposes is still under explored and only a few authors have described their use with real antigens. Experiments on the incorporation of recombinant malaria protein antigen R32NS1 into lipospheres, using a technique which involves the cooling of an emulsion prepared with melted lipids led to an increase in serum specific IgG response after i.m injection. Immune response lasted for at least 12 weeks after primary immunization and it was found to depend on particle composition and particle size, with larger lipospheres (73 μm) being less effective than smaller ones (10 μm)[61]. In a comprehensive study Saraf et al.[62] produced positively and negatively charged lipid microparticles by the solvent evaporation technique (w/o/w) containing protein antigens (HBsAg) for intranasal immunisation against hepatitis B. The mean particle size obtained (1.6 μm) was appropriate for mucosal uptake, which was demonstrated by ex vivo with alveolar macrophages. When given intranasally to rats both anionic and cationic microparticles elicited higher specific mucosal antibodyresponses, when compared with the free and the alum-adsorbed antigen. This response was also higher than that obtained upon i. m. vaccination with the same formulations. The specific anti-HBsAg sIgA levels induced intranasally by the cationic microparticle formulation were the highest in the nasal, pulmonary and salivary glands, as determined in nasal washes, in bronchoalveolar lavages and saliva, respectively. Only in serum, the intramuscularly administered alum-adsorbed antigen resulted in a superior IgG level. A recent report describes the oral immunisation of mice with solid lipid microparticles containing a Japanese encephalitis antigen. The microparticles of 1.6 μm in diameter were prepared by the double-emulsion solvent evaporation method and showed good in vitro uptake by the in estinal M-cells. The oral administration of the vaccine on weeks 0, 1 and 4 induced serum IgG titres higher than the level of protection, up to 14 weeks into the experiment[63]. Although still sparse, the existing information clearly indicates that, as for biodegradable microspheres, lipid microparticles act as effective vaccine carriers with immunoadjuvant properties by parenteral and mucosal routes. Immunity to antigens can be drastically improved through the administration of antigen-containing lipid particles The in vivo deposition observed in the respiratory tract upon intranasal administration[62] particles mechanisms of adjuvanticity is in agreement with the fact that the lung mucosa plays an important role in eliciting immune response to intranasally administered antigens[64,65].

Nanocarriers for nasal vaccination

The use of nanocarriers provides a suitable way for the nasal delivery of antigenic molecules. Besides improved protection and facilitated transport of the antigen, nanoparticulate delivery systems could also provide more effective antigen recognition by immune cells. These represent key factors in the optimal processing and presentation of the antigen, and therefore in the subsequent development of a suitable immune response. In this sense, the design of optimized vaccine nanocarriers offers a promising way for nasal mucosal vaccination[66].

CONCLUSION

Lipid based nanocarriers have the greater importance in the developing field of nanotechnology with several advantages apart from various carriers. Lipid based carriers are a promising nanoscaler delivery system for the pharmceutical industry due to the fact that:

- Large scale production possible, no organic solvents needed
- High concentrations of functional compounds can be achieved
- Lyophilization possible
- Spray drying for lipids with T > 70ºC to yield powders.

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