ISSN : 2320 4850

BI MONTHLY

Asian Journal of Pharmaceutical Research And Development

(An International Peer Reviewed Journal of Pharmaceutical Research and Development)

AJPRD

Volume - 02 Issue - 03 MAY-JUN 2014

website: www.ajprd.com
editor@ajprd.com

Asian Journal of Pharmaceutical Research and Development
(An International Peer-Reviewed Journal of Pharmaceutical Research and Development)
www.ajprd.com
ISSN 2320-4850

Review Article

LIPID NANOPARTICLES: APPLICATION IN CANCER THERAPY

S. M. Salunkhe *, V. M. Gambhire, K. N. Gujar, M. S. Gambhire
Sinhgad College of Pharmacy, Pune- 41

Received: June 2014 ***Revised and Accepted: June 2014***

ABSTRACT

Several obstacles frequently encountered with anticancer drugs, such as normal tissue toxicity, stability, poor specificity and a high incidence of drug resistant tumor cells, are overcome by delivering them using Lipid Nanoparticles (LNs). LNs include Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs), which have been developed by most of researchers for cancer therapy. The SLNs and NLCs because of submicron-sized particulate matter may preferentially extravasate into the tumor and be retained there to achieve passive tumor targeting. The advances in surface-engineering technology modified the surface physico-chemical properties of LNs to target them to the tissue of interest. This maximizes the amount of drug that can reach the targeted tumor sites and minimizes systemic drug toxicity. The emergence of the newer forms of LNs such as polymer– lipid hybrid nanoparticle and long-circulating SLN may further expand the role of this versatile drug carrier in cancer therapy. In the present review, an attempt has been made to highlight the importance of solid lipid nanoparticles and nanostructured lipid carriers in anticancer drug delivery. The discussion includes obstacles in cancer therapy, rationale of using lipid nanoparticles in cancer therapy, significant anticancer activity of LNs -encapsulated cytotoxic drugs, future directions for SLN and NLC- based delivery systems in cancer therapy. In the near future, LNs will be further improved to deliver anticancer compounds in a more efficient, specific and safer manner.

Keywords- *Solid lipid nanoparticles, Nanostructured lipid carriers, Chemotherapy, Obstacles, Anticancer, Cytotoxicity*

INTRODUCTION

Development of new drug alone is not sufficient to ensure progress in drug therapy but development of suitable drug carrier system is a promising strategy to overcome the lacunas of drug therapy. As a drug carrier system, nanomedicine has opened new horizons in cancer therapeutic research. The introduction of nanotherapeutics to cancer treatment is to overcome the inherent limitations of conventional drug delivery systems such as normal tissue toxicity, poor specificity, stability and high incidence of drug resistance to the tumor cells [1].

* Address for correspondence-
S. M. Salunkhe *
Sinhgad College of Pharmacy, Pune- 41
Email- ssalunkhe18@gmail.com
Telephone- +91-9561075834

Nanotherapeutics has come up with various drug delivery systems such as polymeric nanoparticles, nanoemulsions, dendrimers, microemulsions, liposomes and solid lipid nanoparticles [2]. Currently, both clinical and preclinical studies show various types of nanoparticles developed for treatment of cancer. There are many types of nanoparticles currently at an early design step that may progress in the future to preclinical development for cancer imaging and therapy [3]. Here, we would like to highlight the potential of Solid Lipid Nanoparticles (SLNs) and Nanostructured lipid carriers (Second generation SLNs) in Cancer therapy.

In 1990s, three working groups, Muller et al. [4,5], Gasco et al. [6,7] and Westesen [8,9], developed the first generation of lipid nanoparticles, called Solid Lipid Nanoparticles (SLN). In SLN, the oily portion of the emulsion was replaced by a solid lipid or a blend of solid lipids, thus making the lipid

matrix of the SLN which is solid at room as well as body temperature [10]. The SLNs are the submicron colloidal carriers with mean diameter in the range of 50- 1000 nm [11]. Solid lipid nanoparticles represent an alternative carrier system to traditional colloidal carriers, such as emulsions, liposomes and polymeric micro- and nanoparticles. SLN combine advantages of the traditional systems but avoid some of their major disadvantages. SLN provide some advantages like physical stability, protection of the incorporated drug from degradation, controlled release and low cytotoxicity [12]. Ultrasonication or high speed homogenization, High pressure homogenization, Solvent emulsification/evaporation, Micro emulsion based methods, Spray drying are some of the methods employed in formulation of SLNs [13]. The potential disadvantages of SLN are low drug loading capacity, lipid crystalization and drug expulsion during storage [14]. To overcome the SLN related problems, the second generation of lipid nanoparticles, Nanostructured Lipid Carriers (NLC) was developed. NLC possess a solid lipid matrix at room and body temperature that consists of a blend of a solid lipid and oil, preferable in a ratio of 70:30 up to a ratio of 99.9:0.1 [10]. The NLCs which are developed in the early 2000, is embraced to be the successor to solid lipid nanoparticles (SLN) for its superior drug-loading capacity. The NLC has the capability of enhancing the lipophilic drug solubility and providing protection from enzymatic degradation. In the present review, an attempt has been made to highlight the importance of solid lipid nanoparticles and nanostructured lipid carriers in anticancer drug delivery or cancer therapy. The discussion includes obstacles in cancer therapy, rationale of using lipid nanoparticles in cancer therapy, significant anticancer activity of SLN-encapsulated cytotoxic drugs, future directions for SLN and NLC- based delivery systems in cancer therapy.

OBSTACLES TO CONVENTIONAL CANCER THERAPY

The application of SLN formulations for anticancer drug delivery has overcome many obstacles commonly observed with the conventional cancer chemotherapy, such as limited or non specificity, high toxicity, and tendency of drug resistance towards tumor cells [15, 16]. Different research groups have studied obstacles in conventional cancer therapy and tried to overcome them by novel formulations [17-20]. Conventionally administered cytotoxic agents often extensively bind to body tissues and serum protein in a highly unpredictable manner. Because of this reason, only small fraction of the drugs reach the site of tumor, which may both reduces the therapeutic efficacy and increases systemic drug toxicity. Conventional administration routes (e.g. intravenous route and oral) have shown relatively low tumor uptake [21], due to the hindrance of drug-loaded formulation to access the solid tumor [22], and low circulation time, due to the fast clearance by reticulo-endothelial system, which in turn decreases the specific drug targeting effect. For this reason, various alternative application routes, such as duodenal, subcutaneous, and pulmonary routes have been tried to incorporate drug into novel formulations such as SLNs and NLCs. Some of the problems faced during conventional cancer therapy are listed below-

I. Non-specific chemotherapy - Chemotherapy is a group of highly toxic chemical drugs that were developed to kill the cells which are fast-replicating. They are non-specific (not able to distinguish between diseased tumor cells and healthy cells) and are designed to kill all fast-replicating cells [15]. Even though cytotoxic drugs ideally should kill only cancer cells, in reality they are also toxic to healthy cells, especially to rapidly dividing cells, e.g. bone marrow cells and cells of the gastrointestinal tract [16].
II. Combination therapy with so many chemotherapies in together: The available cancer protocols of combination chemotherapy involve the infusion of several different cytotoxic drugs over the course of time to kill cells at different levels of development [15]. But in one of the study on solid Ehrlich tumor, it is concluded that, the combination of MTX and Atorvastatin had a better effect than

each of MTX or Atorvastatin alone against solid Ehrlich tumor in mice [23].

III. Side-Effects of currently available treatments- The commonly observed side effects with cytotoxic drugs are loss of hairs (alopecia), loss of dignity, constant nausea, mouth sores and vomiting: Because hair and the gastrointestinal tract also consist of fast-replicating cells which the chemo/radiation impacts, patient experience hair loss, excruciating mouth and esophageal pain and sores, and constant nausea and vomiting [23].

IV. Loss of energy, anemia, risk of excessive bleeding, recurrent transfusions: Because chemotherapy and radiation therapy also destroys good red blood cells and platelets, patient also suffer anemia and a tremendous loss of energy and risk of excessive bleeding. Because of the loss of red blood cells and platelets the child patient has to endure routine blood transfusions to boost their energy level and must restrict physical activities that might cause excessive bleeding [15].

V. Increased risk of infection that can lead to death: Both chemotherapy and radiation therapy result in a drastic reduction and total elimination of white blood cells which our body need to fight against infections. Accordingly, cancer patients often live in fear of contact with germs and viruses which, while not harmful to other people, could kill them.

POTENTIAL OF SOLID LIPID NANOPARTICLES AND NANOSTRUCTURED LIPID CARRIERS IN CANCER THERAPY

Various drug delivery systems such as liposome, polymer conjugates, dendrimers, polymeric micelles and nanoparticles are being exploited to selectively deliver various anti-cancer drugs to the tumor site [2, 24]. In case of liposome, it requires special storage conditions which lead to increase in cost of processing and stability problems. Due to use of polymers in polymeric nanoparticles the cytotoxicity of drugs gets increased. Camptothecin, Paclitaxel and Doxorubicin have been successfully encapsulated in the dendrimers but again the cost issue arises here [25-27]. However, a need for more efficient and active targeting system does exist to augment intracellular uptake of drug loaded carriers within the cancerous cells. Solid lipid nanoparticles and Nanostructured lipid carriers are novel nanocarriers which consist of biocompatible lipid core and an amphiphilic surfactant at the outer shell. These encompass the advantages of fat emulsions, liposomes, polymeric micelles and nanoparticles while overcome some of their disadvantages. A crucial aspect is that SLNs safeguard drug to a greater extent against chemical degradation as compared to liposomes because there is little access of water to the inner core of lipid particles [28]. Along with this, SLNs and NLCs have advantages like large scale production, ease of modulation of drug release, high drug pay-load and sterilization by autoclaving and gamma irradiation which make them the perfect drug delivery vehicle. The benefit of using SLNs as compared to other polymeric nanoparticles for tumor specific targeting delivery includes the absence of production of toxic metabolites; avoidance of organic solvents [29]. Due to submicron-size of solid lipid nanoparticles, it may preferentially extravasate into the tumor and be retained there for longer time. This is often referred as the “enhanced permeability and retention” (EPR) effect [16, 30]. This EPR effect is helpful in passive tumor targeting. By passive tumor targeting poor tissue specificity problem can be partly solved. Moreover, with the advances in surface-engineering technology (surface modification of SLNs), the biodistribution of SLN can be further manipulated by modifying the surface physico-chemical properties of SLN to target them to the tissue of interest [16, 31]. This maximizes the amount of drug that can reach the targeted tumor sites and minimizes systemic drug toxicity. The nanoparticulate system is hemo-compatible and biocompatible. Various anticancer drugs, including: doxorubicin [17], tamoxifen [1], silibinin [18], emodin [19], baicalein [20], have been incorporated into SLNs and NLCs by different research groups and evaluated.

Mannosylation of solid lipid nanoparticles facilitates targeted delivery of anti-cancer drug

to tumor sites, with reduced access to non-tumor tissues. Thus optimal therapeutic response, improved therapeutic efficacy may be attained with the interception of minimal side effects. By in-vitro studies (Fig.1), and cellular uptake study (Fig.2), A. Jain et al. [29] described the sustained release nature of mannosylated solid lipid nanoparticles of anticancer drugs. They investigated the tumor targeting potential of surface tailored solid lipid nanoparticles (SLNs) loaded with an anti-cancer drug doxorubicin HCl (DOX). In vitro studies concluded that mannose-conjugated SLNs to be least hemolytic and suitable for sustained drug delivery. Mannosylated SLNs were most cytotoxic and were preferably taken up A549 tumor cells as evaluated against uncoated SLNs and plain DOX. Pharmacokinetic studies revealed improved bioavailability, half life and mean residence time of DOX upon mannose conjugation. The biodistribution pattern exhibited that mannosylated SLNs were able to deliver a higher concentration of DOX in the tumor.

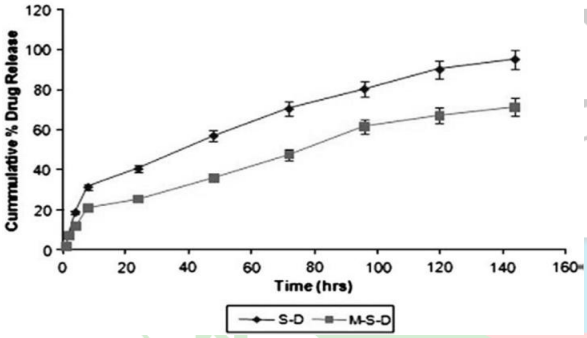

Figure1: In vitro drug release study of Mannosylated SLNs in PBS [29]

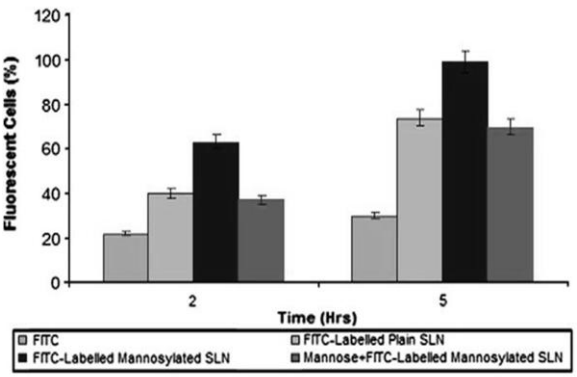

Figure 2: Cellular uptake study of mannosylated SLNs on A549 cell lines [29]

Solid lipid nanoparticles and Nanostructured lipid carriers are known to enhance the bioavailability of drugs with poor oral bioavailability (due to low solubility in GI tract or pre-systemic hepatic metabolism) allowing transportation into the systemic circulation via the intestinal lymphatics and bypassing first-pass metabolism [32]. The reason for why SLNs get preferentially absorbed by lymphatic route is that they could stimulate chylomicron formation with a hydrophobic core and a more hydrophilic surface, and ultimately carry the entrapped lipophilic drugs by following the classical transcellular mechanism of lipid absorption [33]. The poor orally available lopinavir was successfully encapsulated in glyceryl behenate based solid lipid nanoparticles (Lo-SLN) by Alex et al. [34] for its ultimate use to target intestinal lymphatic vessels in combined chemotherapy [Highly Active Anti-Retroviral Therapy (HAART)]. They got the SLNs with mean particle size of 230nm (polydispersity index, PDI < 0.27) and surface electrical charge of approx. −27 mV, by hot homogenization process followed by ultrasonication. From the intestinal lymphatic transport study it became evident that SLN increased the cumulative percentage dose of lopinavir secreted into the lymph, which was 4.91-fold higher when compared with a conventional drug solution in methyl cellulose 0.5% (w/v) as suspending agent (Lo-MC). The percentage bioavailability was significantly enhanced. The AUC for the Lo-SLN was 2.13-fold higher than that obtained for the Lo-MC of similar concentration.

Mussi et al. [35] has given new approach to improve encapsulation and antitumor activity of doxorubicin loaded in solid lipid nanoparticles. Their aim is to develop solid lipid nanoparticles loaded with doxorubicin and evaluating the influence of docosahexaenoic acid (DHA), a polyunsaturated fatty acid that enhances the activity of anticancer drugs, on drug encapsulation efficiency. The in vitro studies (Fig. 3) showed the higher cytotoxicity (Fig.4) of doxorubicin-DHA-loaded SLN than free doxorubicin and DHA on human lung tumor cell line (A549) and the improved cellular uptake achieved with the drug encapsulation.

They hypothesized that encapsulation of amphiphilic doxorubicin is usually low and the formation of an ion pairing with a lipophilic anion, such as the DHA is an alternative to increase encapsulation and also enhancing doxorubicin cytotoxicity.

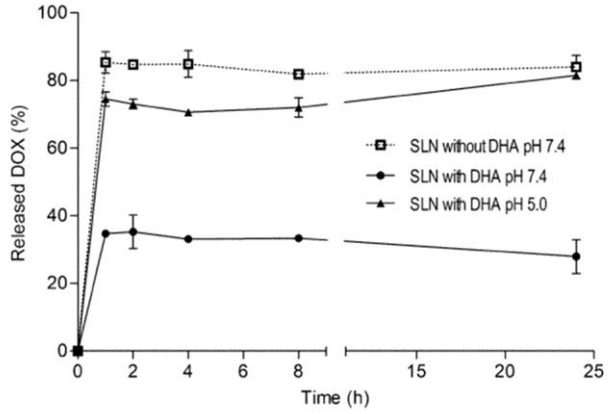

Fig.3: Drug release from doxorubicin-loaded SLN with or without DHA in isotonic PBS (pH-7.4) or in isotonic acetate buffer (pH 5.0) at 37^{0}C. [35]

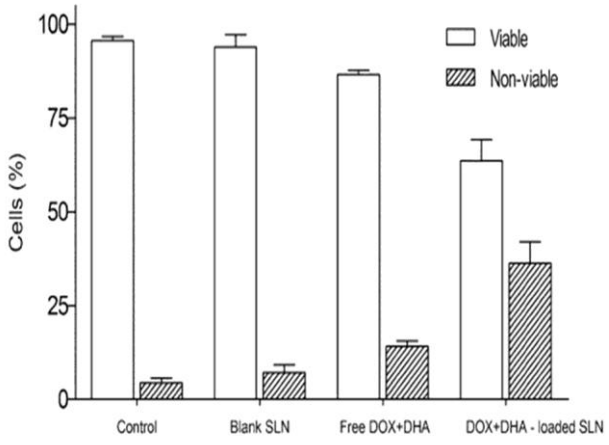

Figure 4: Cytotoxicity studies, as evaluated by TB exclusion assay, of blank SLN, free doxorubicin + DHA and doxorubicin-DHA-loaded SLN (DOX + DHA-loaded SLN) against A549 cell after 48 h exposure [35].

SIGNIFICANT ANTICANCER ACTIVITY OF LNs- ENCAPSULATED CYTOTOXIC DRUGS

The goal of cancer therapy is to kill as many cancer cells as possible by leaving the healthy cells unaffected. To predict the cytotoxicity of a SLN encapsulated drug in cancer cells, just by determining the chemical stability of the encapsulated drug is usually insufficient. In many SLN formulations less than half of the loaded drug is released, but in the cytotoxicity studies, these formulations are sometimes more cytotoxic to the cancer cells than the corresponding free drug. Means the cytotoxic compounds that remain unreleased and associated with the nanoparticles also appear effective. Their anticancer activities may be different from the free drug, even though the chemical identity of the drug is unaltered after encapsulation [16].

Luan et al. [36] developed polyethylene glycol-coated Amoitone B-loaded nanostructured lipid carriers (AmB-PEG-NLC) for parenteral delivery of Amoitone B to prolong drug circulation time in body and enhance the bioavailability. The NLCs were prepared by emulsion–evaporation and low temperature-solidification method. The drug release pattern with burst release initially and prolonged release afterwards was obtained in vitro for AmB-PEG-NLC. The AmB-PEG-NLC exhibited prolonged MRT and higher AUC compared with AmB-NLC as well as Amoitone B solution. They concluded that AmB-PEG-NLC could be a promising delivery system for Amoitone B to prolong the circulation time in body and thus improve its bioavailability.

Tumor metastasis is the leading cause of breast cancer-related mortality and remains to be the main obstacle for the successful chemotherapy of breast cancer. To block metastasis of breast cancer, Xu et al. [37] prepared Silibinin-loaded SLNs containing TPGS and phosphatidylcholine by a thin-film hydration method. The optimized SLNs were approximately 45 nm in particle size with high stability in serum, which were further demonstrated to be efficiently up taken by MDAMB- 231 breast cancer cells. Importantly, the SLNs could accumulate within tumor tissues with high efficiency and amounts. The systematic in vivo evaluations demonstrated that SLNs treatment group resulted in 67% and 39% less pulmonary metastases formation than saline treatment group in the spontaneous and blood vessel metastasis models, respectively. The interesting thing they observed is that, the blank lipid nanoparticles without silibinin were also found, for the first time, to possess

the efficient anti-metastatic capabilities to some extent. Biocompatibility assay in two mouse models reveals that SLNs treatment did not exhibit obvious systemic toxicity. Therefore, SLNs are the promising delivery systems against metastasis of breast cancer cells.

Martins et al. [38] reported about the preparation of camptothecin-loaded SLN by hot high pressure homogenization for the purpose of brain delivery upon intravenous injection. Camptothecin was encapsulated in the hydrophobic and acidic environment of SLN matrix to stabilize the lactone ring, which is essential for its antitumor activity, and for avoiding premature loss of drug so as to target it to the brain. In vitro release studies in plasma, showed a prolonged release profile of camptothecin from SLN, confirming the physical stability of the particles under physiological pH. The prepared SLN showed higher affinity to the porcine brain capillary endothelial cells (BCEC) in comparison to macrophages. MTT studies in BCEC revealed a moderate decrease in the cell viability of camptothecin, when incorporated in SLN compared to free camptothecin in solution. The fluorescently labeled SLN were detected in the brain after i.v. administration after in vivo studies in rats.

Taratula and group [39] developed multifunctional tumor targeted system capable of delivering an anticancer drug simultaneously with siRNA specifically to the lung tumor. They used nanostructured lipid carriers as a vehicle to achieve this goal. In this system, NLC was used as carriers, an anticancer drug served as cell death inducer and siRNA as a suppressor of cellular drug resistance in cancer cells. The prepared NLCs were tested in vitro using human lung cancer cells and in vivo utilizing mouse orthotopic model of human lung cancer. After inhalation, the proposed NLCs effectively delivered its payload into lung cancer cells leaving healthy lung tissues intact and also significantly decreasing the exposure of healthy organs when compared with intravenous injection. When compared with intravenous treatment, the prepared NLCs also showed enhanced antitumor activity. The data obtained by them demonstrated high efficiency of proposed NLCS for tumor-targeted local delivery of anticancer drugs by inhalation and mixture of siRNAs specifically to lung cancer cells and, hence efficient suppression of tumor growth and prevention of adverse side effects on healthy body organs. Large number of compounds has been tried in SLN formulations for its delivery with anticancer properties [Table 1]

Table 1: Summary of the significant works on SLN formulations used for delivery of drugs with anticancer properties

Anticancer compound	**Research group**	**Area of studies**
Camptothecin	Susana Martins and group [38]	Brain delivery of Camptothecin by means of solid lipid nanoparticles
Quercetin	Liang Liu and group [40]	In vivo Biodistribution of quercetin-loaded cationic nanostructured lipid carriers
Curcumin	Jiabei Sun and group [41]	Blending of liquid lipids with solid lipids to encapsulate curcumin in solid lipid nanoparticles
Cholesteryl butyrate, Doxorubicin and Paclitaxel	L. Serpe and group [42]	Evaluation of antiproliferative effect of SLN formulations versus conventional drug formulations on HT-29 cells.
Doxorubicin	Akhtar Siddiqui and group [17]	Overcome multidrug resistance in adriamycin resistant ovarian cancer cells by Doxorubicin and MBO-as GCS oligonucleotide loaded lipid nanoparticles

Emodin	Shengpeng Wang and group [19]	HPH method for preparing high quality Emodin-SLNs and to enhance aqueous solubility
Doxorubicin and Paclitaxel	Oleh Taratula and group [39]	Multifunctional nanostructured lipid nanocarriers-based system(NLCS) for pulmonary co-delivery of anticancer drugs and siRNA by inhalation
Docetaxel	Donghua Liu and group [43]	Modified film ultrasonication–dispersion method for parenteral delivery of Docetaxel- NLCs
Vinorelbine bitartrate	Jian You and group [44]	Effect of enhancement of lecithin content in lipid matrix on particle size
Amoitone-B	Jingjing Luan and group [36]	Development of polyethylene glycol-coated Amoitone B-loaded nanostructured lipid carriers to prolong drug circulation time in body by parenteral delivery
Simvastatin and Tocotrienol	Hazem Ali and group [45]	Combination of simvastatin and tocotrienol rich fraction for potential anticancer therapy.
Silibinin	Pengfei Xu and group [37]	Synergistic inhibition of breast cancer metastasis by silibinin SLNs containing TPGS

FUTURE DIRECTIONS FOR SLN AND NLC- BASED DELIVERY SYSTEMS IN CANCER THERAPY

Tumor-specific targeting with SLNs and NLCs for cytotoxic drug delivery will be beneficial in case of anticancer drugs. In order to increase cancer cell-selective cytotoxicity, one strategy that is gaining attention is to surface-engineer drug delivery systems for molecular targeting. A folate receptor targeted SLN system has been developed for the delivery of a paclitaxel prodrug (paclitaxel-2′-carbonylcholesterol) [46]. The gene therapy is becoming a promising strategy for cancer management in the past few years. Recent emergence of cationic SLN may make it possible to deliver the genetic materials in cancer treatment. Cationic SLN can incorporate cationic lipids like liposomal systems. Using Cos-1 monkey kidney fibroblast-like cells, SLN and liposomes formulated from the same cationic lipids demonstrated equipotent in vitro transfection efficiencies [47]. The Gene therapy is proposed to be used alone or in combination with cytotoxic drug treatment. Simply administering the cytotoxic drugs in the form of SLN may be useful but is unlikely to solve the whole drug resistance problem. So to solve the drug resistance problem cytotoxic drugs are given in combination with chemosensitizers. A few SLN formulations of chemosensitizers have been prepared and studied. SLN of cyclosporin-A [48] and polymer lipid hybrid nanoparticle (PLN) of verapamil [50] were successfully formulated, although they have not been tested upon any biological systems. Ho Lun Wong in their study which involves the use of a PLN system co-loaded with doxorubicin and verapamil [49,50], demonstrated that this system may deliver both of the cytotoxic (doxorubicin) and chemosensitizing (verapamil) agents

simultaneously, and the release profiles of the two drugs apparently did not interfere each other.

CONCLUSION

The anticancer drugs, particularly cytotoxic drugs are more diverse in terms of molecular structure and physicochemical properties and they are also much more reactive, toxic and unstable. The SLNs and NLCs due to their tremendous flexibility and favorable qualities, it will be not surprising if this relatively new class of drug carriers is quickly being adopted for the delivery of various anticancer compounds. There are so many anticancer drugs which have already tried for SLN based drug delivery systems. Few of them are Camptothecin, Quercetin, Curcumin, Cholesteryl butyrate, Doxorubicin, Paclitaxel, Emodin, Docetaxel, Vinorelbine bitartrate, Amoitone-B, Silibinin, Simvastatin and Tocotrienol. Very soon it is expected that modified forms of SLN such as NLC, LDC, PLN, stealth SLN, targeted-SLN, and SLN loaded with drug combinations will be perfected and utilized to further improve the efficacies and side effect profiles of chemotherapeutic drugs for anticancer treatment.

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