Liposomal Paclitaxel: Recent Trends and Future Perspectives

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ABSTRACT

One of the most promising anti-cancer agents paclitaxel, especially effective for treatment of ovarian and breast cancer, suffers from the problems like poor water solubility and low bioavailability. The current formulation available in a non-aqueous vehicle containing Cremophor EL® (polyethoxylated castor oil), on aqueous dilution, when given intravenously, may cause allergic reactions and precipitation. The lack of any appropriate delivery vehicle limits and delays the extensive clinical use of this drug. Hence, the development of alternate paclitaxel formulation with good aqueous solubility and lesser side effects is highly recommended. Researchers have so far explored different techniques including co-solvents, emulsions, micelles, liposomes, microspheres, nanoparticles, cyclodextrans, pastes, and implants. Among all these formulations, liposomes have been found to be highly effective against cancer. The current review encompasses the recent advancements in the delivery of paclitaxel by using liposomes. The focus is on searching future prospective for the safe and effective delivery of paclitaxel.

Keywords: Liposomes, Paclitaxel, PEGylation, Targeting.

INTRODUCTION

Paclitaxel (PTX), a diterpenoid pseudo alkaloid (Figure 1), is the first of a new class of microtubule stabilizing agents, isolated from natural source. The molecular formula of C_{47}H_{51}NO_{14}, corresponding to the molecular weight of 853 Da. PTX confers favorable therapeutic effects to treat many tumors, including breast, lung, ovarian, colon, head, and neck cancers. The generally accepted dose is 200–250 mg m⁻² and is administered as a 3 and 24 h infusion every 3 to 4 weeks, thus producing a complete or partial response in patients with ovarian and metastatic breast cancer.

![Figure 1: Chemical structure of paclitaxel (5β,20-Epoxyp-1,2α,4,7β,13α-hexahydrotax-11-en-9-one4,10-diacetate2-benzoate13-ester with (2R,3S)-N-benzoyl-3-phenyllisoserine)](image)

Available Dosage Forms of Paclitaxel And Existing Limitations

Paclitaxel is poorly soluble in an aqueous medium, but can be dissolved in organic solvents. Solution of paclitaxel can be prepared in a millimolar concentration in a variety of alcohols such as methanol, ethanol, tertiary-butanol and DMSO. Moreover, paclitaxel lacks functional groups that are ionisable in a pharmaceutically useful pH range and therefore manipulation in pH does not enhance its solubility. Furthermore, common approaches to overcome solubility limitations like addition of charged complexing agents or producing alternate salts of paclitaxel are not feasible. Paclitaxel is currently formulated in a vehicle composed of 1:1 blend of Cremophor EL® and ethanol which is diluted 5–20-fold with normal saline or dextrose solution (5%) for administration. The side effects associated with Cremophor EL® include hypersensitivity reactions, hyperlipidaemia, abnormal lipoprotein patterns, aggregation of erythrocytes and peripheral neuropathy. Cremophor EL® also influences the function of the endothelial and vesicular muscles and causes vasodilation, labored breathing, lethargy and hypotension. Premedication with corticosteroids (dexamethasone) and antihistamines (both H1 and H2-receptor antagonists) enhances the safety and reduces the intensity and the incidence of serious hypersensitivity reactions, associated with paclitaxel in Cremophor EL®. Paclitaxel has short-term physical stability, as some particles slowly tend to precipitate out of the aqueous media. Over a period of 24 h, small number of particles was observed in the diluted form and the number of particles appeared to be greater in the solution containing more than 0.6 mg/ml of paclitaxel. In addition to the possibility of metabolic interactions, paclitaxel is highly bound in the blood (about 90–95%). Such binding is concentration independent and none of the agents used for prophylaxis alter the binding capacity significantly. However, the protein binding decreases the cellular uptake of paclitaxel.
Need of Liposomes

Liposomes are spherical vesicles with several concentric membranes. Liposomes are composed of polar lipids which are characterized by a lipophilic and hydrophilic group on the same molecule. They encapsulate a fraction of the solvent for encapsulating hydrophilic drugs. Lipophilic drugs, on the other hand, can be incorporated in their lipid bilayer. Liposomes (Figure 2) are biodegradable and are essentially non-toxic vehicles for both hydrophilic and hydrophobic moieties.

Table 1: Stages in the development of paclitaxel as an anticancer drug

<table>
<thead>
<tr>
<th>Year</th>
<th>Progress</th>
</tr>
</thead>
<tbody>
<tr>
<td>1962-68</td>
<td>NCI screening of cytotoxic drugs from natural products</td>
</tr>
<tr>
<td>1967</td>
<td>Antitumor activity detected</td>
</tr>
<tr>
<td>1969</td>
<td>Pure paclitaxel isolated</td>
</tr>
<tr>
<td>1971</td>
<td>Structure elucidated</td>
</tr>
<tr>
<td>1983</td>
<td>Phase I studies Initiated</td>
</tr>
<tr>
<td>1986</td>
<td>Hypersensitive reactions observed</td>
</tr>
<tr>
<td>1988</td>
<td>NCI suggests premedication regimen</td>
</tr>
<tr>
<td>1989</td>
<td>Proved effective against ovarian cancer</td>
</tr>
<tr>
<td>1991</td>
<td>Proved effective against breast cancer</td>
</tr>
<tr>
<td>1992</td>
<td>Proved effective against non-small cell lung cancer</td>
</tr>
<tr>
<td>1992</td>
<td>Approved by US FDA for ovarian cancer</td>
</tr>
<tr>
<td>1992</td>
<td>Approved by US FDA for breast cancer,</td>
</tr>
<tr>
<td></td>
<td>Total synthesis by Nicolaou and Holton,</td>
</tr>
<tr>
<td></td>
<td>independently</td>
</tr>
<tr>
<td>1994</td>
<td>Approved in India for ovarian cancer</td>
</tr>
<tr>
<td>1995</td>
<td>Approved in India for breast cancer</td>
</tr>
<tr>
<td>2000</td>
<td>Available in generic form</td>
</tr>
<tr>
<td>2001</td>
<td>Approved in the UK for ovarian, breast and lung cancers and Kaposi’s sarcoma</td>
</tr>
<tr>
<td>2005</td>
<td>Approved by US FDA in the form of Albumin-bound paclitaxel (trade name Abraxane®)</td>
</tr>
<tr>
<td>2012</td>
<td>Abraxane® in combination with carboplatin</td>
</tr>
<tr>
<td></td>
<td>approved by US FDA, for the initial treatment non-small cell lung cancer (NSCLC)</td>
</tr>
</tbody>
</table>

Figure 2: A. Graphical representation of liposome. B. Transmission electron microscopic photograph of liposomes

The main advantages of using liposome include:

- Bio-compatible
- Simple method of preparation
- Versatile nature of the liposomes allows the loading of hydrophilic, amphiphilic and lipophilic compounds
- Most conventional liposomes are trapped by the reticulo endothelial system (RES) hence, delivery to the RES can be achieved easily
- Targeting principles can effectively be applied to liposomes

Liposomes, because of their biphasic character, can act as carriers for both lipophilic and hydrophilic drugs. Depending upon their solubility and partitioning characteristics, the drug molecules are located differently in the liposomal environment and exhibit different entrapment and release properties. Based on these parameters, the drugs can be divided into four classes (1) highly hydrophilic, (2) highly lipophilic, (3) amphiphilic drugs that exhibit good biphasic solubility, (4) drugs that exhibit biphasic insolubility. Highly hydrophilic drugs like cytosine and 5-FU are located exclusively in the aqueous compartments of the liposomes. Highly lipophilic drugs are entrapped almost completely within the lipid bilayer of the liposomes, e.g. cyclosporine. Since they are very poorly soluble in water, problems like loss of entrapped drug on storage are minimal with this class of drugs. Drugs with intermediate partition coefficients pose a major problem because they partition easily between the lipid and aqueous phases and are easily lost from the liposomes. Examples are mitomycin C, actinomycin D and vinblastine. Such molecules form stable liposomal stems only when they form complexes with the membrane lipids. However, the most problematic candidates for liposomal entrapment are the drug molecules which have poor biphasic solubility. Being insoluble in either aqueous or lipid phase, they show low uptake by the liposomes. Typical examples include 6-mercaptopurine and azathioprine. For these liposomal candidates, the lipophilic drugs have been proved to be the best in the terms of cost, stability and utility.
LIPOSOMES IN CANCER CHEMOTHERAPY

Drug delivery systems based on liposomes enhance the therapeutic efficacy of anti-cancer agents, either by increasing the exposure of tumor cells to the drug, decreasing the normal tissues damage, employing the enhanced permeability and retention effect (EPR) phenomenon or by utilizing the targeting principles.\(^{23}\) Phospholipids are the key components along with cholesterol to formulate liposomes. Due to their unique features, liposomes enhance the performance of products by increasing the ingredient solubility, improving its bioavailability, enhancing the intracellular uptake and altering the pharmacokinetics. Thus, liposomes improve the overall in vitro and in vivo performance. Being encapsulated in liposome, the drug is protected from metabolism and inactivation in the plasma and due to reduced volume of distribution, less drug accumulates in the healthy tissues.\(^{24}\) Extravasations into the interstitial space from the bloodstream is the general pathway of liposome to reach their site of action for anti-cancer therapy. Both active and passive targeting strategies can be designed with liposomes. The vesicular size is the most significant physical aspect in the clinical success of liposomes. Liposomes of size ranging from 50-100 nm are the most suitable candidates for cytotoxic drug delivery.\(^{25}\) Liposomes can be easily manipulated by anchoring additional molecules in the form of ligand to the outer surface of the lipid bilayer. Coating of liposomal membrane with polyethylene glycol (PEGylation) reduces opsonization, thus reducing the clearance by the RES, eventually improving the circulation half-life. Formulations of anticancer drugs based on liposome have been already approved for human use.\(^{26,27}\)

Conventional Liposomes of Paclitaxel

Current formulation of paclitaxel comprises of a 1:1 blend of Cremophor EL\(^{®}\). However, this vehicle is associated with several side effects. Paclitaxel liposome (Lipusu\(^{®}\)) developed by Sike Pharmaceutical Co. Ltd., Nanjing, Jiangsu, P.R. China, was the first paclitaxel liposome injection that came to the clinical market in China in 2006. In order to reduce the vehicle related toxicity and to enhance the therapeutic benefits, newer recommendations for the premedication of Lipusu\(^{®}\) in the treatment of solid tumors were advocated. These include (1) methylprednisolone 40 mg administered intravenously 30 min before Lipusu\(^{®}\), and granisetron 30 min before chemotherapy, (2) dexamethasone 2.25-3 mg taken orally 12 h and 2 h before Lipusu\(^{®}\), and granisetron 30 min before chemotherapy.\(^{28}\) To eliminate the need of Cremophor EL\(^{®}\) vehicle, an attempt was made by Zhang and co-workers to develop a well characterized novel lyophilized liposome-based paclitaxel (LEP-ETU) formulation. Because LEP-ETU is not formulated in a Cremophor EL\(^{®}\) vehicle, hypersensitivity reactions were avoided. Preliminary data showed that LEP-ETU was well-tolerated at doses up to 325 mg/m\(^2\) compared to the recommended dose of 175 mg/m\(^2\).\(^{29}\) Phospholipids (mainly phosphatidylcholine) and cholesterol are the key components to formulate conventional liposomes. Recently, liposomes composed of naturally unsaturated and hydrogenated phosphatidylcholines were formulated and characterized. The liposomes exhibited a 15% paclitaxel to phospholipid molar ratio on phospholipid bilayer without precipitates during preparation. The liposomal formulation was found to be stable in liquid form at 4°C for at least 6 months. The median lethal dose (LD\(_{50}\)) for intravenous bolus injection in mice exceeded by 40 mg/kg, as observed in the acute toxicity test. The liposomal formulation manifested significantly reduced acute toxicity in mice compared to the current Cremophore EL\(^{®}\)/alcohol formulation.\(^{30}\)

PEGylated Liposome- Based Paclitaxel

Liposomes with particle size > 200 nm are directly taken up by the RES. Longer circulation half life is required for improved biodistribution and greater cytotoxic effect. One of the effective methods to achieve this is size reduction of liposomes and the other proven method involves coating of liposomal membrane with polyethylene glycol (PEG) like hydrophilic polymers.\(^{25}\) In order to improve the solubility and circulation half life of liposomes, Yang and co-workers formulated PEGylated liposomes of paclitaxel. The biological half-life of prepared PEGylated liposomal paclitaxel increased from 5.05±1.52 h to 17.8±2.35 h compared to the conventional liposomes in rats. Significantly reduced uptake in RES-containing organs like liver, spleen and lung was further established by the biodistribution studies in breast cancer xenografted nude mouse model.\(^{26}\) Recently, for the improvement of in vivo disposition and anti-tumor efficacy, PEG liposome was formulated by Yoshizawa and co-workers. The PEGylated liposomal formulation showed 3.6 times higher Area under curve (AUC) than that of naked liposome following intravenous injection into normal rats. The effect could be due to the lower uptake in RES-containing organs like liver, spleen and lung. Significantly (p < 0.05) larger amount of paclitaxel delivered to tumor tissue to obtain improved anti tumor effect was further confirmed by in vivo studies utilizing Colon-26 solid tumor-bearing mice.\(^{31}\) Multidrug resistance (MDR), associated with the overexpression of drug efflux transporter pumps such as P-glycoprotein, is one of the major obstacles to get successful cancer chemotherapy. Long circulatory liposomes were formulated to overcome this problem. A highly efficacious third generation P-gp inhibitor tariquidar was also delivered with paclitaxel. To reduce the exposure to normal tissue and to enhance their co-localization in the tumor cells, both were loaded in the long circulatory liposomes. The formulation showed an increased accumulation of paclitaxel in the tumor cells and improved resensitization was evident even in resistant variant for paclitaxel.\(^{32}\)

Targeted Liposome-Based Paclitaxel

Recent reports have proved that the therapeutic potential of chemotherapy agents can be significantly enhanced by
tumor cell targeting. The targeting principle provides certain specific properties like minimal exposure to nontargeted tissues or organs, efficacy and safety due to high selectivity towards a biological target, overall higher concentrations of drug molecules within tumor area, protection from enzymatic degradation, and improved biocompatibility due to low immunogenicity. A specific expression pattern of receptors, membrane transport systems or adhesion molecules is associated with the tumor cells. These molecules can be easily and effectively targeted with suitable ligands. The molecules that selectively recognize and bind to a target antigen selectively expressed by the tumor cells can be used as a ligand. Antibody molecules or their fragments, naturally occurring or synthetic ligands such as peptides, carbohydrates, glycoproteins or receptor ligands can be explored as targeting groups. Different receptors like folate, HER-2, peptide and hyaluronan were investigated for this purpose. A novel liposomal formulation of paclitaxel targeting the folate receptor (FR) was prepared and characterized. An over expressed folate receptor on epithelial cancer cells were targeted with this formulation. The formulation was composed of DPPC/DMPG/DSPE-mPEG/DSPE-PEG-folate (molar ratio, 85.5:9.5:4.5:0.5) and had a drug-to-lipid molar ratio of 1:33. The liposomes developed by the membrane extrusion method had a mean particle size of the 97.1 nm and were found to be stable for at least 72 h at 4 °C. Cellular uptake studies revealed a 3.8-fold greater cytotoxicity with FR-targeted liposomes as compared to the non-targeted control liposomes in KB oral carcinoma cells. A remarkably longer terminal half-life (12.33 h) was observed for FR-targeted liposomes compared to paclitaxel in Cremophor EL* (1.78 h). The breast cancer cells were targeted on human epidermal growth factor receptor-2 (HER-2) for the delivery of PTX by liposomes. A PEGylated paclitaxel-loaded liposome was developed and coupled to thiolated Herceptin to target human breast cancer cells. Cellular uptake study was performed with over-expressing HER2 breast cancer cells BT-474 and SK-BR-3 and PEGylated immunoliposome showed twice higher uptake than PEGylated liposome. In another study, paclitaxel-loaded PEGylated targeted liposome was investigated for uptake and cytotoxicity in breast cancer cell lines while in vivo antitumor efficacy was performed in the xenograft nude mouse model. Cellular uptake was increased with PEGylated targeted liposome compared to non targeted liposome. Moreover, higher tumor tissue distribution of paclitaxel in the BT-474 xenograft model with PEGylated targeted liposome as compared to non targeted liposome was further confirmed in the in vivo study. Vascular targets expressed by the endothelial cells like the vascular endothelial growth-factor receptors such as neuropilin-1 (NRP-1) and alpha V integrins were effectively targeted. To use AlphaV integrins as a target that is overexpressed on the surface of new formed tumor vessels, PEGylated liposomal formulation of paclitaxel was prepared. The Arg-Gly-Asp (RGD) sequence was integrated in to lipid bilayers. The purpose was to improve the solubility and specific targeting ability of paclitaxel to tumor vasculature. More than 95% drug entrapment and less than 100 nm size were achieved with this formulation. The drug was found to be stable in vitro and higher concentration of paclitaxel was obtained in the plasma distribution study as compared to the Taxol™-treated group. Cellular uptake was enhanced in presence of integrin-targeted liposomal paclitaxel-treated group compared to the Taxol™-treated group. Treatment of mice bearing the human lung carcinoma cell line A549 with the integrin-targeted paclitaxel liposomes resulted in greater cytotoxicity i.e. a lower tumor microvessel density and ligand mediated internalization compared to the Taxol™-treated group. With the use of similar strategy, a novel dual targeted liposome anchored with an alpha V integrin specific ligand and a neuropilin-1 specific motif was formulated. Binding affinity was increased compared to the single targeted liposome. Improved cellular uptake and significantly increased tumor suppression were observed with studies performed on A549 (human lung carcinoma) and HUVEC (human umbilical vein endothelial cells). Promising behavior of this dual target was further confirmed by better tumor growth inhibition in the tumor xenograft models compared to the general paclitaxel injections (Taxol™) and single-targeted paclitaxel liposomes. Another example of receptor is represented by the fibroblast growth factor receptors. Variety of tumor cells and tumor neovascularity overexpress this receptor on the surface, thus representing a suitable target for tumor and vascular targeting. To explore this receptor, a novel truncated basic fibroblast growth factor peptide anchored cationic liposomal paclitaxel was formulated. Pharmacokinetic parameter such as the AUC value was found to be 1.38-fold better in comparison to free paclitaxel. In the biodistribution study, significantly higher accumulation in the tumor cells was observed in comparison with free paclitaxel and cationic liposomal paclitaxel. Another newer approach represented by the folic acid and modified transactivating transcriptional activator peptide (TATp) conjugated PEGylated polymeric liposomes and both are attached to the octadecyl-quaternized lysine-modified chitosan. With a particle size of 60 nm, this formulation showed an instant internalization by folate receptor-overexpressing KB human nasopharyngeal carcinoma cells. In vitro cytotoxicity was better in this formulation as compared to Taxol™ and the antitumor efficacy in murine models bearing nasopharyngeal carcinoma of this novel formulation was found to be comparable to Taxol™. Beside all these receptors, CD44 receptor that binds hyaluronic acid (hyaluronan or HA) was found to be upregulated in many cancers of epithelial origin. Two different types of target CD44 on cancer cells and HA in the matrix were also explored for this purpose.
NEWER TARGETS VS NEWER OPPORTUNITIES

Based on physiology

Some of the vascular barriers of tumors are represented by heterogeneous blood flow, expression of efflux transporters, high intercapillary distance, and extracellular matrix components. The key problem with the use of any nanocarrier is to effectively deliver them to and into the tumors or even inside the individual tumor cells. EPR effect is the physiology-based principal that is directly related to the anatomical and pathophysiological difference from the normal tissues. The EPR effect is characterized by leaky vasculature and lack of lymphatic drainage, resulting in enhanced accumulation of particulate and macromolecules at the tumor site. Drug loaded nanocarriers enter into the interstitial space of tumor by leaky vasculature and are retained by the impaired lymphatic system. One of the prime requirements for a suitable EPR-based drug delivery system is its long circulation time in the blood. To achieve sufficient accumulation at the tumor site, they should stay for an extended period of time in the blood. Significant uptake in reticuloendothelial system (RES)-containing organs, including the liver, spleen and lung is predominantly responsible for the early clearance of drug delivery systems. To achieve this requirement, size reduction of liposome and PEGylation of liposomal membrane are the effective methods. Long plasma half-life with reduced uptake by RES has been demonstrated by PEGylated liposomes, which has proved them as a suitable candidate for the delivery of anticancer drugs. Some serious limitations involved in EPR effect-based drug delivery include the presentation of great pathophysiological heterogeneity by tumors and absence of EPR effect particularly in the central area of the metastatic cancers. To overcome these limitations, several attempts like elevation in blood pressure for hydrodynamic enhancement of drug delivery and by generation of N.O. (nitric oxide) with (N.O.-)-releasing agents are being made. To summarize, the EPR-mediated drug delivery is currently considered as an effective means to bring drugs to and into the tumors.

Based on formulation

Different drug delivery systems like PEGylated and targeted liposomes are already proved as suitable drug delivery systems for anti-cancer drugs. Currently stimuli sensitive approaches (pH, temperature, ultrasound, etc.) are gaining attention. Thermosensitive formulation was prepared by PTX-containing liposomes dispersed in Pluronic® F127 gel. A higher in vitro drug release was observed with gel loaded liposome in comparison to liposome and commercial formulation Taxol®. In experiments performed with KB cells, cytotoxicity and drug concentration were higher in case of gel loaded liposome. It has been established that PEGylated liposomes offer a stable and long circulatory formulation capable of reaching the tumor site. They are effective in minimum release of drug during circulation but must release their content upon reaching the tumor site. Presence of PEG interferes in this process and hence there is a need to find suitable and effective method for triggered release of reagents from PEGylated liposomes. Light activation can be considered as a promising method for triggering vesicle content release since it provides a broad range of adjustable parameters (such as pulse duration, intensity, pulse cycle, and wavelength) that can be optimized for biologic compliance. The majority of these approaches utilize visible or ultraviolet light. Recent evidence has demonstrated that even far-red excitation (760 nm) can be used to alter membrane permeability.

CONCLUSION

Paclitaxel (Taxol®), the first taxane in clinical trials, is active against a broad range of cancers that are generally considered to be refractory to conventional therapy. Several studies have implicated that liposomal delivery system is safer and effective as compared to Taxol®. Formulation like Lipusu® has been already commercialized. Recent formulations in the form of ligand targeted liposomes are under various developmental stages of clinical trials. However, the safety, efficacy and economics of liposomes would influence the development of an ideal dosage form that would bypass the present limitations and provide a desirable means of cure.

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