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Review

Pharmaceutical aspects of paclitaxel

Dedicated to: The author dedicates this review to his *Guru*, Guide and Philosopher Professor Wolfgang A. Ritschel, University of Cincinnati, on his 65th birthday.

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Abstract

Paclitaxel is one of the most important lead compounds to emerge from a natural source. Because of the complex and unusual chemistry of paclitaxel, it is mainly extracted from the bark of a slow growing Western yew. Although total chemical synthesis of paclitaxel has been achieved, it may not be feasible commercially. Paclitaxel has a low therapeutic index: it is highly lipophilic and practically insoluble in water. The commercially available injection preparation is a sterile solution of the drug in Cremophor® EL and dehydrated alcohol. Present-day cancer chemotherapy with paclitaxel frequently causes hypersensitivity reactions. The major hurdles for successful therapy with paclitaxel are the availability of the drug and its delivery. The importance of developing an improved delivery system for paclitaxel is obvious from the problems seen from present-day therapy. Hence, the current approaches are mainly focused on: (1) developing formulations that are devoid of Cremophor® EL, (2) the possibility of large-scale preparation; and (3) stability for longer periods of time. The path to identify new molecules with better therapeutic efficacy will continue to be an integral part of health care systems, but the author is emphasizing the importance of 'better delivery of drugs' which is going to further refine the therapy. The different approaches investigated so far have shown much promise in replacing the Cremophor® based vehicle for paclitaxel delivery. However, the final product for human use is still far away. Therefore this review is the first comprehensive account of the pharmaceutical aspects of paclitaxel, with special emphasis on its delivery.

Keywords: Drug delivery; Paclitaxel; Pharmaceutical aspects

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1. Introduction

Cancer is a general term for a group of diseases caused by abnormal and unrestricted growth of cells. It has high morbidity and mortality, being the second most common cause of all deaths (about 20–25%), after cardiovascular diseases, in the Western developed countries. Traditional and folk medicine have long used plant products for therapeutic purposes. Treatment of 'gulma' (cancer) by using herbs was described in the first surgical treatise from India, *Sushruta Samhita*, as far back as 2500 BC, and Ayurveda (the Indian system of medicine) also described treatment of cancer with certain plants (Chunekar and Pandey, 1990). *Eber Papyrus* described the same in 1500 BC (Hartwell and Schrecker, 1951). Since then, the pioneering research of Gilman and Farber in the 1940s demonstrated that patients with certain cancers could be cured by systemic chemotherapy, if diagnosed early (Gilman and Philips, 1946, Farber et al., 1948). At present, it is possible to obtain remission in patients with advanced stage of disease because of new agents, combination therapy and novel delivery of drugs.

Paclitaxel was discovered as part of the new cancer drugs screening and discovery program of the National Cancer Institute in the 1960s. In this program many plant extracts were screened for anticancer activity, which included a crude extract from the bark of *Taxus brevifolia* (Pacific or Western yew). This crude extract showed antitumor activity against several cancer cell lines and the chemical structure of the active ingredient of the extract was identified as paclitaxel (Wani et al., 1971). No other chemotherapeutic agent other than penicillin has generated so much interest as paclitaxel since its unique mode of action was discovered in 1979 (Schiff et al., 1979). Paclitaxel is one of the most important lead compounds to emerge from a natural source. After about two decades, since its identification as an anticancer agent from *Taxus brevifolia* (Rowinsky and Donehower, 1991; Suffness, 1993), paclitaxel has been an approved drug for ovarian and breast cancer in the USA (Fig. 1). Paclitaxel is effective against several murine tumors and is one of the most exciting anticancer molecules currently available. The advances achieved during the last two decades have resulted in the successful treatment of certain cancers. The objective of this review is to focus attention on various pharmaceutical aspects of paclitaxel with major emphasis on its delivery.

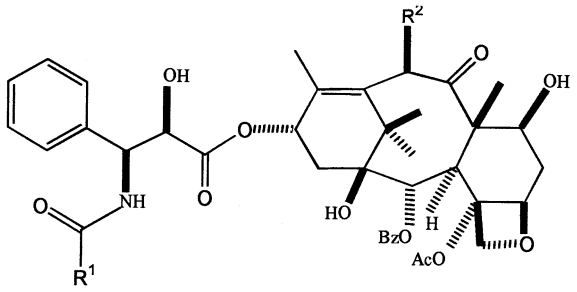
2. Chemistry of paclitaxel

Paclitaxel (Fig. 2), a diterpinoid, is an important new anticancer drug first isolated from Western yew, *Taxus brevifolia* (Wani et al., 1971). The chemical name of paclitaxel is 5β,20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one-4,10-diacetate-2-benzoate 13 ester with (2*R*, 3*S*)-*N*-benzoyl-3-phenylisoserine (molecular formula $C_{47}H_{51}NO_{14}$; molecular weight 853.9). Paclitaxel differs structurally from other currently available antineoplastic agents (Kingston, 1991, Gregory and DeLisa, 1993).

Because of the complex and unusual chemistry of paclitaxel, it is mainly extracted from the bark

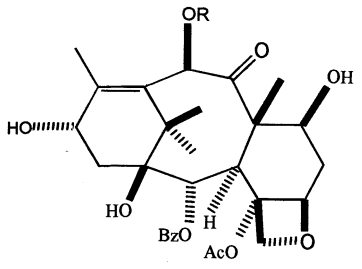
1962-68	NCI screening of cytotoxic agents from natural products
1967	Antitumor activity detected
1969	Pure paclitaxel isolated
1971	Structure elucidated
1983	Phase I studies initiated
1986	Hypersensitive reactions observed
1988	NCI suggests premedication regimen
1989	Proved effective against ovarian cancer
1991	Proved effective against breast cancer
1992	Proved effective against non-small cell lung cancer
1992	Approved by US FDA for ovarian cancer
1994	Approved by US FDA for breast cancer
	Total synthesis by Nicolaou and Holton, Independently
1994	Approved in India for ovarian cancer
1995	Approved in India for breast cancer

Fig. 1. Stages in the development of paclitaxel as an anticancer drug.



Paclitaxel : R^1 = Ph; R^2 = OAc

Docetaxel : R^1 = ^{t}BuO; R^2 = OH



Baccatin III; R = Ac
10-deacetylbaccatin III(10-DAB); R = H

Fig. 2. Chemical structures of the taxoid drugs licensed so far, and baccatins.

of a slow growing Western (Pacific) yew, and yields are about 0.01% of the dry weight of bark (Whiterup et al., 1990). Newer methods of extraction of paclitaxel in large-scale application using chloroform from a single reverse-phase column has increased yields to 0.04% (Rao et al., 1995). Approximately 3000 trees must be sacrificed in order to obtain 1 kg of paclitaxel and is sufficient to treat about 500 patients; present-day protocol prescribes about 2 g for total treatment (Blume, 1989).

An alternative method allowing preparation of the drug in larger yields (i.e. a semisynthetic method using a precursor extracted from needles and twigs of a more prevalent yew) has been developed (Nicolaou et al., 1994a). Docetaxel (Fig. 2), a semisynthetic taxoid, is produced by attaching a semisynthetic side-chain to 10-deacetylbaccatin III, which is readily available, in yields of about 1 kg per 3000 kg of needles, from *Taxus baccata* (Colin et al., 1990). Although total synthesis of paclitaxel was achieved (Holton et al., 1994, Nicolaou et al., 1994b), the total chemical synthesis of paclitaxel on an industrial scale is very difficult and may not be feasible commercially (Jenkins, 1996).

In the treatment of cancer using paclitaxel, the availability of the drug to meet the growing demands appears to be one of the limiting factors, in addition to delivery problems (Anon., 1996). However, it appears that *Taxus* cell culture, by a fungal endophyte, *Taxomyces andreanae*, for biosynthesis of paclitaxel might be a solution (Strerle et al., 1993), even though the amount produced is very low. A recent report indicates the possibility of increasing the production of paclitaxel and baccatin III in *Taxus* cell suspension cultures by addition of methyl jasmonate, which plays an important role in signal transduction processes (Yukimune et al., 1996). Sustained production of paclitaxel by semicontinuous perfusion nodule cultures appears to be another approach to produce large quantities (Ma et al., 1994, Ellis et al., 1996). Preliminary results indicate that the concentration of sucrose in the culture enhances paclitaxel production. On the other hand, the concentration of precursors and elicitors had no effects on paclitaxel production. Although the results are very promising, large-scale production with commercial feasibility needs to be established. Additional means of paclitaxel or baccatin III production through biotechnological methods involving plant tissue culture appears to be bright prospect in unfolding the fascinating story of paclitaxel.

Paclitaxel is a white to off-white crystalline powder. It is highly lipophilic and practically insoluble in water. Although paclitaxel analogs appear be another approach to overcome the problem of availability, the limiting factor is the construction of the taxane framework with its four-membered oxetane ring and a homochiral ester side-chain at C13 which becomes a challenge for synthetic chemists. Extensive studies have indicated that an intact taxane ring and an ester side-chain are essential for cytotoxic activity

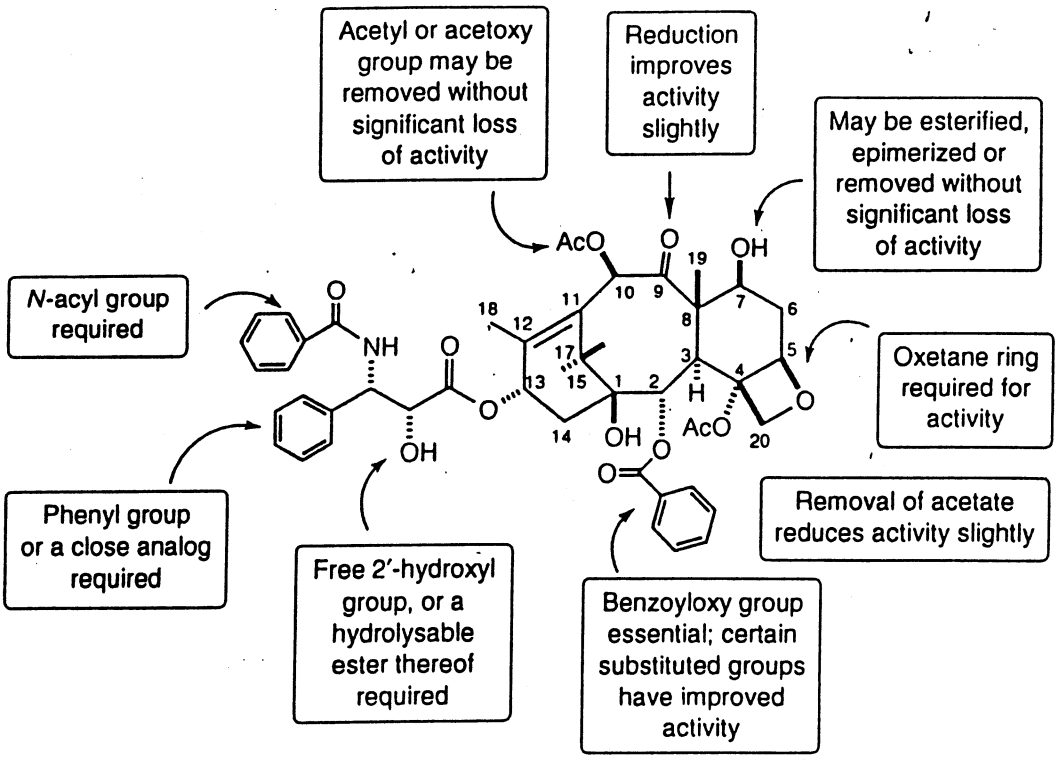


Fig. 3. Structure–activity relationships of paclitaxel (from Kingston, 1994, with permission).

(Kingston, 1994). In addition, it was shown that the presence of an accessible hydroxyl group at position 2″ of the ester side-chain enhances the cytotoxic activity of the drug (Guenard et al., 1993). Modification of the side-chain has resulted in a more potent analog, docetaxel (Fabre et al., 1995). These structure–activity relationships are very important in the development of paclitaxel analogs (Fig. 3). Paclitaxel undergoes epimerization in culture media and forms 7-epitaxol, which is as effective as paclitaxel (Ringel and Horwitz, 1987). The importance of structure–activity relationships and conformational studies of derivatives of paclitaxel have been reviewed (Hepperle and George, 1994).

3. Mechanism of action

Microtubules are cylindrical structures (made up of proteins, mainly tubulin) that are involved in various cellular functions such as movement, ingestion of food, controlling the shape of cells, sensory transduction and spindle formation during cell division (Rowinsky et al., 1990, Horwitz, 1992). Paclitaxel has a unique mechanism of action and differs from that of other currently available anticancer agents (Kuhn, 1994). It aids polymerization of tubulin dimers to form microtubules, even in the absence of factors that are normally required for microtubule assembly (e.g. guanine triphosphate, GTP) (Fig. 4), and then stabilizes the microtubules by preventing depolymerization (Schiff et al., 1979, Schiff and Horwitz, 1980). Paclitaxel mainly binds to microtubules, rather than to tubulin dimers (Parness and Horwitz, 1981). The binding site for paclitaxel is the N-terminal 31 amino acids of the β-subunit of tubulin in the microtubule (Rao et al., 1994), unlike the binding sites of colchicine, vinblastine and podophyllotoxin for GTP (Parness and Horwitz, 1981). The microtubules formed due to pa-

clitaxel action are not only very stable but are also dysfunctional, leading to cell death (Rowinsky and Donehower, 1995). While the precise mechanism of action of the drug is not understood fully, paclitaxel disrupts the dynamic equilibrium within the microtubule system and blocks cells in the late G2 phase and M phase of the cell cycle, thereby inhibiting cell replication.

4. Dose, dosing and problems

Paclitaxel has a low therapeutic index, and the therapeutic response is always associated with toxic side-effects (Weiss et al., 1990, Nightingale, 1992, Anon., 1993). It should be only used when the potential benefits of paclitaxel therapy outweigh the possible risks. An excellent review describing different clinical aspects of paclitaxel was recently published (Rowinsky and Donehower, 1995). Table 1 summarizes the toxicities encountered with paclitaxel therapy.

Paclitaxel is extremely hydrophobic; therefore, the commercially available injection is a sterile solution of the drug in Cremophor® EL (polyethoxylated castor oil) and dehydrated alcohol (Horwitz, 1992, Mead Johnson Oncology Products, 1997). Paclitaxel is generally given at a dose of 135 or 175 mg/m^2 as a 3- or 24-h infusion, every 3 weeks (Kramer and Heuser, 1995). Larger dosages and longer infusion periods have also been used (Fields et al., 1996). A recent report indicates that gut P-glycoprotein is involved in low peroral bioavailability of paclitaxel (Sparreboom et al., 1997).

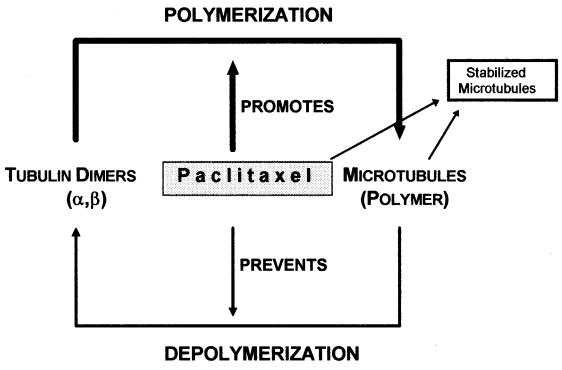


Fig. 4. Mechanism of action of paclitaxel.

Table 1
Summary of therapeutic efficacy and toxicities

(a) Tumors responding to paclitaxel	Ovarian cancer, breast cancer, head and neck cancer, small cell lung cancer, colon cancer, multiple myeloma, melanoma, Kaposi's sarcoma
(b) Dose-limiting toxic effects	Neutropenia, mucositis, neurotoxicity, hypersensitivity
(c) Different systems	
Cardiovascular	Asymtomatic bradycardia, atrioventricular conduction blocks, atrial arrhythmias, ventricular tachycardia, ischemia
Hematological	Neutropenia, thrombocytopenia
Hypersensitivity	Dyspnea with bronchospasm, urticaria, hypotension
Neurotoxicity	Peripheral neuropathy, transient myalgia, scintillating scotamata
Gastrointestinal tract	Mucosities, nausea, vomiting, diarrhea
Hepatotoxicity	Elevation of liver function tests
Others	Alopecia, myopathy, fatigue, pulmonary lipid embolism

The maximum tolerated dose (MTD) of paclitaxel administered by a 3-h infusion to patients with solid tumors was found to be 225–240 mg/m^2 without any hypersensitivity reactions but resulted in hypotension (Kramer and Heuser, 1995). Increased peak plasma concentrations and AUCs, and decreased clearance and volume of distribution with increasing dose were observed, which suggests that paclitaxel follows non-linear pharmacokinetics when given by 3-h infusion (Tamura et al., 1995). There is no evidence that efficacy and tolerability are schedule dependent (Capri et al., 1996). The MTD of paclitaxel was further increased in combination therapy with high-dose cyclophosphamide and cisplatin, followed by autologous hematopoietic progenitor-cell support (Stemmer et al., 1996). Co-administration of R-verapamil at the MTD has resulted in hypotension and bradycardia, and pharmacokinetic analysis has revealed that R-verapamil decreases paclitaxel clearance and increases mean peak paclitaxel concentrations (Berg et al., 1995, Tolcher

et al., 1996). The addition of R-verapamil significantly alters the toxicity and pharmacokinetics of paclitaxel. This alteration may complicate the interpretation of response and toxicity data from clinical trials of this drug combination. In addition, use of cyclosporine (Jachez et al., 1993) and other strategies involving multidrug resistance (MDR) are well addressed (Seidman, 1995). Further, Cremophor also to some extent reverse MDR in preclinical systems (Woodcock et al., 1990). Acquired resistance to paclitaxel is an established fact (Rowinsky et al., 1990). Two mechanisms of resistance are well characterized, so far, including the impaired ability of α and β polymerization into microtubules and P-glycoproteins acting as drug efflux pumps (Rowinsky and Donehower, 1995).

Paclitaxel frequently causes hypersensitivity reactions, which can be severe (Rutherford, 1994). Premedication is mandatory prior to paclitaxel administration in order to prevent severe reactions (Rowinsky and Donehower, 1991, Dabur Pharmaceuticals, 1994, Mead Johnson Oncology Products, 1997). These hypersensitivity reactions may result from the Cremophor® EL, rather than the drug itself (Gregory and DeLisa, 1993). To prevent hypersensitivity reactions, patients should be pretreated with corticosteroids (e.g. dexamethasone), diphenhydramine, and H2-receptor antagonists (e.g. cimetidine, ranitidine) before giving paclitaxel. In cases of severe hypersensitivity reactions, it has been advised that paclitaxel should be discontinued immediately (Gregory and DeLisa, 1993, Kohler and Goldspiel, 1994) and patient treated with epinephrine, intravenous fluids, and additional doses of antihistamine (e.g. diphenhydramine) and corticosteroids as clinically indicated. There is no known antidote for paclitaxel overdose.

Paclitaxel is administered by intravenous infusion after diluting the paclitaxel concentrate for injection with 0.9% sodium chloride injection or 5% dextrose injection or 5% dextrose and 0.9% sodium chloride injection, or 5% dextrose in Ringer's injection to a final paclitaxel concentration of 0.3–1.2 mg/ml (Waugh et al., 1991, Kramer and Heuser, 1995, Mead Johnson Oncology Products, 1997). The stability of 0.1 and 1 mg/ml of paclitaxel in 5% dextrose injection and 0.9% sodium chloride injection is about for 3 days (Xu et al., 1994).

Contact of undiluted paclitaxel concentrate for injection with plasticized polyvinyl chloride (PVC) equipment or devices used to prepare solutions for infusion is not recommended (Waugh et al., 1991). Cremophor® EL causes leaching of diethylhexylphthalate (DEHP) from PVC containers. This leaching of DEHP is substantial and occurs in a concentration-dependent manner; it is also dependent on the type of administration set used (Allwood and Martin, 1996). DEHP leaching was found to increase with time and was independent of the brand of PVC infusion material (Mazzo et al., 1997). In addition to plastic surfaces, rapid and non-specific adsorption of paclitaxel also occurs with glass surfaces (Song et al., 1996). This problem can be overcome, to some extent, by increasing the organic component of the solvent system or using organic solvents. To minimize exposure of patients to leached DEHP, diluted paclitaxel solutions should preferably be stored in glass or polypropylene bottles or in plastic (polypropylene or polyolefin) bags, and administered through polyethylene-lined administration sets (Pfeifer and Hale, 1993, Allwood and Martin, 1996).

Paclitaxel concentrate is a clear, colorless to slightly yellow viscous liquid (Dabur Pharmaceuticals, 1994). After dilution in an infusion solution, the drug may exhibit haziness due to the surfactant content of the formulation vehicle rather than precipitation of paclitaxel. Paclitaxel in aqueous solutions is chemically stable for 27 h or longer (Waugh et al., 1991, Mead Johnson Oncology Products, 1997). A hydrophilic, microporous inline filter of a pore size not more than 0.22 μm is necessary during paclitaxel infusion (Waugh et al., 1991, Dabur Pharmaceuticals, 1994).

5. Paclitaxel delivery

A drug delivery system is defined as 'one in which a drug (one component of the system) is integrated with another chemical, or drug admin-

istration device, or a drug administration process to control rate, release site, or both'. It is very critical in the overall scheme of 'rational drug design'. The impetus for the development of newer/novel drug delivery systems (NDDS), apart from therapeutic efficacy, is the cost. The developmental cost of a new drug may be about $250–300 million and takes about 12–15 years to reach the market place, whereas an existing drug molecule can get a second life with NDDS that can be developed in half the time and at 20% of the cost of a new drug discovery.

The strategies for NDDS center around knowledge of the relationships among physiological barriers, disease characteristics, physicochemical properties, pharmacokinetics and pharmacodynamics of the drug, and as well as material science. The cost per milligram of drug delivered through NDDS is more expensive than conventional peroral administration and can only be justified if NDDS improves therapeutic efficacy and patient compliance and reduces toxic/side-effects.

The major hurdles for successful therapy with paclitaxel are the availability of drug and its delivery. Hence, effective therapy using paclitaxel is dependent on the development of NDDS. Since paclitaxel is poorly soluble in water and peroral delivery is not effective, it is mainly given by intravenous administration. The importance of developing an improved delivery system for paclitaxel is obvious from the problems seen in present-day therapy. Hence, the current approaches are mainly focused on developing formulations that are devoid of Cremophor® EL, the possibilities of preparation on a large scale and stability for longer periods of time. Several attempts have been made to deliver paclitaxel by newer methods such as nanocapsules (Bartoli et al., 1990), liposomes (Onyuksel et al., 1994, Sharma and Straubinger, 1994), enzyme-activatable prodrugs in conjugation with antibodies (Rodrigues et al., 1995b), albumin conjugates (Dosio et al., 1997), parenteral emulsion (Tarr et al., 1987), water-soluble prodrugs (Greenwald et al., 1994, 1996) and microspheres (Burt et al., 1995), but with limited success. None of these alternatives has reached the stage of replacing Cremophor based vehicle in the clinical situation. Another approach getting attention is that paclitaxel complexes with cyclodextrins (Sharma et al., 1995b).

5.1. *Liposomes, micelles and niosomes*

Liposomes, microparticulate lipoid vesicles, represent versatile drug carrier systems for a wide range of drugs. Recent advances in this area have lead to the development of some products for human use and many others are in various stages of clinical trials (Sharma and Sharma, 1997). Drugs with various lipophilicities can be encapsulated into liposomes. In addition to stability and elimination of non-aqueous vehicle, the drawing force for the development of liposomal delivery systems for paclitaxel is the flexibility and greater compatibility with intravenous administration sets, which would help to overcome the problems associated with current paclitaxel therapy (Sharma and Straubinger, 1994). Preliminary studies indicated that paclitaxel liposomal delivery is not only feasible but also as effective as Cremophor® based formulation. Although nanocapsules developed by the same group have shown activity similar to that of liposomes, further investigations were not undertaken because of toxicity of the nanocapsules (Bartoli et al., 1990).

Physical aspects of paclitaxel partition into lipid bilayers have been well studied, including thermodynamic characterization, which has implications in the development of stable formulations (Wenk et al., 1996). The reaction is enthalpy-driven, and easily explained by van der Waal's interactions between the hydrophobic drug and the hydrophobic region of lipid bilayer. The partition equilibrium is temperature dependent, and a 10°C increase in temperature reduced the paclitaxel solubility in the lipid phase by a factor of four. Particulate drug carriers formed from non-ionic surfactant vesicles (niosomes; size 40 μm) were studied for increasing the solubility of paclitaxel (Uchegbu et al., 1996). The paclitaxel was found to be solubilized as the level of PEG-24 cholesteryl ether increased with the absence of cholesterol, and an isotropic liquid of mixed micelles was produced when heated to 35°C.

A mixed micellar formulation of paclitaxel has resulted in well-defined small unilamellar liposomes upon dilution (Onyuksel et al., 1994). Although a stable freeze-dried formulation of paclitaxel less toxic than the standard Cremophor® EL based vehicle formulation was developed, further evaluation of its cytotoxic potential needs to be evaluated at the same solubilizing potential of bile salts. The MTD of paclitaxel in liposomes was developed without formation of paclitaxel crystals upon dilution and was evaluated in an animal model (Sharma and Straubinger, 1994). At the mentioned dose, pharmaceutically acceptable, lyophilized formulations were successfully developed in a reproducible manner, which are chemically and physically stable for more than 2 months at 4°C. These liposome formulations when evaluated in human ovarian tumor xenografts indicated that the formulations are well tolerated and easily administered intravenously when compared to standard Cremophor® vehicle based paclitaxel formulations (Sharma et al., 1995a, 1997). In addition, these formulations were well tolerated at doses greater than or with the same MTD as free paclitaxel, and when evaluated in taxol-resistant murine tumor models have shown a better in vivo therapeutic profile (Sharma et al., 1993). However, further evaluation in platinum- and/or multidrug-resistant models, and also at higher doses, is necessary before initiating clinical studies.

5.2. Polymeric delivery systems

Poly(∈-caprolactone) (PCL), a biodegradable and biocompatible polymer, is gaining importance in the development of paclitaxel delivery systems. PCL microspheres with paclitaxel were developed and evaluated in a chick chorioallantoic membrane (CAM) model for inhibition of angiogenesis (Dordunoo et al., 1995). These microspheres (5% paclitaxel) were effective in induction of vascular regression and marked inhibition of angiogenesis. These microspheres were improved upon by incorporating poly(*dl*-lactic acid) and non-biodegradable ethylenevinyl acetate for controlled delivery of paclitaxel (Burt et al., 1995). About 95% encapsulation efficiency was achieved and 10–15% paclitaxel was released in 2 months by diffusion and polymer erosion mechanisms (Zhang et al., 1996b). Increased cytotoxic effect was observed in case of faster paclitaxel releasing formulations. In addition to be effective in inhibition of angiogenesis, these microspheres has shown lot of potential for the development of paclitaxel targeted systems to tumor via chemoembolization.

PCL based surgical paste of paclitaxel was developed with methoxypolyethylene glycol and was evaluated for antiangiogenic activity by CAM model (Winternitz et al., 1996). Sustained release of paclitaxel at a concentration of 0.1% was observed and 3 mm surgical paste pellets have shown antiangiogenic activity. Paclitaxel release from this formulation was further increased by incorporation of water-soluble additives (Dordunoo et al., 1997).

These formulations have been effective both in vitro and in vivo, when evaluated in CAM model and in mice, for tumor regression and inhibition of angiogenesis, respectively. Application of this type of formulation in the molten state via syringes to tumor recession sites has opened up a many opportunities for localized delivery of antineoplastic agents (Kubo et al., 1994). The above point was further emphasized by successful interstitial delivery of paclitaxel in a biodegradable polymer matrix to malignant glioma model in rats (Walter et al., 1994).

Polymeric micelles of paclitaxel (PMT) were developed by using amphiphilic diblock copolymers (polymeric surfactants), poly(D,L-lactide)-block-methoxypolyethylene glycol (PDLLA-MePEG) (Zhang et al., 1996a). Dissolution studies of PMT in water, 5% dextrose or normal saline have shown no precipitation of paclitaxel. PMT has shown in vivo activity including plasma paclitaxel concentrations as well as lipoprotein distribution similar to that of Cremophor® based vehicle formulation (Ramaswamy et al., 1997). Of the plasma lipoproteins, paclitaxel is mainly associated with the high-density lipoproteins but its clinical/pharmacological implications are yet to be investigated.

5.3. Cyclodextrins

Cyclodextrins (CDs) belong to a family of cyclic oligosaccharides with a hydrophilic outer and a lipophilic cavity in the center. The most commonly used CDs are α-CD, β-CD and γ-CD, consisting of six, seven and eight linked glucose units, respectively. Chemically modified CDs overcome the inherent low solubility characteristics of natural CDs, toxicity, and can act as hosts for a wide variety of lipophilic drugs (Szeltli, 1994). These CDs are known to form non-covalent inclusion complexes with a wide variety of guest molecules (in both solid and aqueous state) and have an annular cavity of 5–8 Å (Uekama et al., 1994).

CDs and chemically modified CDs are well studied and used in many fields (Uekama et al., 1994, Stella and Rajewski, 1997). Encapsulation of guest molecules in CDs leads to a change in their physicochemical properties and can result in increased stability, solubility, bioavailability and tolerability (Jarho et al., 1995), as well as elimination of negative side-effects of drugs (Szeltli, 1994). In addition to these advantages, the majority of drugs form 1:1 complexes with various CDs (Stella and Rajewski, 1997). Interaction of anticancer drugs with α-CD (Cserhati et al., 1995) and hydroxypropyl-β-CD (Cserhati and Hollo, 1994) was studied by means of charge transfer chromatography in order to understand the role of molecular parameters in the formation of inclusion complexes. These studies are preliminary in nature but the results indicate the formation of inclusion complex with CDs, thereby enhancing the solubility of paclitaxel. In addition, a linear relationship between the complex hydrophobicity index and the complex-forming capacity of anticancer drugs was also observed. This relationship could be helpful to predict the complex-forming capabilities of paclitaxel analogs with CDs. The intensity of the interaction significantly depended on the hydrophobicity of the drug studied (Cserhati and Hollo, 1994).

The feasibility of paclitaxel administration with chemically modified CDs has already been shown (Sharma et al., 1995b). Although the solubility of paclitaxel was increased with CD concentration, precipitation of paclitaxel was observed upon dilution. However, CD complexes did not alter the cytotoxic properties of paclitaxel and physical stability and MTD is very much less when compared to Cremophor® based vehicle, when evaluated in healthy BALB/C mice. From the available literature, among the CDs studied so far, hydroxypropyl-β-cyclodextrin (HPβCD) appears to be promising as an agent to improve the solubility of paclitaxel, and the paclitaxel inclusion complexes of HPβCD are more stable compared to complexes with other CDs (Dordunoo and Burt, 1996).

The interactions of paclitaxel with CDs were also investigated in order to assess the physical stability of complexes and also to understand the enhanced solubility of paclitaxel in CD solution (Vander Velde et al., 1993, Balasubramanian et al., 1994). However, in CD solutions, paclitaxel undergoes aggregation depending on the polarity and concentration of solvents. In addition, a model was proposed in which paclitaxel molecules are held together in stacks by intermolecular hydrogen bonds. Although these studies have provided reasons for the precipitation of paclitaxel upon dilution due to weak complex formation in solutions, further systematic investigations need to be carried out and a lot more solid-state properties of paclitaxel CD inclusion complexes need to be studied (Sharma et al., 1995b). The role of pH and temperature on the stability and solubility of paclitaxel has been investigated (Dordunoo and Burt, 1996). At the same pH, solubility and stability was greater in cyclodextrin solutions than in buffer solution. These paclitaxel solutions were stable for more than 1 month at 37°C and the increase in solubility is about five times (in case of HPβCD) when compared to that in buffer solutions. Further increase in solubility of paclitaxel was observed upon addition of co-solvent (ethanol); however, dilution with normal saline or 5% dextrose solutions resulted in precipitation of paclitaxel. These results are very promising and indicate a potential for the development of non-Cremophor® based formulation. However, the dose-limiting toxicity of these CDs needs to be addressed before initiating any clinical studies in human subjects.

5.4. Macromolecule conjugated delivery systems

Other than prodrugs, another approach to improve chemotherapeutic potency and water solubility of paclitaxel involves conjugation with stable macromolecular delivery systems such as monoclonal antibodies (Duncan, 1992, Panchagnula and Dey, 1997). Macromolecules such as albumin, globulins and other, synthetic polymers accumulate in tumor tissue due to increased tumor vascular permeability (Jain, 1990). The advantages of macromolecular conjugated delivery systems include improved pharmacokinetic behavior (Duncan and Spreafico, 1994) and overcoming drug resistance (Fiume et al., 1988, Ohkwada et al., 1993).

Recently, a humanized antibody (humAB4D5-8) was developed against human cancers that overexpress $p185^{HER2}$, and is currently under clinical trials (Carter, 1992). β-Lactamase, a fusion protein in conjugation with humanized antibody was successfully used in targeted delivery of cephem based prodrug (Rodrigues, 1995). β-Lactamase mediated delivery of prodrug of paclitaxel, PROTAX, was developed and evaluated in vitro and compared with that of paclitaxel. Preliminary results indicate that PROTAX and enzyme activatable prodrug delivery systems are about 10-fold less toxic than paclitaxel (Rodrigues et al., 1995).

Paclitaxel–albumin conjugates have been prepared and up to 30 molecules of paclitaxel conjugated to each albumin molecule (Dosio et al., 1997). These conjugates are stable and show linear paclitaxel release in the presence of proteases or liver extracts in vitro and in vivo. High cytotoxicity of conjugates was observed with efficient cell wall binding and internalization, and sustained release of paclitaxel was shown upon pharmacokinetic evaluation. Even at a dose of 70 mg/kg IV, administration toxicity was not observed, in contrast to 30 mg/kg from a Cremophor® based vehicle.

5.5. Prodrugs and modified taxoids approach

Prodrugs are therapeutically inactive derivatives of therapeutically active drug. A prodrug undergoes metabolism (bioconversion) by hydrolysis or enzymatic degradation to produce therapeutically active drug in biological environment. A basic requirement for the development of a prodrugs based approach for effective delivery is the ready availability of a chemical derivative type applicable to the 'class of drugs' in question and satisfying the prodrug requirements (Bundgaard, 1992). If both these requirements are met, then a series of prodrugs can be developed which may have different physicochemical properties in order to meet the therapeutic needs. An appropriate mix of formulation techniques and prodrugs approach offers much promise for improved delivery of drugs with solubility problems (for both hydrophilic and lipophilic drugs).

Aqueous paclitaxel formulations are not possible due to very poor solubility, and paclitaxel lacks functional groups that would allow formation of salts. Although the complex structure of paclitaxel has limited the structure–activity studies, the prodrug approach to improve solubility of paclitaxel is slowly gaining attention. Esterification of C2′ (side-chain) and/or C7 (see Fig. 3) has been shown to improve the water solubility (Deutsch et al., 1989). The sulfonate group used to modify paclitaxel improved its water solubility, but resulted in reducing its cytotoxic activity compared to paclitaxel (Zhao and Kingston, 1991). Amino acid derivatives of paclitaxel have shown potential to be developed as prodrugs. Results indicate that modification at the 2′-position gives compounds that show activity similar to that of paclitaxel, whereas modification at the 7-position results in compounds with only 50% activity of paclitaxel (Mathew et al., 1992). Although 2′- and 7-phosphates of paclitaxel showed improved solubility, the resulting derivatives exhibited decreased cytotoxicity when evaluated in vivo (Vyas et al., 1993).

The taxoids synthesized from 14β-hydroxy-10-deacetylbaccatin have activity similar to paclitaxel but solubility problems are yet to be assessed (Ojima et al., 1994). Deoxygenation of paclitaxel has resulted in a product that is more cytotoxic than paclitaxel, indicating that the hydroxyl group at position 7 as suggested by earlier reports to be important for cytotoxicity may not actually be so (Chaudhary et al., 1993).

Polyethylene glycol (PEG), an amphiphilic molecule, is known to increase the solubility of conjugates of hydrophobic compounds. Therefore, PEG appears to be an excellent candidate to conjugate to paclitaxel. PEG(5000)-carbolic acid conjugated to paclitaxel has resulted in stable conjugates with markedly improved solubility, which also showed slow release of paclitaxel at physiological pH. Although 2′- and 7- esters with PEG have shown cytotoxicity, 7-esters of PEG are not as active as paclitaxel (Greenwald et al., 1994). On the other hand, the 2′-ester conjugate of PEG has an increased the solubility of about 650 mg/ml with cytotoxicity similar to that of paclitaxel.

The 2′-paclitaxel esters which produce paclitaxel on hydrolysis at physiological pH retain the cytotoxicity of paclitaxel but have shown only modest improvement in solubility (Nicolaou et al., 1993). The same group has developed prodrugs based on enzymatic hydrolysis, in which the conjugates have shown improved solubility (1.5 mM) with cytotoxicity similar to paclitaxel (Nicolaou et al., 1994c). In order to achieve any meaningful therapeutic benefit from PEG based prodrugs, the circulation half-life must always be greater than the hydrolysis half-life of PEG-prodrugs of paclitaxel. PEG based paclitaxel has shown potential for improved delivery of paclitaxel; however, at the molecular weight of PEGs (2000–5000) the percentage of paclitaxel that can be incorporated needs further investigations, since only 4% (by weight) of paclitaxel was conjugated with PEG of molecular weight of 40 (Greenwald et al., 1996). Another point that also needs attention, before the introduction of PEG based prodrugs, is the dose-limiting toxicity of PEG itself.

5.6. Others

Stable parenteral emulsions of paclitaxel were prepared in 50% triacetin at concentrations of 10–15 mg/ml (Tarr et al., 1987). At 10 mg/ml, no precipitation of paclitaxel was observed. However, triacetin at a concentration required to administer therapeutic doses of paclitaxel is toxic when administered intravenously to mice. The same group also developed a ternary co-solvent system using pluronic L-64, ethanol and polysorbate 80 for injectable formulation of paclitaxel (Tarr and Yalkowsky, 1987). When diluted in water for injection, paclitaxel solutions were physically stable for 3 days (Tarr and Yalkowsky, 1987).

Polyvinylpyrrolidine nanoparticles of paclitaxel prepared by reverse emulsion type gave a product with particles in the size range of 50–60 nm (Sharma et al., 1996). These nanoparticles are stable, non-toxic and effective when evaluated in murine melanoma transplanted mice, unlike the other nanoparticles which were toxic due to the materials used although effective in vivo (Bartoli et al., 1990).

A protein based injectable gel formulation of paclitaxel has been used for the first time against human pancreatic adenocarcinoma (Smith et al., 1995). This intratumoral delivery system has resulted in sustained release of paclitaxel and is very effective in mice when compared to conventional systems of chemotherapy or intraperitonial delivery of paclitaxel solutions.

A nanocrystalline dosage for parenteral delivery of paclitaxel, strictly speaking not a NDDS, was developed under aseptic conditions by wet milling technology; thus, milling of an aqueous suspension of paclitaxel (2% w/v) and surfactant (1% w/v) provided a paclitaxel formulation as a stabilized nanocrystalline drug suspension that retained biological effectiveness following intravenous administration (Merisko-Liversidge et al., 1996). These nanocrystals are very stable (4 weeks) and paclitaxel has not shown any particle aggregation and/or agglomeration upon dilution with plasma at 37°C. At MTD, nanocrystals have been shown to be more effective than Cremophor® based formulation when evaluated in vivo in tumor-implanted mice.

6. Future trends

The development of new taxanes with improved pharmacological activity and pharmaceutical properties appears to be an important aspect of cancer chemotherapy. However, the possibility of developing a new molecule that may not be struc-

turally similar to paclitaxel by computer-aided drug design and from natural sources can not be ruled out (Cowden and Paterson, 1997). However, these approaches are time consuming because of the processes involved in the introduction of new chemical entities and are also expensive. The path to identify new molecules with better therapeutic efficacy will continue to be an integral part of health care systems but the author emphasizes the importance of 'better delivery of drugs', which is going to further refine the therapy.

Even partial success in the development of any new delivery systems of paclitaxel that replace Cremophor® and decrease its systemic clearance is going to improve significantly cancer therapy with paclitaxel. Although the different approaches investigated so far have shown a lot of promise to replace Cremophor® based vehicle for paclitaxel delivery, the final product for human use is still far away. For localized targeting, PCL based paclitaxel surgical pastes appear to have lot of potential to reach the clinic in the near future. Liposome formulation of paclitaxel is the other approach which has maximum potential for systemic therapy. Current limitations such as amount of paclitaxel delivered and clearance can be overcome by improving the encapsulation efficiency and longer-circulating liposomes, respectively. The optimistic view expressed by the author is based on ever increasing numbers of liposomal formulations under clinical trials, and advances seen in the last decade in liposome technology.

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