

Liposomal, Nanoparticle, and Conjugated Formulations of Anticancer Agents

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Major advances in the use of liposomes, conjugates, nanoparticles, and microspheres as vehicles delivering pharmacologic agents and enzymes to sites of disease have occurred in the past 10 years (1–3). Pegylated-STEALTH liposomal doxorubicin (Doxil, Caelyx) was the first liposomal anticancer drug to be approved by the Food and Drug Administration, whereas paclitaxel albumin-bound particle suspension (ABI007, Abraxane) was recently approved for the treatment of metastatic breast cancer (4–6). The theoretical advantages of liposomal-encapsulated and carrier-mediated drugs are increased solubility, prolonged duration of exposure, selective delivery of entrapped drug to the site of action, improved therapeutic index, and potentially overcoming resistance associated with the regular anticancer agent (1, 2). The process by which these agents preferentially accumulate in tumor and tissues is called the enhanced permeation and retention effect (7). Although pegylated-STEALTH liposomal doxorubicin and paclitaxel albumin-bound particle suspensions are the only such agents that are approved in the United States, there are >50 other agents that are in preclinical and clinical development (Table 1). Newer generations of liposomes containing two anticancer agents with a single liposome and antibody-targeted liposomes that may improve selective toxicity are in preclinical development (8–10). In addition, antiangiogenesis agents and antisense oligonucleotides each represent rational candidates for liposomal formulations (9).

The pharmacokinetic disposition of liposomal and nanoparticle agents is dependent on the carrier and not the parent drug until the drug is released from the carrier (10). Thus, the pharmacology and pharmacokinetics of these agents are complex and detailed studies must be done to evaluate the disposition of the encapsulated or conjugated form of the drug and the released active drug (11). The factors affecting the pharmacokinetic and pharmacodynamic variability of these agents remain unclear; however, it most likely include the reticuloendothelial system, which has also been called the mononuclear phagocyte system (12–14).

Systemic and Tissue Disposition of Liposomes

Liposomes are microscopic vesicles composed of a phospholipid bilayer that are capable of encapsulating the active drug. Whether the drug is encapsulated in the core or in the bilayer of the liposome is dependent on the characteristics of the drug and the encapsulation process (15). In general, water-soluble drugs are encapsulated within the central aqueous core, whereas lipid-soluble drugs are incorporated directly into the lipid membrane. Liposomes can alter both the tissue distribution and the rate of clearance of the drug by making the drug take on the pharmacokinetic characteristics of the carrier (1, 2, 15, 16). Pharmacokinetic variables of the liposomes depend on the physiochemical characteristics of the liposomes, such as size, surface charge, membrane lipid packing, steric stabilization, dose, and route of administration (16). The primary sites of accumulation of conventional liposomes are the tumor, liver, and spleen compared with nonliposomal formulations (1, 12, 13, 17–20). The development of STEALTH liposomes was based on the discovery that incorporation of polyethylene glycol (PEG)-lipids into liposomes yields preparations with superior tumor delivery compared with conventional liposomes composed of natural phospholipids (1, 17, 18, 21). Incorporation of PEG-lipids causes the liposome to remain in the blood circulation for extended periods of time (i.e., $t_{1/2} > 40$ hours) and distribute through an organism relatively evenly with most of the dose remaining in the central compartment (i.e., the blood) and only 10% to 15% of the dose being delivered to the liver (17–20). This is a significant improvement over conventional liposomes where typically 80% to 90% of the liposome deposit in the liver.

The clearance of conventional liposomes has been proposed to occur by uptake of the liposomes by the reticuloendothelial system (RES) (Fig. 1; refs. 1, 17). The mononuclear phagocyte system uptake of liposomes results in their rapid removal from the blood and accumulation in tissues involved in the RES, such as the liver and spleen. Uptake by the RES usually results in irreversible sequestering of the encapsulated drug in the RES, where it can be degraded. In addition, the uptake of the liposomes by the RES may result in acute impairment of the mononuclear phagocyte system and toxicity. Sterically stabilized liposomes, such as STEALTH liposomes, prolong the duration of exposure of the encapsulated liposome in the systemic circulation (2, 14). The presence of the PEG coating on the outside of the liposome does not prevent uptake by the reticuloendothelial system, but simply reduces the rate of uptake (Fig. 1; ref. 17). The exact mechanism by which steric stabilization of liposomes decreases the rate of uptake by the reticuloendothelial system is unclear (1, 2, 14, 22).

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Table 1. Summary of carrier-modulated chemotherapy

Liposomal agents		Nanoparticles	
Conventional	Pegylated (sterically stabilized)	Microspheres	Conjugates
LE-SN38	Doxil/Caelyx	Abraxane (AB1007)	Xyotax (PPX)
Lurtotecan/OSI-211	S-CKD602	Paclimer	DHA-paclitaxel
9NC		TOCOSOL-Paclitaxel	PEG-doxorubicin
Irinotecan			PEG-methotrexate
Paclitaxel			PEG-INF
Doxorubicin			PEG-camptothecin
Daunorubicin			20-Carbonate-camptothecin
Cytarabine			
Topotecan			
Vincristine			
FRL-doxorubicin:vincristine			
FRL-daunorubicin:cytarabine			
FRL-cisplatin:irinotecan			

Abbreviations: FRL, fixed-ratio liposomes; DHA, docosahexaenoic acid; PPX, paclitaxel polyglumex.

Tumor Delivery of Liposomal Agents

Solid tumors have several potential barriers to drug delivery that may limit drug penetration and provided inherent mechanisms of resistance (23). Moreover, factors

affecting drug exposure in tissue, such as alteration in the distribution of blood vessels, blood flow, capillary permeability, interstitial pressure, and lymphatic drainage, may be different in tumors and the surrounding normal tissue (23, 24).

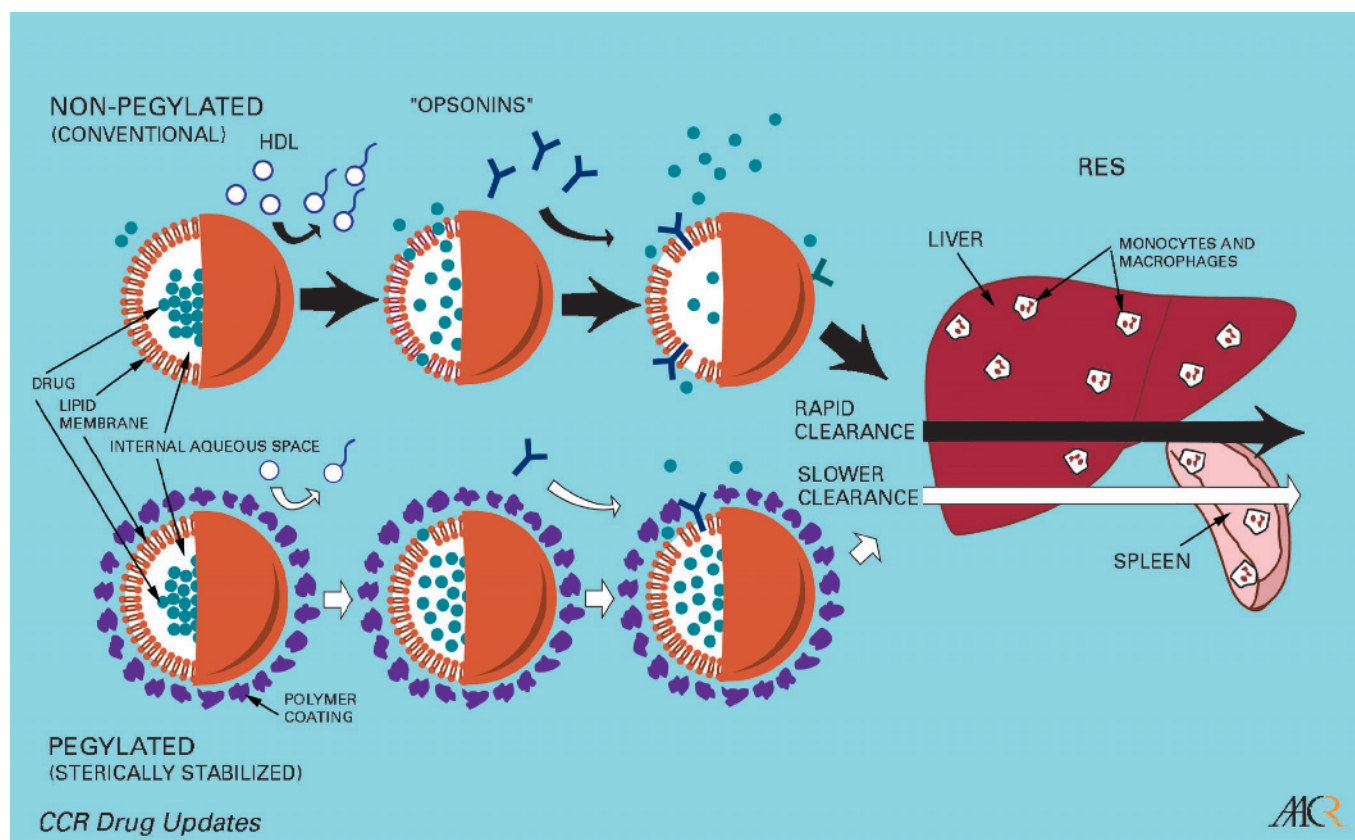


Fig. 1. Clearance of pegylated (sterically stabilized) and nonpegylated (conventional) liposomes via the reticuloendothelial system (RES) in the liver and spleen. Nonpegylated liposomes undergo greater breakdown in blood and more rapid clearance via the RES compared with pegylated liposomes.

Once in the tumor, standard liposomes are localized in the extracellular fluid surrounding the tumor cell but do not enter the cell (25–27). Thus, for the liposomes to deliver the active form of the anticancer agent, such as doxorubicin, the drug must be released from the liposome into the extracellular fluid and then diffuse into the cell (11). As a result, the ability of the liposome to carry the anticancer agent to the tumor and release it into the extracellular fluid are equally important factors in determining the antitumor effect of liposomal-encapsulated anticancer agents. In general, the kinetics of this local release are unknown as it is difficult to differentiate between the liposomal-encapsulated and released forms of the drug in solid tissue; however, with the development of microdialysis, as discussed below, local release may be studied (11).

Several preclinical studies have shown extensive tumor targeting and prolonged exposure of Doxil in tumors, which is consistent with the increased antitumor activity in preclinical models compared with doxorubicin and with clinical activity in patients with refractory ovarian cancer and Kaposi sarcoma (19). In studies comparing STEALTH liposomal cisplatin (SPI-77) and cisplatin tumor disposition in murine colon tumor xenografts, the platinum (Pt) exposure was four-fold higher and prolonged after SPI-77 compared with cisplatin administration (20). However, because the Pt exposure was measured in tumor extracts, it is unclear whether the Pt measured was SPI-77 (i.e., liposomally encapsulated Pt), protein-bound Pt, or unbound-Pt. Moreover, although there is a four-fold higher exposure of total-Pt in tumors after SPI-77 compared with cisplatin, this has not translated into antitumor response in clinical trials (28, 29).

One possible explanation for the inconsistency between the high tumor exposure and low antitumor effect could be the lack of release of active unbound cisplatin from the liposome into the tumor extracellular fluid. We evaluated the exposure of unbound cisplatin in tumor extracellular fluid using microdialysis after administration of SPI-77 and compared these results to the tumor extracellular fluid exposure after cisplatin administration (11). The results of this study suggest that SPI-077 distributes into tumors but release significantly less Pt into tumor extracellular fluid, which results in lower formation of Pt-DNA adducts compared with cisplatin. The clinical importance of these studies is underscored by the need to select liposomal anticancer agents with high tumor penetration and delivery of the active drug to the tumor.

Modification of Toxicity with Liposomal Agents

Liposomal formulations can also modify the toxicity profile of a drug (e.g., Ambisome; ref. 30). This effect may be due to the alteration in tissue distribution associated with liposomal formulations (11, 17, 19, 20). Anthracyclines, such as doxorubicin, are active against many tumor types, but cardiotoxicity related to the cumulative dose may limit their use (31). Preclinical studies determined that liposomal anthracyclines reduced the incidence and severity of cumulative dose-related cardiomyopathy while preserving antitumor activity (31). There is also clinical evidence suggesting that Doxil is less cardiotoxic than conventional doxorubicin (31, 32). Direct comparisons between Doxil or Caelyx and conventional doxorubicin showed comparable efficacy but significantly lower risk of cardiotoxicity with the STEALTH liposomal formulations

of doxorubicin (31). In addition, histologic examination of cardiac biopsies from patients who received cumulative doses of Doxil from 440 to 840 mg/m², and had no prior exposure to anthracyclines, revealed significantly less cardiac toxicity than in matched doxorubicin controls ($P < 0.001$; ref. 33). Administration of a drug in a liposome may also result in new toxicities (34–36). The most common adverse event associated with Doxil is hand-foot syndrome (also known as palmar-plantar erythrodysesthesia) and stomatitis, which have not been reported with conventional doxorubicin (34). The exact mechanisms associated with these toxicities are unknown, but are schedule and dose dependent. In general, Doxil is generally well tolerated and its side effect profile compares favorably with other chemotherapy used in the treatment of refractory ovarian cancer. Proper dosing and monitoring may further enhance tolerability while preserving efficacy; however, there is still a need to identify factors associated with hand-foot syndrome, which can be dose limiting in some patients.

Other Liposomal Agents in Development

Some other liposomal anticancer agents that are currently in development are SN-38 (LE-SN38; refs. 37–40), lurtotecan (OSI-211; refs. 41–44), 9NC (45–47), irinotecan (48, 49), STEALTH liposomal CKD-602 (S-CKD602; ref. 50), paclitaxel (LEP-ETU; ref. 51), and doxorubicin (52). Liposomal encapsulation of camptothecins is an attractive formulation due to the solubility issues associated with most camptothecin analogues and the potential for prolonged exposure after administration of a single dose (37, 41, 50). As compared with pegylated or coated liposomes, conventional liposomal formulations of camptothecin analogues, such as LE-SN38 and OSI-211, may result in the rapid release of the drug from the liposome in blood and thus act more as a new i.v. formulation rather than a tumor-targeting agent (37–42). However, studies evaluating encapsulated and released drug in plasma and tumor have not been reported (11).

Future generations of liposomes may contain targeting antibodies, two anticancer agents combined within a single liposome, or liposomes that are thermosensitive (8–10, 52). Immunoliposomes combine antibody-mediated tumor recognition with liposomal delivery and are designed for target cell internalization and intracellular drug release (10). There are several liposomal formulations that contain fixed ratios of two anticancer agents, such as doxorubicin:vincristine, daunorubicin:cytarabine, and cisplatin:irinotecan, which are currently in preclinical development (8, 53). Thermosensitive liposomes may provide a means of improving the tumor-specific delivery of anticancer agents by rapidly releasing drug from the liposome when hypothermia is applied to the tumor area (52).

Nanoparticle, Microsphere, and Conjugate Formulations

ABI-007 is the first protein-stabilized nanoparticle approved by the Food and Drug Administration (3, 6, 54). ABI-007 is an albumin-stabilized nanoparticle formulation of paclitaxel designed to overcome the solubility issues associated with paclitaxel that require the need for solvents such as cremophor, which have been associated with infusion-related reactions and require the need for premedication. Cremophor may also be incompatible with certain i.v. bags or tubing (3, 54). The

albumin-stabilized nanoparticle results in a more rapid distribution out of the vascular compartment and provides a tumor-targeting mechanism. The albumin receptor-mediated transport through the endothelial cells within blood vessels facilitates the passage of ABI-007 from the bloodstream into the underlying tumor tissue (3, 54).

Similar to liposomal agents, the dosage of ABI-007 is determined by the paclitaxel content of the formulation (3, 54). The approved regimen for ABI-007 is 260 mg/m² i.v. over 30 minutes every 3 weeks, which is higher than the usual dose range for paclitaxel (i.e., 135-200 mg/m²; refs. 3, 6). In addition, there was a lower incidence of myelosuppression after administration of ABI-007 than previously seen with similar doses of paclitaxel (54). The remainder of the toxicities associated with ABI-007 were similar to high-dose paclitaxel, including sensory neuropathy and mucositis. Keratopathy, a relatively uncommon toxicity, was also associated with ABI-007 (54). Thus, as with liposomal formulations, administration of a drug in a nanoparticle formulation can alter the pharmacokinetics, tissue and tumor distribution, and toxicity pattern. Also, similar to liposomal agents, the mechanism by which the albumin-stabilized nanoparticle is catabolized and paclitaxel is released is unclear.

Additional nanoparticle-formulations of paclitaxel are in clinical and preclinical development. Paclitaxel poliglumex (Xyotax), a macromolecular drug conjugate that links paclitaxel with a biodegradable polymer, poly-L-glutamic acid, has completed phase 1 studies (55). Paclitaxel poliglumex is a water-soluble formulation that also eliminates the need for cremophor in the formulation. Paclimer, a microsphere formulation of paclitaxel, is currently in preclinical development (56). Paclimer microspheres contain paclitaxel in a polylactofate polymer microsphere and is designed to continuously deliver low-dose paclitaxel. Other conjugates of paclitaxel have been stopped in clinical development and have been associated with potential pharmacologic and pharmacokinetic problems (57, 58). Docosahexaenoic acid-paclitaxel, a novel conjugate formed by covalently linking the natural fatty acid docosahexaenoic acid to paclitaxel, was designed as a prodrug targeting intratumoral activation (57). At the maximum tolerated dose of docosahexaenoic acid-paclitaxel (1,100 mg/m²), paclitaxel represented only 0.06% of the docosahexaenoic acid-paclitaxel plasma exposure (58). However, the paclitaxel concentrations remained >0.01 μmol/L for an average of 6 to 7 days and the paclitaxel area under the curve was correlated with neutropenia. The results of this study suggest that most of the drug remained in the inactive prodrug conjugated form and that significant toxicity only occurred when released paclitaxel reached clinically

relevant exposures. This depicts the need to perform detailed pharmacokinetic studies of conjugated and released drug in plasma and tumor.

During the past 10 years, there has been a renaissance in the field of PEG-conjugated anticancer agents (59). This new development has been attributed to the use of higher-molecular-weight PEGs (>20,000) and especially with the use of PEG 40,000, which has an extended *t*_{1/2} in plasma and potential selective distribution to solid tumors (59). Various PEG conjugates of anticancer agents, such as doxorubicin (60), methotrexate (61), IFN (62, 63), and camptothecin analogues (64, 65), are currently in development (60-65). PEG- and 20-carbonate conjugates of camptothecin analogues are especially interesting as the conjugated prodrug forms highly water soluble agents and significantly extend the duration of exposure after a single dose (64-66). Hyaluronic acid conjugates of anticancer agents are also in development. Carrier-mediated conjugates of anticancer agents also have the same pharmacologic issues (the need to evaluate the pharmacokinetics of the prodrug conjugate and released drug) as liposomal and nanoparticle formulations and the overall clinical benefit of these agents remains unclear.

Conclusion

Liposomes may be an effective carrier to deliver anticancer agents to tumors (1, 2, 11, 17, 18). However, for anticancer agents encapsulated in pegylated and nonpegylated liposomes to be an effective treatment in patients with solid tumors, the active form of the anticancer agent must be released from the liposome into the tumor extracellular fluid and then penetrate into the cell (11). New liposomal and nanoparticle anticancer agents should be evaluated in preclinical models and early clinical trials to ensure that adequate release of drug occurs at its site of action. Immunoliposomes that contain an antibody conjugated to a liposome are being developed to provide targeted delivery to cancer cells expressing specific proteins (8, 67). Future studies need to evaluate the mechanism of clearance of liposomal and nanoparticle drug formulations and the factors associated with pharmacokinetic variability (19, 37, 41, 42, 50, 67). In addition, additional preclinical models are needed for toxicity, efficacy, and pharmacokinetic studies, especially because liposomes may not be allometrically scaled across species and toxicity in certain species may not predict human toxicity (50, 68).

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