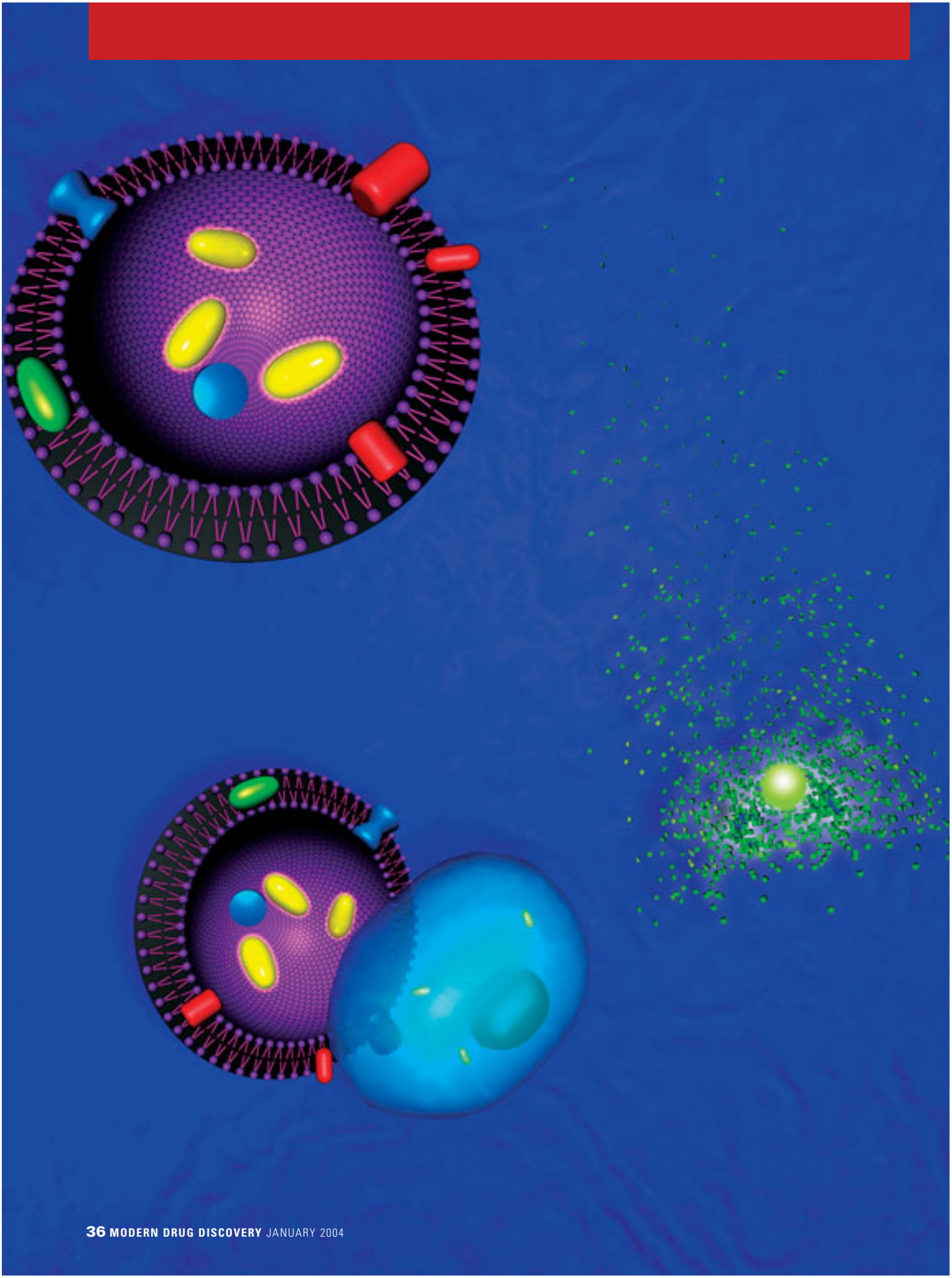

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LIPOSOMES: THE NEXT GENERATION

New lipid technologies are improving drug shelf life, efficacy, and delivery.

BY HARIS JAMIL, SAIFUDDIN SHEIKH, AND IMRAN AHMAD

Liposomes are microscopic particles composed of lipids. The most common constituent of a liposome is phospholipid that spontaneously forms closed structures in aqueous solution (*1*). The phospholipid is a double fatty acid chain, which is primarily responsible for bilayer formation (Figure 1). Liposomes can be classified into three groups: multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), and large unilamellar vesicles (LUVs).

MLVs are generally heterogeneous in nature and may have several compartments. They are easy to prepare and can vary in size from 0.5 to 5 µm. SUVs range in size from 20 to 50 nm, have a spherical shape, and are homogeneous in nature. Finally, LUVs are larger (200–1000 nm), giving them greater space for encapsulation of aqueous medium.

Liposomes interact with the cell surface by two main mechanisms: adsorption and endocytosis. Liposomes can be adsorbed to a cell surface directly (nonspecifically) or by specific interaction with a cell-surface receptor.

To achieve specificity, liposomes have been derivatized with protein, antibody, or carbohydrate moieties or with other polymers. In endocytosis, however, the liposomes are internalized actively by the target cell.

In addition to adsorption and endocytosis, there are two other categories of liposome interaction with the cell surface: fusion of the cell with a vesicle and lipid exchange.

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Drug delivery

Liposome systems became a popular drug delivery platform for several reasons. Liposomes are naturally occurring lipids from living systems, making them nontoxic and biodegradable. Because the drug can be entrapped in liposomes, it is not exposed to the metabolic machinery of the body, preventing degradation and dilution. Liposomes can be modified to release the drug slowly, resulting in prolonged exposure and thereby increasing the therapeutic efficacy of the compound. In cancer, liposomes preferentially accumulate at tumor sites as a result of their ability to negotiate their way (extravagate) through pores in the capillary endothelium. In addition, because cancer cells must consume large amounts of fat to sustain rapid growth, they recognize liposomal drugs as a potential source of nutrition. The liposome-entrapped drug is engulfed by the tumor cells, and the drug is released, eventually destroying the tumor cells (see art, opposite). Furthermore, liposomes are unique because they provide an environment that can enclose both hydrophilic and hydrophobic molecules. Hydrophobic molecules are intercalated within the bilayer membrane, and hydrophilic molecules can be entrapped in the internal aqueous region.

Essential factors

The important factors in liposome drug development are drug entrapment efficiency, size, stability, and the ability to scale up the process for manufacturing. Producing liposome-based drugs in large quantities and adhering to the specifications traditionally have been a challenge for pharmaceutical companies (*2*). Earlier liposomal formulations were not successful because of instability and poor control over sustained release of the drugs in the physiological system.

Liposomes are primarily taken up by the cells of the reticuloendothelial system (RES), which includes the liver, spleen, kidney, and lung. The liver and spleen are the organs where most of the liposome uptake occurs because their cells have the ability to sequester inert particles, including macrophages. Because of this, monocyte-phagocyte-system (MPS) directed delivery is useful for treating intracellular infections, in which liposomes are rapidly removed from the blood by the phagocytic cells of the RES sys-

tem, which is mediated by direct interaction of cells with liposomes.

The rate at which liposomes are cleared depends on their size, surface charge, and stability. In general, unmodified large liposomes are cleared more rapidly than small, neutral, or positively charged liposomes. The clearance rate also depends on the route of administration of the liposomes. Most intravenously injected liposomes are rapidly taken up by the RES and thus are cleared quickly. One of the major steps in improving circulation time was the introduction of ganglioside or poly(ethylene glycol) modification of the liposomes (*1, 3*). These modified liposomes are sterically stabilized, which allows them to circulate for a longer time with reduced uptake by the RES.

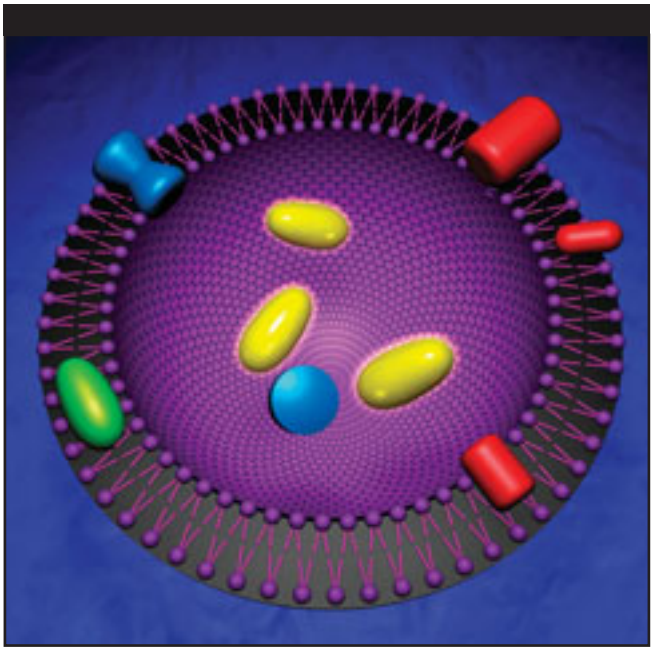

Figure 1. Schematic representation of a liposome. The liposome can entrap lipid-soluble or water-soluble therapeutic agents in the phospholipid bilayer or aqueous cavity, respectively. (Image courtesy of NeoPharm, Inc.)

NeoLipid technology

First-generation liposomes were difficult to formulate and manufacture consistently. Only a limited number of compounds were feasible for entrapment, and the "leaky" liposome phenomenon created unstable in vivo formulations. Thus, none of the first-generation liposome products were able to reach the market.

To overcome these problems, researchers have developed next-generation technologies. For instance, NeoPharm, Inc. (*www.neophrm.com*), uses novel proprietary lipids and improved procedures to overcome major drawbacks of the early liposomal formulations. The company's NeoLipid technology allows a broadened range of drugs, including genes and antisense molecules, to be entrapped. Depending on the properties of the drug, each formulation is designed to create small (<200-nm particle size), homogeneous, and stable liposomes.

Cardiolipin, also known as diphosphatidylglycerol, is a key component contributing to the success of NeoPharm's liposomal technology. The compound belongs to a class of complex anionic phospholipids that are typically purified from cell membranes of tissues associated with high metabolic activity, including those of the mitochondria of heart and skeletal muscles. Cardiolipin contains almost exclusively 18-carbon fatty acids (linoleic acid 18:2) in animal tissues, 16:1 and 18:1 fatty acids in yeast, and 14- to 18-carbon unsaturated monoenoic fatty acids in bacterial membranes. It plays a vital role in the mitochondrial electron transport system and signal transduction. It also appears to have a role in restoring normal cell function, including apoptosis, which may enable cardiolipin to be used as a bioactive lipid (*4, 5*).

Cardiolipin has two negatively charged phosphate groups, which generate a negative charge in liposomes. Liposomes can be neutral or have a negative or positive charge, depending on the type of lipid used in the formulation. Negatively charged liposomes can be formed from solutions containing phosphatidylcholine and cardiolipin. NeoPharm researchers have used negatively charged cardiolipin to entrap anticancer compounds such as paclitaxel, mitoxantrone, and active metabolite of Camptosar (SN-38). Liposomal formulations of SN-38 (LE-SN38), mitoxantrone (LEM), and paclitaxel (LEP) have produced small (<200 nm) and stable liposomes with high entrapment efficiency and a long shelf life.

NeoPharm researchers have also used a cationic lipid for liposomal formulation of negatively charged cRaf antisense, which is used to treat various cancers. Initial formulation of cRaf antisense (LErafAON) contained dimethyldioctadecyl ammonium bromide (DDAB) with a large particle size, and this formulation required sonication prior to administration (*6*).

Recently, NeoPharm discovered cationic cardiolipin, in which phosphate groups were replaced with quaternary ammonium groups, resulting in a positive charge (*4*). To improve cRaf antisense formulation, cationic lipid DDAB has been replaced with a newly developed cationic cardiolipin analogue, which enhances stability for drug delivery systems to give improved drug storage, better delivery to target sites, superior pharmacologic efficacy, and fewer side effects. The liposome formulation of cRaf-containing cationic cardiolipin is stable, homogeneous, and smaller in size (<200 nm) and can be administered directly to patients without preparation. Cationic cardiolipin also has potential for the delivery of biological agents, including genes, proteins, and peptides, to intracellular targets (*4*).

Liposome formulations

SN-38 (camptothecin) is the active metabolite of CPT-11 (irinotecan), a known topoisomerase I inhibitor. CPT-11 is recommended for the treatment of various solid tumors, including colon cancer; however, the response rate of treatment is only 20%. CPT-11 is a prodrug, and its antitumor activity depends on its conversion to SN-38, which occurs on a cellular level where only 6% is actually converted. SN-38 is approximately 100 to 1000 times more cytotoxic than CPT-11, but its poor solubility has limited its use as an anticancer agent.

Using liposomal technology, researchers were able to develop a formulation of SN-38 that results in stable liposomes with uniform size and high entrapment efficiency. LE-SN38 enhances the solubility of SN-38 and provides protection from rapid drug degra-

Table 1

Companies involved in liposomal drug delivery

Company	Website	Compound	Target	Status
ALZA	www.alza.com	Doxi	Ovarian cancer	Market
Antigenics Inc.	www.antigenics.com	Aroplatin	Colorectal cancer	Phase II
		ATRA-IV	Acute promyelocytic leukemia	Market
Celsion	www.celsion.com	ThermaDox	Prostate cancer	Phase I
Gilead Sciences	www.gilead.com	AmBisome	Fungal infection	Market
		DaunoXome	Karposi's sarcoma	Market
Inex Pharmaceuticals	www.inexpharm.com	OligoVax	Cancer	Research
		OligoVax	Infectious disease	Research
NeoPharm	www.neophrm.com	LE-SN38	Various solid tumors	Phase I/II
		LEM	Prostate cancer	Phase I/II
		LE-AON	Various solid tumors	Phase I/II
		LEP	Various solid tumors	Phase I/II

dation. It is hypothesized that LE-SN38 may broaden antitumor efficacy because conversion from the inactive product to the active product is not necessary. LE-SN38 may also permit dose intensification with an increased safety profile because of the liposome protection.

LE-SN38 can be stored as a lyophilized powder at 4 °C and reconstituted with lactate buffer prior to administration. Lyophilization and reconstitution have no effect on drug morphology or potency. The formulation achieves a reconstitution process in less than a minute and results in a liposome size of less than 200 nm. Preclinical studies have demonstrated antitumor activity at very low doses in multiple tumor cell lines and human and murine cancer models (*7–10*). Phase I/II clinical trials are in progress (*11*). The primary objectives in the Phase I/II clinical trials is to determine the maximum tolerated dose, dose-limiting toxicities, and pharmacokinetics of LE-SN38 in patients with advanced local or metastatic solid tumors who have failed to respond to conventional therapy.

In summary, NeoLipid technology can be used for improving the formulations of existing chemotherapeutic agents and biologics, including bioactive lipids, where lipids and/or liposomes are being used as the active agent. NeoPharm's research to date has shown that NeoLipid formulations can reduce the toxicity of potent chemotherapeutic drugs, creating an opportunity for a more comfortable-to-administer, well-tolerated, and safer treatment for cancer patients.

The future of liposome technology

The future of next-generation liposomal drug delivery technology is promising (Table 1). For example, it can be used when clinical development of a therapeutic agent is impeded by its low solubility, low bioavailability, and difficult delivery to the target tissues. A large number of pharmaceutical companies with vast chemical libraries have to put many of their drugs on the shelf because they cannot formulate them suitably for delivery, or the drugs lose their potency before reaching their target sites. Efforts to improve the therapeutic index of these molecules are plagued by the lack of availability of a suitable formulation and delivery system. Liposome technology creates an ample opportunity for formulation and delivery of a wide variety of difficult-to-deliver therapeutic agents, including genes, peptides, siRNA or RNAi, protein, and growth hormones.

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