

1

**DRUG DELIVERY FORMULATIONS AND TARGETING****CROSS REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of priority under 35 USC §119(e)(1) of Provisional Application No. 60/216,173 filed Jul. 6, 2000.

**STATEMENT AS TO FEDERALLY SPONSORED RESEARCH**

This invention was made with Government support under Outstanding Investigator Grant R35-CA-56591 awarded by the National Institutes of Health. The Government may have certain rights in the invention.

**FIELD OF THE INVENTION**

The invention relates to drug delivery.

**BACKGROUND OF THE INVENTION**

The efficiency of drug transport to solid tumors can vary as a function of host microenvironment, tumor type, and growth. Several barriers have been shown to impede delivery of therapeutics to solid tumors in clinically relevant concentrations (Jain, *Scientific American*, 271(1):58–65, 1994). These barriers include a heterogeneous blood supply, elevated tumor interstitial pressure (Jain, *Nature Medicine*, 6:655–657, 1998), and a dynamic range of tumor blood vessel pore sizes (Hobbs et al., *Proc. Natl. Acad. Sci. USA*, 95:4607–4612, 1998). Delivery of drugs to solid tumors can thus present a formidable challenge.

Studies have shown anatomical and morphological differences between normal and tumor blood vessels (Baldwin et al., *Microvascular Research*, 42:160–178, 1991). Most tumors are angiogenesis-dependent (Folkman, *Journal of the National Cancer Institute*, 82(1):4–6, 1989), requiring the development of new blood vessels. The glycoprotein layer of the vascular endothelium of angiogenic blood vessels is composed primarily of negatively charged functional groups (Baldwin et al., *Microvascular Research*, 42:160–178, 1991). These functional groups can facilitate molecular interactions with positively charged macromolecules (Dellian et al., *British Journal of Cancer*, 82:1513–1518, 2000). Accordingly, it was hypothesized that cationic liposomes could be used to target tumor vessels where established glycoprotein layers had formed. Indeed, it was found that cationic liposomes are taken up by tumor vessels to a greater extent than by the normal vascular endothelia. Cationic liposomes have thus been used to target anionic endothelial cells in tumors and chronic inflammation in mice, with some success (Thurston et al., *Journal of Clinical Investigation* 101:1401–1413, 1998; Roberts et al., *Cancer Research* 57:765–772, 1997).

In addition to tumor growth, numerous other disease states are associated with abnormal angiogenesis. Increased angiogenesis in the bones, joints, skin, liver, kidney, lung, ear, nerves, heart, skeletal muscles, adipose tissue, peritoneum pleura, endocrine organs, hematopoiesis, lymph, and other organs and systems, for example, is associated with tumors and chronic inflammation in those organs, as well as obesity, warts, uterine bleeding, and respiratory disease. On the other hand, vascular insufficiency is associated with diseases such as aseptic necrosis, impaired healing of fractures, decubitus or stasis ulcers, gastrointestinal ulcers, pulmonary and systemic hypertension, placental

2

insufficiency, stroke, vascular dementia, Alzheimer's disease, CADASIL, ischemic heart and limb disease, and thyroid pseudocysts. Other vascular abnormalities have been implicated in psoriasis, diabetes, and hypertension.

**SUMMARY OF THE INVENTION**

The invention is based on the discovery that angiogenic vessels have heterogeneous surface charge and that cationic liposomes actually target human tumor blood vessels only in irregularly shaped patches. The invention thus features methods for delivering therapeutic compounds to angiogenic vascular endothelial surfaces using a mixture, or "cocktail", of positively charged and neutral liposomes. The new methods can be used to target multiple regions on the same tumor vessel and/or clusters of vessels within the same tumor. Liposomes with different chemical and/or physical properties (e.g., charge, stability, solubility, diameter) can be delivered simultaneously, and can target tumor vessels and other angiogenic vessels with greater efficiency compared to cationic liposomes alone.

In general, the invention features a formulation that includes cationic liposomes, containing a first therapeutic agent, and electrostatically neutral liposomes, containing a second therapeutic agent, where the first and second therapeutic agents can be the same or different. Each therapeutic agent can include, for example, one or more compound that have, or are suspected to have, some biological activity, as well as other additives and excipients. Preferably, such additives or excipients are substantially non-toxic at the concentration and quantity employed. The cationic and neutral liposomes can be present in various ratios (e.g., between about 1:9 and 9:1, between about 1:3 and 3:1, or between about 2:3 and 3:2, such as about 1:1. Either or both of the first and second therapeutic agents can be, for example, an anti-tumor drug (e.g., a chemotherapeutic agent such as paclitaxel, doxorubicin, or other plant alkaloids, antibiotics, alkylating agents, antimetabolites, or miscellaneous agents), a nucleic acid, or another natural or synthetic therapeutic agent. The cationic lipids can include, for example, dioleoyltrimethyl-ammonium propane (DOTAP), N-[1-(2,3-dioleoyloxy)-propyl]-N,N,N-trimethylammonium chloride (DOTMA), dimethyldioctadecylammonium bromide (DDAB), 1,2-dimyristyloxypropyl-3-dimethylhydroxyethyl (DMRIE), dioleoyl-3-dimethylammonium propane (DODAP), N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC), or N-(1-(2,3-dioleoyloxy)propyl)-N-(2-(spermincarboxamido)ethyl)-N,N-dimethyl ammonium trifluoroacetate (DOSPA), or any other natural or synthetic cationic lipids. The neutral liposomes can include, for example, dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), distearylphosphatidylcholine (DSPC), dimyristoylphosphatidylcholine (DMPC), or 1,2-sn-dioleoylphosphatidylcholine (DOPE), or any other natural or synthetic electrostatically neutral lipids.

The invention also features several methods of administering a drug to a patient. One method includes the steps of providing a formulation as described above (i.e., where at least one of the first and second therapeutic agents includes the drug to be administered), and administering the formulation to the patient (e.g., intravenously or intraarterially or by any other suitable route). The patient can be, for example, a patient suspected of having a tumor. The patient can be, for example, a mammal such as a human, a mouse, a rat, a sheep, a goat, a dog, a cat, a pig, a cow, or other animal used for research, human companionship, agriculture, or consumption. Another method includes administering to the