Editorial

Stealth Liposomes and Tumor Targeting: One Step Further in the Quest for the Magic Bullet

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At the turn of the 20th century, the German bacteriologist Paul Ehrlich coined the expression "magic bullets" in his search for chemotherapeutic agents with specific affinity for diseased tissues. Understanding target structure and function, developing drug delivery strategies to achieve controlled release, and targeting of drugs to specific tissues of the body have been a major focus of research in the last decades in an attempt to improve selectivity in cancer treatment. Shortly after they were first characterized (1), liposomes were proposed as drug carriers in cancer chemotherapy by Gregoriadis *et al.* (2). Since then, interest in liposomes as devices for drug delivery gradually increased, and they have been one of the main players in the cancer drug delivery arena for the last 25 years. However, the early enthusiastic notes on liposomes and cancer drug delivery were strongly criticized (3, 4) when it became apparent that liposomes are rapidly recognized and removed from the circulation by the RES,[2] thus nullifying any possible chance of substantial localization in tumors. Moreover, the initial steps of the liposome approach were tainted by a nebulous scientific rationale: why should liposomes home to tumors? Why should liposomes spare some healthy tissues? To deal with these issues, a rational approach, linking liposome formulation with liposome pharmacology and its implications on biodistribution and extravascular transport, was needed.

Clearly, if liposomes are to be used for targeting to extra-RES tissues, a key issue is to reduce the rate of uptake by the RES so as to enable them to remain in the circulation longer. The effect of particle size in favor of small vesicles was recognized early (5). Thereafter, during the 1980s, liposome composition was found to play an important role in circulation time, and the key factors involved were characterized. High-phase-transition temperature phospholipids, a high fraction of cholesterol, and a small fraction of some specific glycolipids (*e.g.*, monosialoganglioside and hydrogenated phosphatidylinositol) imparting a weak surface negative charge were recognized as factors contributing to the longer circulation half-lives of liposomes (6–9). About a decade ago, this liposome engineering process culminated with the observation that coating of liposomes with PEG, a synthetic hydrophilic polymer, would improve their stability and lengthen their half-lives in circulation (10–13), rendering the use of glycolipids obsolete. PEG coating inhibits protein adsorption and opsonization of liposomes, thereby avoiding or retarding liposome recognition by the RES. These PEG-coated liposomes are also referred to as sterically stabilized, or Stealth liposomes. The PEG stabilizing effect results from local surface concentration of highly hydrated groups that sterically inhibit both hydrophobic and electrostatic interactions of a variety of blood components at the liposome surface (14, 15). Although PEG is the most common polymer used for liposome coating, other polymers have also been shown to protect liposomes from opsonization and prolong their circulation time (16).

The rationale for long-circulating liposomes in cancer drug delivery was based on data revealing a strong correlation between liposome residence time in blood and their uptake by implanted tumors in mice (8). We hypothesized then that liposome extravasation in tumors is the result of passive convective transport through a leaky endothelium. A longer blood residence time will result in repeated passages through the tumor microvascular bed of high concentrations of vesicles and, consequently, in a greater efficiency of extravasation per unit volume of convective transport. The physiopathological changes underlying the high permeability of tumor microvessels to liposomes, other nanoparticles, and macromolecules include large interendothelial fenestrations, discontinuous basement membranes, and a high rate of trans-endothelial transport (17) and appear to be secondary to the neoangiogenic stimulus caused by factors secreted by tumors cells such as vascular endothelial growth factor, formerly referred to as vascular permeability factor (18). An additional factor contributing to liposome accumulation is the lack of a functional lymphatic drainage in tumors, thus creating a "dead-end" for extravasated liposomes. The enhanced permeability and retention model, which has been proposed to explain the preferential accumulation of macromolecules in tumors (19, 20), is also applicable to liposomes. Morphological studies with colloidal gold-labeled Stealth liposomes (21) and *in vivo* dynamic observations in the skin-fold chamber model with fluorescent labels (22) indicate that liposomes remain in the tumor interstitial fluid in close vicinity to tumor vessels. Drug molecules are released from the extravasated liposomes as a consequence of poorly understood processes that may include chemical disruption of the gradient retaining the drug, as in the case of doxorubicin (23), and enzymatic breakdown of the liposome membrane.

In this issue of *Clinical Cancer Research*, Harrington *et al.* (24) present important observations on the pharmacokinetics, biodistribution, and imaging of radiolabeled, drug-free Stealth liposomes in cancer patients. In this regard, this is probably one of the most complete studies on Stealth liposomes in humans. One of the strengths of the study by Harrington *et al.* (24) is the labeling methodology, which is based on the formation of an intraliposomal ^{111}In-DTPA chelation complex (25). This complex, as also shown for ^{67}Ga-deferoxamine (26), is highly stable

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[2] The abbreviations used are: RES, reticuloendothelial system; PEG, polyethyleneglycol; DTPA, diethylenetriaminepentaacetic acid.

with negligible levels of transchelation of the isotope to transferrin and other serum proteins. Therefore, any extracellular leakage of ^{111}In-DTPA from liposomes will be rapidly followed by renal excretion of the intact chelation complex. In addition, the investigators took care to add EDTA to the preparation to chelate any unencapsulated ^{111}In and ensure its rapid urinary excretion after i.v. injection. As a result, the data and images obtained are an accurate representation of the tissue distribution of liposomes in patients.

Not surprisingly, the average circulation half-life of these PEG-coated radiolabeled liposomes in patients was ~3 days (76 h), remarkably close to what has been reported for Doxil (27, 28), a formulation of Stealth liposomal doxorubicin with the same lipid composition and a highly stable drug retention during circulation. The fact that tumors were visualized in 15 of 17 patients is confirmatory evidence of the preclinical findings on tumor targeting of Stealth liposomes and has significant implications for the clinical use of Doxil and perhaps future Stealth liposome-entrapped agents. Two recent reports from Koukourakis *et al.* (29, 30), using a direct labeling procedure of Doxil with ^{99m}Tc-DTPA to image patients receiving Doxil treatment, also point to a high tumor accumulation of Stealth liposomes, although these patients were scanned 2–10 h after injection when there is still a high blood background.

Another important observation by Harrington *et al.* (24) is a trend to higher liposome uptake in smaller tumors, consistent with previous findings in animal tumor models (31). It is remarkable that in five of six tumors <100-cm^3 volume, the fraction of injected dose/kg tumor was >20% (Ref. 24; Fig. 7), based on region of interest analysis.[3] Except for the spleen, these values are greater than for many well-perfused organs such as liver, lung, and kidney. A report pointing to tumor size as an important prognostic factor for response to Doxil in ovarian cancer (32) suggests that the tumor volume dependence of liposome uptake is clinically relevant. Indeed, hypovascular areas and increased interstitial pressure in large tumors will interfere with extravascular convective transport and decrease liposome uptake (33). More clinical information is still needed on other factors that may affect liposome accumulation in solid tumors, such as tumor types, anatomical location, primary *versus* metastatic tumors, irradiation, and hyperthermia.

What can we learn from our growing knowledge on the biodistribution of Stealth liposomes in cancer patients? Because of the expanding use of Doxil in cancer chemotherapy (34), studies that will attempt to correlate liposome tumor targeting with antitumor response may be extremely valuable because they may help us to select those patients who are more likely to benefit from therapy. In fact, a targeted drug carrier system is likely to be ineffective and even detrimental to patients in whom the tumor is not targeted, as compared with a carrier-free drug. A region of interest analysis of radiolabeled liposome biodistribution will also give us an opportunity to make an estimate of the amount of liposome-delivered drug to an individual patient's tumor, provided that there is no significant drug leakage in circulation. Tumor drug levels are one of the best predictors of antitumor response. Therefore, this approach, short of functional imaging, should give a better pharmacodynamic prediction than dose or plasma levels.

From the point of view of targeting, Stealth liposomes are to a large extent a simple and passive system, devoid of any specific ligands, exploiting basically the differences in microvascular permeability between tumor and other normal tissues. Although the quest for the magic bullet continues, the Stealth liposome approach represents a realistic compromise for selective drug delivery in cancer and opens up new avenues in therapeutic applications.

[3] For comparison, the fraction of injected dose/kg tumor of free doxorubicin is ~2% only, based on tumor drug concentration in breast carcinoma patients 30 min after injection of 25 mg/m^2 (35).

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