Animal Experiments—An Essential Component for the Development of Liposomal Anticancer Agents

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During several years, in our institute more than a dozen of established or novel anticancer compounds have been encapsulated in liposomes and their pharmacological behavior has been tested in in vitro and in vivo experimental models.

It was revealed, that for each substance a tailored liposomal system had to be developed. Animal experiments designed to determine both the antitumor activity and side effects of liposomal in comparison to the free drugs have shown that in the majority of cases a benefit for the vesicular formulation could be obtained. In 7/12 liposomal compounds tested (Bleomycin, Daunorubicin, Cisplatin, Carboplatin, Cyclophosphamide, CCNU, Alkylphospholipids) a substantial decrease of toxicity, mainly due to changed pharmacokinetic data could be observed. The therapeutic efficacy could be increased by use of liposomes for Bleomycin, Taxol, and Mitoxantrone while in other examples no change (Daunorubicin, Methotrexate, TNF) or even a decrease of activity (Cisplatin, Cyclophosphamide, CCNU) was registered.

Carboplatin is one example in which by liposomal encapsulation the pharmacological properties were decisively changed. While the free drug leads to leuko- and thrombopenia, the Carboplatin-liposomes (CPL) revealed after only one i.p. or i.v. injection into mice a substantial and long-standing leukocytosis. That effect was paralleled by a release of cytokines from macrophages into the serum, an increased number of peripheral blood stem cells and colony forming activity. The anticancer activity of carboplatin was remarkably improved especially in breast cancer xenografts by using the liposomal formulation. We hypothesise that CPL of specific size and constitution are efficiently taken up by macrophages/monocytes.
That leads to the induction of growth factors inducing secondarily a stimulation of haematopoiesis.

Another example is the encapsulation of Tamoxifen (Tam), an antiestrogen used mainly as first line therapy in estrogen receptor positive breast cancer. Tamoxifen-containing LUVETs prepared from egg phosphocholine, dicetylphosphate and an alkylphospholipid (OPP) had a higher in vitro cytototoxicity both in Tam-sensitive and –resistant breast cancer lines. In vivo testing in xenografts with inherited or acquired Tam-resistance showed that in 2/4 models resistance could be overcome by an oral treatment with appropriate liposomes.

These both examples impressively document that only by inclusion of a consequent in vivo testing procedure the surprising pharmacological effects of liposomal anticancer agents can be revealed.

Emerging Role of Liposomal Drug Carrier Systems in Cancer Chemotherapy

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The rationale for the use of liposomes in cancer drug delivery is based on the following pharmacological principles: 1) Slow drug release; 2) Site avoidance of specifically sensitive tissues; 3) Accumulation in tumors for liposomes with prolonged circulation time owing to increased tumor vascular permeability.

Tumor localization of long-circulating liposomes, such as pegylated liposomes (sometimes referred to as Stealth or stERICALLY-stabilized), is a passive targeting effect which may enable substantial accumulation of liposome-encapsulated drug in the interstitial fluid at the tumor site. This is followed by gradual release of drug in situ and subsequent diffusion to the intracellular tumor compartment. This rationale is the basis for the development of pegylated liposomal doxorubicin for cancer therapy. Pegylated liposomal doxorubicin (doxil, caelyx) is a formulation of doxorubicin in long-circulating, polyethylene (glycol)-coated (stealth) liposomes with a prolonged circulation time and unique toxicity profile.

Preclinical experiments indicate that Stealth liposomal delivery of anthracyclines decreases the cardiotoxic effect, enhancing anti-tumor activity, and improving the overall therapeutic index. In contrast to the short distribution half-life of free doxorubicin (~5 min), doxil is cleared from plasma with a half-life of 2–3 days. A slow plasma clearance and a small volume of distribution are characteristic of Doxil. In addition, data from imaging studies in cancer patients with radiolabeled Stealth liposomes, as well as data from drug levels in tumor biopsies, point to a selective accumulation of doxil in some human tumors. In extensive studies with AIDS-related Kaposi’s sarcoma patients, doxil appears to be the most active therapy
available for this disease. In studies evaluating patients with solid tumors, cutaneous and mucosal toxicities were recognized as the two main dose-limiting factors of doxil. In Phase II-III studies in recurrent ovarian cancer, doxil has significant antitumor activity and a favorable safety profile which have led to its recent approval as second line chemotherapy in ovarian cancer. Doxil shows anti-tumor activity in a variety of other neoplastic conditions, particularly breast cancer, and is being extensively screened as single agent or in combination chemotherapy.

Additional Stealth liposome formulations with cytotoxic drugs (topo I inhibitor, cisplatin) are on preclinical or clinical development. Although their clinical potential is still unclear, one important observation is that the rate of drug release is critical for bioavailability and therapeutic activity. In the case of stealth liposomal cisplatin, the kinetics of drug release is extremely slow resulting in poor anti-tumor activity, despite great liposomal drug accumulation in tumors. Non-pegylated liposomal formulations of anthracyclines have also been approved for clinical use in AIDS-related Kaposi’s sarcoma (daunoxome) and breast cancer (myocet). Although no direct comparison has been made with doxil, it is clear that both formulations have shorter half lives in circulation than doxil. In addition, non-pegylated liposomal formulations of mitoxantrone, vincristine, and topo I inhibitors are in different phases of preclinical and clinical development.

Virosomes, a New Liposome-like Vaccine Delivery System

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The immunopotentiating reconstituted influenza virome (IRIV) vaccines combine several components known to promote immunostimulatory processes. IRIV’s are prepared by detergent removed influenza surface glycoproteins and a mixture of natural and synthetic phospholipids. The influenza virus glycoproteins incorporated into the virosomal bilayer membrane are thought to facilitate the binding to the receptors of the antigen presenting cells, the endocytosis and the fusion with the endosomal membrane thus mediating the rapid release of the transported antigen into the membranes of the target Th-cells. Other immunostimulatory effects of the hemagglutinins and neuraminidase incorporated into the IRIV membrane are also discussed.

Preclinical experiments with a virosomal hepatitis B vaccine containing a B-cell epitope from the S region as well as a Th-cell epitope from the pre-S1 region showed that the pre-S1 Th-cell epitope was only efficient when the two peptide antigens were placed on the same vesicle. Other preclinical experiments performed in mice with virosomal influenza vaccines revealed immunogenic superiority when compared with the effect of an aqueous vaccine and, when applied by intranasal administration, the capacity to induce an excellent systemic and local immunity which provided protection from virus challenge. Extended investigations performed in mice with hepatitis A IRIV vaccine demonstrated that the incorporation of influenza hemag-
glutinins and neuraminidase as well as kephalin is indispensable for obtaining an optimal immune response. Further experiments in mice showed that priming with H1N1 influenza antigen had an enhancing effect on the immune response to the vaccination of the animals with an IRIV-SPf (66)n malaria antigen. This confirmed the importance of incorporating influenza virus antigen into the IRIV vaccines.

A test series with adult human volunteers failed to demonstrate any induction of antibodies against the phospholipids contained in the IRIV’s.

Another vaccination trial showed that an aqueous solution of hepatitis A IRIV vaccine stored for more than 2 years had not lost any of its immunogenic activity. It could clearly be established that an IRIV vaccine yielding formalin-inactivated hepatitis A virions bound to the bilayer surface was not only more immunogenic than a fluid vaccine with the same antigen content but that after the injection of an unique vaccine dose the hepatitis A antibodies appeared much earlier (i.e., on day 14 vs. day 28) at a protective level and also persisted at a tenfold higher level after one year when compared with the antibody levels induces by a doubled hepatitis A antigen dose adjuvanted by aluminium hydroxide. Thus, large clinical trials showed that with the IRIV vaccine seroconversion rates of 91–100% can be obtained already on day 14 and of 98–100% on day 28, and protective serum antibody titres were found in 94–100% one year after a single vaccination. A booster injection performed after one year induces a 20- to 30-fold antibody titre increase which evokes a memory T-cell mediated response. Local adverse reactions, i.e., pain, induration or swelling, were highly reduced when the IRIV vaccine was used instead of aluminium vaccine. It was also demonstrated that the simultaneous administration of immunoglobulin would not substantially impair the vaccine induced antibody productions. In a further study it was demonstrated that the hepatitis A antibody titres induces by the IRIV vaccine could be determined as easily by neutralization test (NT) as by the most usual enzyme immuno-assay (EIA) suggesting the antibodies to confer protection and to have a high affinity to hepatitis A virus.

A trivalent IRIV influenza vaccine yielding the appropriate H1N1, H3N2 and B strain assayed in a community of home inmates aged 63 to 102 years (average 78 years) partially showed a significantly higher immunogenic potential specially in the elderly who before vaccination lacked protective antibody levels when the seroconversion rates induces by a whole virus or a subunit vaccine were compared.

In view of the multitude of vaccinations required already in early life and later on, investigations with an IRIV vaccine combining 5 viral and 2 toxoid antigens were undertaken with student volunteers. The combined IRIV vaccine contained formalin-inactivated hepatitis A virions (500 RIA units), hepatitis B surface antigen (HbsAg; 10 μg), diphtheria toxoid (1 Lf), tetanus toxoid (10 Lf) and 3 influenza virus antigens (H1N1, H3N2, B, 15 μg each). The antibody titres induced were compared with those elicited by the corresponding monovaccines injected simultaneously at different sites. It resulted that the hepatitis A antibody production was significantly delayed in the combined vaccine group. Hepatitis B antibodies remained absent in either group thus confirming the necessity of repeated doses for providing protection against the disease. Toxoid as well as influenza antibody levels all showed to be unaffected by the IRIV antigen combination. A complementary vaccination series with a vaccine containing a mixture of hepatitis A virion IRIV’s, diphtheria toxoid IRIV’s and tetanus toxoid IRIV’s failed to indicate any impairment of the immune
response. By this, an epitope suppression seemed to be excluded. However, when volunteers were immunized with an IRIV vaccine combining the hepatitis A virion with diphtheria and tetanus toxoid amounts reduced by 50%, the immune response to all 3 antigens proved to be rapid and complete. This observation indicated that the reduction of the hepatitis A antibody formation observed before was due to an antigenic suppression by the higher yield of toxoid antigens. Thus, it is obviously possible to develop fully effective IRIV based combined vaccines which could play a major role in future vaccination activities.

Uptake and Intracellular Processing of PEG-Liposomes and PEG-Immunoliposomes by Kupffer Cells In Vitro

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Specific targeting of drugs to for instance tumors or sites of inflammation may be achieved by means of immunoliposomes carrying site-specific antibodies on their surface. The presence of these antibodies may adversely affect the circulation kinetics of such liposomes as a result of interactions with cells of the mononuclear phagocyte system (MPS), mainly represented by macrophages in liver and spleen. The additional insertion of poly(ethylene glycol) (PEG) chains on the surface of the immunoliposomes may, however, attenuate this effect.

We investigated the influence of surface-coupled rat or rabbit antibodies and of PEG on the uptake of liposomes by rat Kupffer cells in culture with 3H-cholesteryloleyl ether as a metabolically stable marker. Additionally, we assessed the effects of surface-bound IgG and PEG on the intracellular processing of the liposomes by the Kupffer cells, based on a double-label assay using the 3H-cholesteryl ether as an absolute measure for liposome uptake and the hydrolysis of the degradable marker cholesteryl-14C-oleate as relative measure of degradation.

Attachment of both rat and rabbit antibodies to PEG-free liposomes caused a several-fold increase in apparent size. The uptake by Kupffer cells, however, was 3–4 fold higher for the rat than for the rabbit IgG liposomes. The presence of PEG drastically reduced the difference between these lipidosome types. Uptake of liposomes without antibodies amounted to only about 10% (non-PEGylated) or less (PEGylated) of that of the immunoliposomes.

In contrast to the marked effects of IgG and PEG on Kupffer cell uptake, the rate of intracellular processing of the liposomes remained virtually unaffected by the presence of these substances on the liposomal surface.
These observations are discussed with respect to the design of optimally formulated liposomal drug preparations, combining maximal therapeutic efficacy with minimal toxicity.

Koning GA, Morselt HWM, Kamps JAAM, Scherphof GL. J Liposome Research 2001; 11:195–209.

Dendritic Cells Capture and Efficiently Present Antigen Encapsulated in Liposomes to T Cells In Vivo

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Dendritic cells (DC) are potent inducers of immune responses. DC are widely distributed in the body and are specialized to take up and process antigen (Ag) and to present it in association with the Major Histocompatibility Complex (MHC) molecules they express, activating both naive and mature T cells. T cells are the major regulatory and effector cells of immunity, so for vaccine development it is important to understand how DC recognize and acquire Ag in vivo. DC populations present in lymphoid organs are heterogeneous with respect to expression of cell surface markers. However, we have more information concerning the phenotypic heterogeneity of DC than its functional consequences. We need to know if different populations of DC generate different kinds of effector T cells. Among these are cells that promote humoral or cytotoxic responses, as well as regulatory T cells that can modify ongoing immune responses. If this is the case, it is important to determine whether these different populations may be selectively targeted by Ag. We investigated the relationship between the form of Ag and its capacity to be taken up by DC and presented to T cells. We compared free Ag and Ag encapsulated in liposomes, in an experimental system allowing determination of the efficiency of Ag presentation by DC.

We injected different forms of Ag subcutaneously in mice. At intervals, cells in the draining lymph nodes were used as antigen presenting cells (APC) to stimulate, ex vivo, T cells from mice transgenic for an Ag-specific T cell receptor. Ag delivered in liposomes, but not free Ag, was efficiently presented. To characterize the APC responsible for Ag presentation, we injected Ag-containing fluorescent liposomes. After 24 h, cells that were fluorescent by virtue of the uptake of these liposomes represented about 3% of the total cells in the lymph node. These cells expressed DC-restricted markers and high levels of MHC and costimulatory molecules required for T cell activation. A confocal microscope analysis of the lymph node suggested that fluorescent DC arrived via the afferent lymphatic vessels into the subcapsular sinus and were also present in T cell zones. Fluorescent DC were purified with a fluorescence-activated cell sorter. These DC were highly efficient in stimulating, ex vivo, naive Ag-specific T cells.
Next, we adoptively transferred Ag-specific T cells labeled with CFSE, an agent that permits quantification of cell division using the cell sorter. Mice were immunized with free Ag, alone or in the presence of either Freund’s adjuvant or LPS, or with Ag encapsulated in liposomes. We determined the limiting Ag doses inducing specific T cell proliferation, in draining and distal nodes. At low doses (0.5 ng), only Ag-containing liposomes stimulated T cell proliferation. For the free form of Ag the limiting dose was 500 ng, which could be reduced in the presence of LPS to 50 ng. Ag emulsified in Freund’s adjuvant was sequestered at the site of injection as demonstrated by the absence of response in distal nodes.

These results demonstrate that liposomes are highly effective at in vivo Ag loading and activation of DC inducing T cell responses. Further studies of different populations of DC with respect to Ag uptake and presentation and the consequences for the activity of responding T cells are in progress.

From Properties to Performance: The Mechanochemistry of Lipid Vesicles with Implications for Drug Carrier Design

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When systemic chemotherapy is used in the treatment of solid tumors, it is almost impossible to achieve therapeutic levels of drug at the tumor site without damaging healthy organs and tissues. Lipid-based drug carrier systems have been developed that can retain drugs, evade the body’s defenses, and passively target the interstitial tissue of tumors. In order to correlate performance of carriers with more than just their composition it is necessary to determine detailed relationships between the materials structure and properties (1,2). Our main research focus for the past 15 years has been to develop various micropipet methods to specifically study the mechanochemical features of lipid bilayer vesicles. We are now in a position to use this information not only to characterize the membrane and its intermembrane interactions from a fundamental materials science perspective, but also to provide essential materials property data that are required for the successful design and deployment of lipid vesicle capsules in applications such as drug delivery. Here, the strength and compliance of the membrane, its interfacial interactions, its thermal transition properties, and its exchange with drugs and other molecules are of particular interest. How drug delivery functions are related to the materials composition, structure, and property will be described to help provide a more rational approach to drug carrier design. Several examples will be given that link composition to structure to properties to performance, including a formulation that allows release of encapsulated drug only at the diseased site and at controllable rates triggered by focused heat, i.e., a new thermal-sensitive drug delivery system (2,3,4). We describe a new lipid formulation containing doxorubicin that has been optimized for both mild hyperthermic temperatures (39°C to 40°C) that are readily achievable.
in the clinic, and rapid release times of drug (tens of seconds). The formulation involves incorporating a highly bilayer compatible lysolipid, 1-Palmitoyl-2-Hydroxy-sn-Glycero-3-Phosphocholine (MPPC) into gel phase liposomes composed of 1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC). This compositional modification of the liposomes achieved a significantly enhanced release of entrapped liposome contents at mild hyperthermic temperatures between 39°C–40°C as compared to pure DPPC alone, which released only 20% of contents over a broader range of 40°C–45°C, and the more conventional (DPPC/DSPC-based) temperature-sensitive liposomes that released more slowly in the 43°C–45°C range. This new liposome, loaded with the anti-cancer drug doxorubicin, when tested in a human tumor (FaDu squamous cell carcinoma) xenograft animal model in combination with mild hyperthermia, was found to be significantly more effective than free drug or current liposome formulations at reducing tumor growth producing, 11 out of 11 complete regressions lasting up to 60 days post treatment (4).

1. Needham D. Materials engineering of lipid bilayers for drug carrier function. In: Denis Wirtz, Evan Evans, eds. Materials Science of the Cell: Materials Research Society. MRS Bull 1999; 24(10):32–40.
2. Needham D, Dewhirst MW. The development and testing of a new temperature-sensitive drug delivery system for the treatment of solid tumors. Advanced Drug Delivery Reviews 2001; 53:285–305.
3. Anyarambhatla GR, Needham D. Enhancement of the phase transition permeability of DPPC liposomes by incorporation of MPPC: a new temperature-sensitive liposome for use with mild hyperthermia, J Liposome Research 9:491–506.
4. Needham D, Anyarambhatla G, Kong G, Dewhirst MW. A new concept for triggered drug release: characterization and testing in a tumour model. Cancer Research 2000, March 1; 60:1197–1201.

**Anti-neovascular Therapy by Use of Liposomes Targeted to Angiogenic Vessels**

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Cancer chemotherapy targeted to angiogenic vessels is expected to cause indirect tumor regression through the damage of the neovascularure. To develop a targeting probe for neovascularure, we isolated novel peptides homing to angiogenic vessels formed by a dorsal air sac method from a phage-displayed peptide library. After the determination of the epitope sequences of peptide presented by thus obtained phage clone, we modified liposomes with epitope penta-peptides, APRPG. Liposome modified with APRPG-peptide showed high accumulation in murine tumor, and APRPG-modified liposome encapsulating adriamycin effectively suppressed experimental tumor growth. Furthermore, anti-neovascular therapy by using lipophilic derivative of anti-cancer drug, DPP-CNDAC was examined. Since lipophilic drugs
should be delivered to the cells as liposomal form, the therapeutic efficacy reflects the damage of the cells to which liposome accesses rather than change in local concentration of the agent in tumor tissue. In fact, therapeutic efficacy of APRPG-liposomal DPP-CNDAC is superior to non-modified liposomal DPP-CNDAC, suggesting that the destruction of angiogenic endothelial cells is superior to the direct destruction of tumor cells in the tumor treatment. Finally, specific binding of APRPG-modified liposome to human umbilical endothelial cells, and that of PRP-containing peptide to angiogenic vessels in human tumors, i.e., islet cell tumor and glioblastoma, were demonstrated. The present study provides a novel modality of cancer treatment, anti-neo vascular therapy, and the usefulness of APRPG-modified liposomes as a drug carrier for this treatment.

Mapping Vascular Diversity by Screening Peptide Libraries

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Despite major progress brought about by the Human Genome Project, the molecular diversity of human blood vessels remains largely unexplored. Our research is aimed at targeting diagnostic and therapeutic agents to blood vessels by using probes that can bind to specific vascular addresses. Towards this goal, we developed technologies to identify small peptides that target the endothelium. Different strategies are used to isolate peptides from large libraries displayed in the surface of bacteriophage. Through this platform technology, we have uncovered various tissue-specific and angiogenesis-related vascular addresses. This complex system of ligand-receptor pairs will lead to a better understanding of tumor circulatory microenvironment, changes in blood vessels during tumor progression, and the localization of novel markers in cancer and other diseases with an angiogenesis component. This lecture will review several targeting strategies that may enable the construction of a molecular map outlining vascular diversity in each organ, tissue, or disease.

Magnetofection: Enhancing and Targeting Gene Delivery with Lipid-DNA Vectors by Magnetic Force

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Low efficiencies of nonviral gene vectors, the receptor-dependent host tropism of adenoviral or low titers of retroviral vectors limit their utility in gene therapy. A universal method of minimizing these deficiencies has been lacking so far. Here we present a novel method based on the principle of magnetic drug targeting that potentiates the efficacy of any gene vector, extends the host tropism of
adenoviral vectors to non-permissive cells and compensates for low retroviral titers. We associated gene vectors with superparamagnetic iron oxide nanoparticles that were coated with polyelectrolytes. By applying a magnetic field, the vector dose was accumulated on target cells, reducing the duration of gene delivery to minutes and enhancing vector efficacy up to several thousand fold. Applying magnetofection in vivo, we achieved local accumulation of gene vectors at target sites under the influence of a magnetic field and nonviral, site-specific transfection in the vascular compartment upon intravenous vector administration. Magnetofection provides a novel tool for automated high throughput gene screening in vitro and can help overcome fundamental limitations to ex vivo and in vivo gene therapy.

Transport Properties of Lipid and Polymer-DNA Nanoparticles

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We investigate the structure, phase behavior and molecular order of cationic lipid–DNA composite complexes and discuss their application as supramolecular gene carriers. Recent synchrotron X-ray scattering studies elucidated a series of novel structures with high liquid-crystalline order of alternating lipid–DNA layers. Molecular spacings and intrinsic correlation lengths vary in predictable fashion with composition and temperature. Most importantly topological transitions from lamellar to hexagonal phases are achieved by variation of the mol-percentage phosphatidylethanolamine-helper lipid. The measured phase behavior can be described by theoretical models that take the electrostatic, elastic and entropic contributions to the free energy of a lipid–DNA composite phase into account. Liposomal and polymer-based systems are compared with respect to structure, stability and phase behavior. In particular the interaction of complexes with negatively charged polyelectrolytes is of interest, since in-vivo conditions are rich in highly anionic macromolecules. X-ray data show, that hyaluronic acid and dextrane sulfate dissolve lipid–DNA complexes by forming themselves lamellar lipid–polyelectrolyte complexes. Furthermore the colloidal stability of lipoplexes in salt conditions was studied measuring the size distribution and aggregation behavior using fluorescence microscopy and fluorescence correlation spectroscopy. The number of plasmids per aggregate can be extracted and the growth kinetics be followed. Nano-sized particles are achieved using lipid coatings that inhibit unspecific growth by diffusion limited aggregation. As a particular coating lung-surfactant extracts were used. The lung surfactant coating increases gene transfer for polymer based vectors but inhibits gene transfer for cationic lipid systems. The transport of such nanoparticles in collagen solution was measured.

Rädler JO, Koltover I, Salditt T, Sañinya CR. Science. 1997; 275:810–814.
Zantl RL, Baicu F, Artzner I, Sprenger G. Rapp, Rädler JO. 1999. J Phys Chem 1999; 103:10300–10310.
In Vivo Antigen Loading and Activation of Dendritic Cells via Liposomal Peptide and DNA Vaccine Preparations: Results and Future Prospects

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The initiation of antiviral and antitumor T cell responses is mainly regulated by dendritic cells (DC) transporting antigen from the periphery into organized lymphoid tissues. For the development of T cell vaccines it is therefore important to assess the access of the antigen to DC in vivo and whether DC are activated by the vaccine. We evaluated the immunogenicity of liposomal vaccine formulations using the immunodominant CTL epitope (GP33) derived from the glycoprotein of the lymphocytic choriomeningitis virus (LCMV-GP). Liposome-encapsulated peptides were highly immunogenic when administered intradermally and provided excellent protective antiviral immunity, whereas intravenous or intraperitoneal immunization did not elicit significant CTL responses. Simultaneous liposomal delivery of the LCMV-GP T helper peptide P13 and GP33 further increased GP33-specific CTL responses. After intradermal injection, liposomes formed antigen depots facilitating long-lasting in vivo antigen loading of dendritic cells almost exclusively in the local draining lymph nodes. The immunogenicity of the liposomal peptide vaccine was further enhanced by incorporation of immunostimulatory oligonucleotides (ODN) leading to activation of DC. Furthermore, immunization with the optimized liposomal peptide vaccine containing GP33, P13 and ODN elicited as well protective antitumor immunity and induced CTL responses comparable to adoptively transferred, peptide-presenting DC. Presently, we prepare vaccines with hepatitis C antigen peptides containing immunostimulating molecules such as heat shock proteins and DC specific liposomes. Taken together, our data suggest that direct in vivo antigen loading and activation of DC by liposomal peptide or DNA vaccines represent a practical and efficient approach to elicit antiviral and antitumor immune responses.

Electron Microscopy Techniques to Characterize Lipid-Based Drug/Gene Carriers and Their Interaction with Cells

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Negative staining, freeze-fracture and cryo electron microscopy are the three most common techniques suitable for transmission electron microscopic studies on drug/gene carries such as liposomes, niosomes, cochleate cylinders, nanoparticles, and micelles. Advantages and restrictions of these three techniques will be discussed. Negative staining technique is performed very easily and provides the highest resolution. However, there is a high probability for artifact formation.
because the biological objects are dried out before staining with heavy metal solutions for preparation. Both cryo-fixation techniques, freeze-fracture and cryo electron microscopy, are low in artifact formation but their resolution is in the nanometer range and not as high as obtained with negative staining. While cryo electron microscopy has some advantages in characterizing the inner volume of drug/gene carries, freeze-fracture electron microscopy is a unique technique to investigate the hydrophobic interior of bilayer structures not depending upon whether these bilayers are belonging to cells, liposomes, or cochleate cylinders. Furthermore it is an excellent technique to record bilayer/nonbilayer transformations as well as cell interactions of drug/gene carriers on a nanometer resolution scale.

For Further Reading:
Electron Microscopy in Biology. A Practical Approach edited by J.R. Harris, IRL Press at Oxford University Press, Oxford, New York, Tokyo 1991.
Sternberg B. Freeze-fracture Electron Microscopy of Liposomes In: Gregoriadis G, 2nd Edition. Liposome Technology edited by, CRC Press, Boca Raton, Ann Arbor, London, Tokyo 1993; Vol. I, p. 363–383.

In Memory of Danilo D. Lasic, Passionate Scholar, Scientist, Mentor, and Liposomologist Extraordinaire

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From the first time that I met Dan Lasic, as he introduced himself, until my last email exchange with him shortly before his untimely death, I never ceased to be amazed by his passion for life, and for everything liposomal! Dan literally wrote the book on liposomes (1), single-handedly, a book unmatched before or since. Dan left no aspect of liposomes unexplored. In addition to his scholarly pursuits going as far back in history as libraries can go, Dan participated in and led innovative studies to further our understanding of every kind of lipid system, covering the full range from their most fundamental physics to their most applied possibilities (1,2). Dan’s vast publications numbering nearly 150 in total continue today to play a major role in advancing science. Clearly they illustrate his importance to our history better than anything we can say about him.

Dan’s devotion to liposomes was established long before I met him. He studied liposomes, and all forms of organized lipid systems, without compromise. He not only studied them but strove to teach the world about them, and with great success. He taught on a personal level everyone he encountered and he taught the world through his publications. Publishing over 30 review articles, Dan helped to resurrect the awareness and appreciation of liposomes at a time when they had fallen out of favor with most editors. Dan reviewed liposomes in English such as his review published in American Scientist (3), in French such as his review in La Recherche
(4) which was translated in many languages, in German such as his review in Angewandte Chemie (5), and for the elite of scientists such as his review in Science (6). Dan spoke to everyone, young and old, layperson and scientist, technician and Nobel laureates. One of his great traits was his ability to communicate with everyone and convey his passion for life, and of course liposomes.

Another unmistakable side of Dan was his love of life, family, and friends. Dan was always passionate about whatever he did and the personal relationships with those he interacted. Few of us that worked and played with him can forget our time together. But more importantly than just remembering our time with Dan, we remember his dedication to help those of us surrounding him to achieve our best. I will always be grateful to him for teaching me, pushing me, helping me. And of course, Dan’s passion for life didn’t stop with work. He loved and engaged music, art, philosophy, traveling, etc. It cannot be easily forgotten how, whenever Dan encountered a piano, he was tempted to sit down and play Chopin, often giving in to the pleasure of everyone in earshot. To his family and friends outside of his scientific pursuits, Dan was a friend and mentor. Today, his passionate love of life lives on in the many whose lives he touched.

1. Lasic DD. Liposomes: from Physics to Applications. Elsevier, 1993:600.
2. Barenholz Y, Lasic DD, eds, Handbook of Nonmedical Applications of Liposomes. Boca Raton, Fl: CRC Press, 1995:4.
3. Lasic DD. Liposomes, American Sci 1992; 80:20–31.
4. Lasic DD. Les liposomes. La Recherche 1989; 20:904–913.
5. Lasic DD. Sterisch stabilisierte vesikel. Angewandte Chemie 1994; 106:1765–1779.
6. Lasic DD, Papahadjopoulos D. Liposomes revisited. Science 1995; 267:1275–1276.

NanoCochleate Cylinders for Oral & Parenteral Delivery of Drugs

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The efficacy of a drug carrier depends on many factors, especially its ability to efficiently target and deliver the drug to the site of infection. Nanocochleates cylinders, a well defined, small-sized, lipid-based drug carrier composed of natural phospholipids, showed a potential to deliver Amphotericin B (AmB), a potent antifungal agent, orally and parenterally (1–7).

Nanocochleates containing AmB (CAMB) were efficient in delivering AmB orally in a murine model infected with candidiasis (2,3,5,6), aspergillosis (7) and cryptococcosis; thus offering a new route of delivery for this old drug. In addition, nanocochleates showed recently a potential to deliver Amphotericin B parenterally, at least as efficiently as Fungizone®.

The oral way of delivery, the improved parenteral efficiency by comparison to other lipid systems, combined with a good safety profile, position CAMB as a new
drug that has potential to treat fungal infections. Nanocochleates containing AmB are now in development to enter Phase I clinical trials, for both the oral and parenteral treatment of fungal infections.

REFERENCES

1. Zarif L, Tan F. Cochleates from purified soy phosphatidylserine: their composition, process of preparation, uses for the encapsulation and the delivery of drugs. US Patent Application filed 3/27/2002.
2. Zarif L, Jin T, Segarra I, Mannino RJ. Novel hydrogel isolated cochleate formulations, process of preparation and their use for the delivery of biologically relevant molecules. US Application No 09/613,840 filed 7/11/00. PCT application filed 1/22/2000, WO 01/52817 A2. US Patent 6,153,217, issued Nov 28, 2000.
3. Zarif L. Elongated supramolecular assemblies in drug delivery. Review J Controlled Release 2002. In Press.
4. Segarra I, Movshin D, Zarif L. Pharmacokinetics and tissue distribution after intravenous administration of a single dose of Amphotericin B cochleates, a new lipid-based delivery system. J Pharmaceutical Sciences 2002. In Press.
5. Zarif L, Graybill J, Perlin D, Mannino R. Cochleates: new lipid-based drug delivery system. J Liposome Research 2000; 10(4):523–538.
6. Zarif L, Graybill JR, Perlin D, Najvar L, Bocanegra R, Mannino RJ. Antifungal activity of amphotericin B cochleates against candida albicans in a mouse model. Antimicrobials Agents and Chemotherapy 2000; 44(6):1463–1469.
7. Santangelo R, Paderu P, Delmas G, Chen ZW, Mannino R, Zarif L, Perlin DS. Oral efficacy of cochleate-amphotericin B (CAMB) in a mouse model with systemic candidiasis. Antimicrobials Agents and Chemotherapy 2000; 44(9):2356–2360.