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Enhanced Anticancer Therapy Mediated by Specialized Liposomes

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Abstract

It has been a central aim of experimental and clinical therapeutics to deliver therapeutic agents as close as possible to, or if possible within, a diseased cell. Such targeting achieves two major aims of drug delivery, the maximum dose of therapeutic agent to the diseased cell and avoidance of uptake by and, usually, accompanying side-effects to normal, healthy cells.

Conventional liposomes, originally used for studies in membrane biophysics and biochemistry, have been used in therapy for the past two decades. However, when applied to deliver drugs into cells, conventional liposomes proved inefficient and so novel unconventional or specialized liposomes are constantly being prepared to enhance cell-specific delivery in-vivo. One possible way of achieving better targeting is combination of the positive attributes of more than one specialized type of liposome into one vesicle. Although a limited number of studies has examined the combined effect of such dual-speciality liposomes, more studies are warranted using appropriate models.

Liposomes are composed of one, a few, or many concentric bilayer membranes which alternate with aqueous spaces. The drugs are encapsulated within the aqueous internal volume if they are hydrophilic or in the lipid bilayers if they are hydrophobic (Kim 1993). Liposomes range in size from 25 nm to more than 20 μm (Sugarman & Perez-Soler 1992). Depending on their solubility and method of formulation antimicrobial, cytotoxic and other conventional drugs, hormones, antigens, enzymes, genetic material, viruses and bacteria can be incorporated in either the aqueous or hydrophobic phase.

This review discusses the types and characteristics of non-conventional liposomes used in various modes of cancer therapy, mainly chemotherapy and gene therapy. It concludes with suggestions on improving these novel liposomal to effect better targeting to cancer cells.

Specialized Liposomes

For fabrication of liposomes different from the conventional neutrally charged lipid vesicles, ideas were derived from the field of membrane biophysics. Such designs, tailored for special physiological conditions, include sterically stabilized liposomes, fusogenic liposomes, immunoliposomes, pH-sensitive liposomes, thermosensitive liposomes and cationic liposomes.

Sterically-stabilized liposomes

The vesicle bilayer of sterically-stabilized liposomes contains glycoproteins (example monosialoganglioside GM_1) or lipids conjugated to ethylene glycol, which render a steric barrier outside the membrane. Sterically stabilized liposomes persist in the blood up to one hundred times longer than conventional liposomes and can thus greatly improve the therapeutic effect of delivered drugs (Wang et al 1995). Significant reductions in uptake by the liver and spleen (organs of the mononuclear phagocytic system, MPS) is achieved (Kim 1993).

Increased stability is a direct result of inhibition of interactions with plasma proteins (such as lipoproteins and opsonins) and cell surface receptors by the steric barrier. Prolonged presence of liposomes in the circulation enables them to extravasate into sites with leaky vasculature, such as those supplying tumours (Papahadjopoulos 1995).

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However, a longer circulation period is not without drawbacks. These liposomes are taken up by other tissues and organs at a rate proportional to their surface area and vascularization. Hence, large doses of markers are found in the skin in animal studies (Lasic 1996) and levels are significantly elevated in other organs with a large internal surface areas such as kidneys and lungs (Huang et al 1992). Thus, caution must be exercised when delivering relatively cytotoxic agents such as anticancer drugs.

Fusogenic liposomes

Liposomes coated with Sendal virus (haemagglutinating virus of Japan, HVJ) coat-proteins have an efficiency of more than 95% for introduction of macromolecules into cultured cells (Kaneda et al 1993). This technology relies on the capability of the Sendai virus to fuse readily with almost all types of mammalian cell (Kaneda et al 1995).

These liposomes have also been used in-vivo in rodents with no evidence of toxicity (Yamada et al 1995). Moreover, HVJ is not pathogenic to man and is completely inactivated by UV irradiation without compromising its fusion activity (Okada 1993). Influenza virus glycoproteins are also used to formulate fusogenic liposomes (Lapidot & Loyter 1990). Respiratory syncytial virus proteins can be employed for targeting genes to the lower respiratory tract (Schreier et al 1993).

Liposomal delivery can be enhanced by incorporation of synthetic amphiphilic peptides into the vesicle surface. For instance, lactosylceramide can be used for increasing delivery to hepatocytes with natural galactose receptors (Scherphof et al 1989). Similarly, alveoli type II cell- (Walther et al 1993) and

bone-marrow erythroblast-specific (Stavridis et al 1986) uptake is enhanced by use of fusogenic liposomes, by coupling of surfactant protein A and transferin, respectively.

Fusogenic liposomes comprising the HVJ are currently being evaluated for gene therapy of cardiovascular diseases (Dzau et al 1996). One drawback with these liposomes is their inability to encapsulate DNA quantitatively (Lasic & Pearlman 1996). The past two decades of mammalian cell transfection with these liposomes have led to relatively little progress towards clinical trials.

Immunoliposomes

Liposomes can be targeted by including on their surfaces antibodies that recognize specific cell-surface antigens. Immunoglobulin G (IgG) raised against tumour cells and embedded into liposome membranes enables targeted delivery to tumour cells (Ahmad et al 1993).

Disadvantages of immunoliposomes include their physical diameter, they are too large for extravasation from the circulatory system, and the possibility of immunological recognition of foreign immunoglobulin epitopes that might lead to removal of these vesicles and prevent further administration (Gregoriadis 1995). However, immunological recognition might be overcome by synthesizing fusogenic liposomes from natural ligands such as human desialylated glycoproteins.

pH-sensitive liposomes

It is known that acidification occurs in both endosomes and lysosomes because of the internalization of liposomes into these vesicles (Yoshimura et al 1995). Therefore, pH-sensitive liposomes promise better delivery into the cytoplasm (Chu et al 1990). The liposomal bilayer destabilizes at acidic pH (between pH 5 and 6·3) in the late endosome thereby releasing its contents for entry into the cytoplasm (Legendre & Szoka 1992).

On the basis of the premise that the pH in the vicinity of certain tumours or in pockets of a tumour might be lower than in normal tissues (Murray & Carmichael 1995), liposomes are formulated that leak at lower pH, thereby releasing their contents in the proximity of the affected site. Inadequate perfusion results in hypoxic areas with high reductive potential (Willmott et al 1991). However, the paucity of data on the in-vivo use of pH-sensitive liposomes makes it hard to justify their efficiency in comparison with other types.

Thermosensitive liposomes

Liposomes become more permeable at the phase-transition temperature of the lipids making up the vesicles (Kim 1993). Thermosensitive liposomes are more sensitive to temperatures above 37°C and are, in a way, an extension of the so-called hyperthermic treatment of tumours. This therapy is based on the sensitivity of cancerous cells to temperatures in the range 42–45°C (Yonezawa et al 1996). However, for hyperthermia more research is needed into developing thermo-inductors that are both non-invasive and suitable for deep-seated or small tumours.

Hence, thermosensitive liposomes provide an alternative channel for hyperthermic impact. Liposomal contents leak into the vicinity of the tumour when the temperature in the tumour region is elevated by an external source. The lethal effect is thus doubled. More in-vivo studies are needed to assess the utility of these liposomes in tumour-specific delivery.

A slightly different strategy is used for targeting liposomal agents using photoactivation. Liposomes are formulated from lipids that are altered in response to photosensitization at wavelengths between 680 and 820 nm (Thompson et al 1996). Alteration of these lipids by photons results in permeability of the vesicles to the carried drugs. However, in this case, penetration distance of the wavelengths in question might become a major limiting factor for larger tumours.

Cationic liposomes

Cationic liposomes were developed for delivery of nucleic acids into cells. The anionic nucleic acids initially bind to the surface of the cationic vesicles, forming multilamellar lipid–DNA complexes (Radler et al 1997). These multilamellar vesicles are approximately 1 μm in diameter (Spector & Schnur 1997). DNA persists glued to lipidic molecules with a lipid bilayer surrounding the compacted nucleolipidic particles. In addition to electrostatic attraction, hydrophobic interactions are believed to aid complex formation (Wong et al 1996). For transfection to occur at a reasonable rate, the liposome–DNA complexes must have excess positive charge (Fasbender et al 1995). This enables electrostatic attraction of the complex to the negative surface of cells. Success of cationic liposome-mediated DNA transfer is dependent on factors such as cell type (Jensen et al 1996), whether the culture is primary or subcultures of the primary (Harrison et al 1995), the stage of cell in the growth cycle (Pickering et al 1994), cell seeding densities (Lascombe et al 1996), DNA-to-liposome ratio (Fasbender et al 1995), DNA–liposome complexing volume (Staggs et al 1996), the type and concentration of salts and biomolecules present in the liposome–DNA mixing medium and cell-culture medium (Fasbender et al 1995), size of the liposome–DNA complexes (Behr 1994), the time the liposome–DNA complexes are incubated with cells (Zabner et al 1995), and the lipid components making up the vesicles (Stamatatos et al 1988). These factors contribute to variability in transfection especially in-vivo.

Liposomes are also used to transfer RNA into cells (Glenn et al 1993). Liposomes protect the RNA molecules from nuclease attack and enhance uptake into cells. Similarly, liposomes can be employed for delivery of antisense molecules into cells (Matsubara et al 1996). Liposomal encapsulation of antisense oligodeoxynucleotides (ODNs) protects them from enzymatic degradation and substantially enhances cellular uptake of the antisense molecules (Hughes et al 1996). Ribozymes, belonging to a class of RNA molecules that cleave other RNA sequences enzymatically, have also been delivered by cationic liposomes (Ohta et al 1996).

Dual-speciality Liposomes

Although singly specialized liposomes might be better than conventional liposomes, better targeting might be achieved by combining the positive aspects of at least two types of specialization in the one vesicle. Although the studies listed below have examined the combined effect of such dual-speciality liposomes, more studies using tumour models are warranted.

Sterically stabilized immunoliposomes enhance delivery of doxorubicin in murine squamous lung carcinoma cells in culture (Allen et al 1995) and lung tumours implanted in mice (Ahmad et al 1993). Even at low doses, encapsulated doxorubicin had a potent affect against cultured cancer cells and tumours.

Sterically stabilized immunoliposomes can be used to target pulmonary endothelial cells (Maruyama et al 1990). It is important to note that the targeting ability of immunoliposomes stabilized by either GM_1 or PEG-derivatives is not reduced (Mori et al 1993). In fact, binding to target antigens is enhanced as a result of the prolonged circulation time (Litzinger & Huang 1992).

Sterically stabilized thermosensitive liposomes enhance both delivery and antitumour activity of doxorubicin (Unezaki et al 1994). pH-sensitive immunoliposomes have been employed successfully for in-vivo targeted gene transfer in mice (Wang & Huang 1987). Uptake by cultured human colonic cancer cells was enhanced by use of thermosensitive immunoliposomes (Shinkai et al 1994).

Cationic pH-sensitive liposomes have been formulated to mediate the efficient transfer of DNA into a variety of cells in culture (Budker et al 1996). Acid conditions promoted DNA binding, DNA incorporation, and DNA-induced fusion by these cationic pH-sensitive liposomes. The haemagglutinating antigen from the influenza virus has been used to formulate cationic fusogenic liposomes with enhanced transfecting capacity in-vivo (Mazur et al 1994).

Fusogenic-cationic liposomes were also synthesized by attaching vesicle-coat-cell-specific ligands such as thrombin directed to cells possessing thrombomodulin (TM) such as endothelial cells (Swaim & Dittman 1995). Viral capsids such as that from human papilloma virus or adenoviruses can be used for targeting to cervical and lung cancers, respectively (Cristiano & Roth 1995). However, coating liposomes with proteins such as collagen (Fonseca et al 1996) increases liver uptake and reduces blood circulation time by half. Therefore, caution has to be exercised when using this technique.

All these techniques for preparing dual-speciality liposomes result in enhanced drug delivery in-vitro. These results merit examination of the liposomes in proper animal tumour models. This constitutes the next step towards clinical trials.

Conclusion

The introduction of specialized liposomes has positively altered the area of experimental cancer therapeutics, namely drug targeting. However, the scarcity of data on in-vivo experiments makes it difficult to justify the potential adoption of these vesicles for delivery to tumours in the clinic. More studies are needed to address this issue in depth. There is, however, a contemporary shift towards the combination of different varieties of specialized lipids to formulate vesicles that exploit the positive attributes of each specialized type of liposome in the hope that better targeting to tumours might ensue. Again, more studies are needed for proper assessment of the efficiency of these dual-speciality liposomes compared with that of the specialized predecessors.

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