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Note

Strategies for cytosolic delivery of liposomal macromolecules

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Abstract

Potential approaches to achieve cytosolic delivery of liposomal macromolecules are presented. These approaches include: (1) the co-encapsulation of fusogenic peptides into targeted drug-containing liposomes (2) coupling of the HIV-1-derived cell-penetrating peptide TAT to the surface of liposomes and (3) photochemical internalization, based on photochemically inducible permeabilization of endocytic vesicles.

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1. Introduction

Despite the potential of many macromolecules such as nucleic acids, proteins and peptides to serve as therapeutic agents, the in vivo efficacy can be severely comprised by their unfavorable physicochemical characteristics. One major obstacle is the negative effect of their generally large size and hydrophilic nature on cellular uptake. As the target site of these therapeutics is often located in the cytoplasm, such molecules may not reach their target without a delivery system facilitating cytosolic delivery (Lebleu, 1996). Many different drug delivery systems have been investigated for this purpose. Of these, liposomes have attracted considerable attention. Liposomes are able to provide protection and targeting of the encapsulated macromolecule and may facilitate cellular internalization. Currently, several (targeted) liposome systems are under investigation for this purpose (Simoes et al., 2001; Mandal and Lee, 2002; Mastrobattista et al., 2002; Kakudo et al., 2004).

Fig. 1 illustrates three different pathways by which cytosolic delivery of liposomal macromolecules may be obtained.

Extracellular release and subsequent diffusion of the drug over the plasma membrane (Fig. 1, route 1) are possible for molecules that are able to cross the plasma

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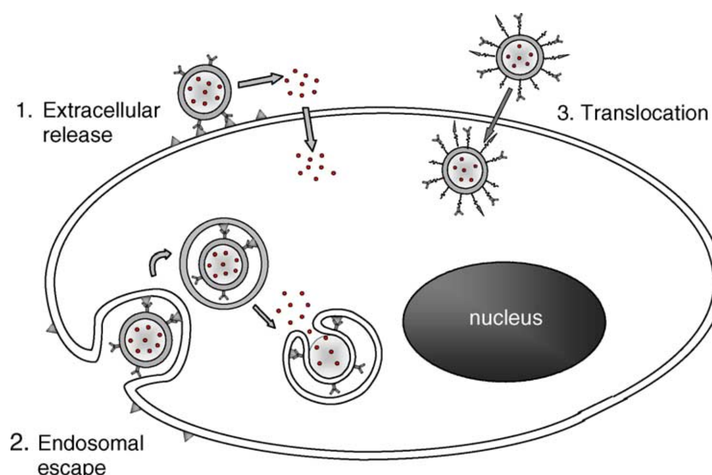


Fig. 1. Schematic representation of potential pathways to achieve cytosolic delivery of liposomal macromolecules.

membrane. Generally, this route is, as just discussed, not an option for macromolecules.

Cellular uptake of liposomes can occur via endocytosis after which they end up in the lysosomes. In the lysosomal compartment, the liposomes will be subjected to the acidic environment and degrading enzymes present there, resulting in degradation of the liposomes including their macromolecular contents. For that reason, utilization of mechanisms that allow endosomal escape may provide an effective way to achieve cytosolic delivery (Fig. 1, route 2).

The so-called cell-penetrating peptides (CPP) have been reported to accomplish direct cytosolic delivery when attached to various cargoes including liposomes (Torchilin et al., 2001; Tseng et al., 2002), as illustrated in Fig. 1, route 3. CPPs are supposed to be able to translocate the cargo over the plasma membrane thereby circumventing endocytosis. This results in direct cytosolic delivery (Lindgren et al., 2000).

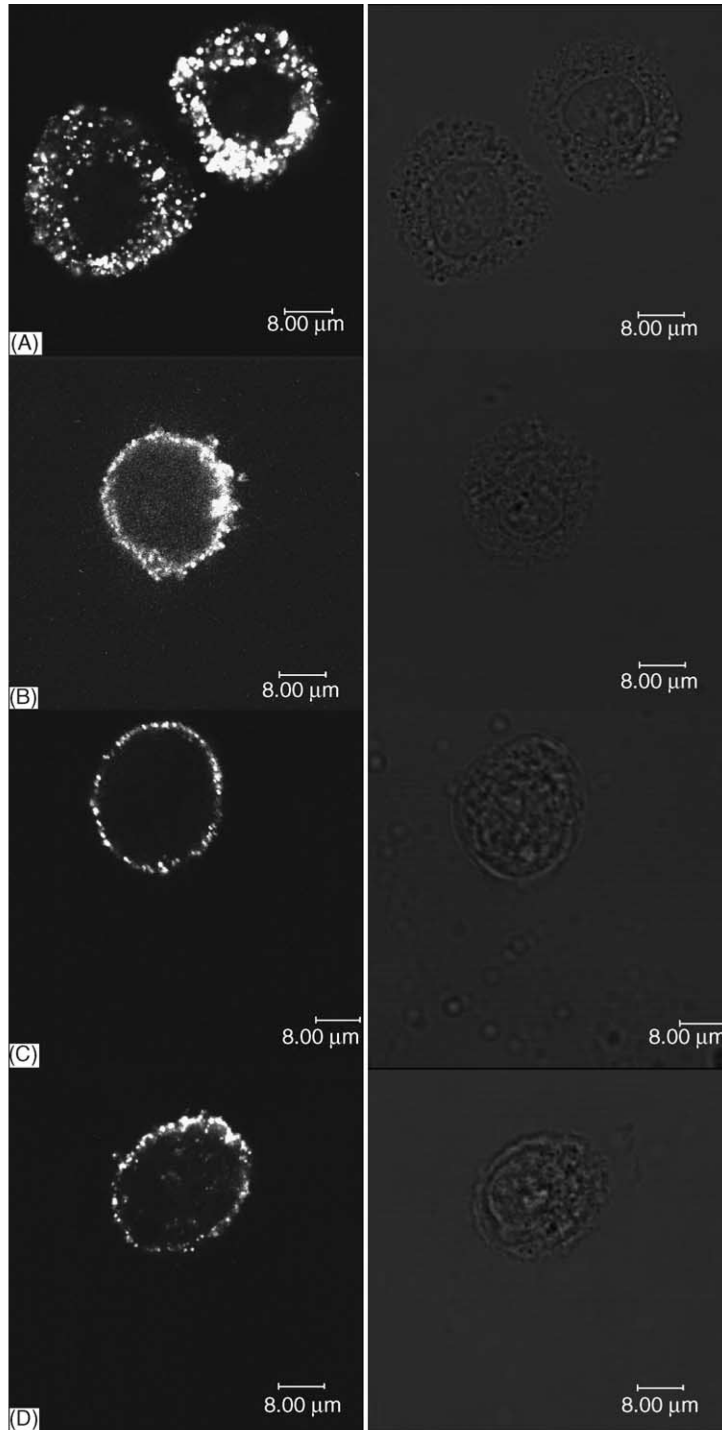
Here we present approaches studied in our laboratory to achieve cytosolic delivery of liposomal macromolecules, including co-encapsulation of fusogenic peptides, surface coupling of CPP and photochemical internalization.

2. Co-encapsulation of fusogenic peptides

By destabilizing the endosomal membrane, endocytosed màterial may be released in the cytoplasm. This endosomal escape mechanism is, for example, exploited by certain viruses, e.g. the influenza virus (White, 1990). After endocytosis of the influenza viral particle, the N-terminal domain of viral protein hemagglutinin subunit HA2 induces membrane destabilization in the lysosomes. Upon acidification, this peptide domain becomes protonated, causing a conformational change from random coil to alpha helix (Skehel et al., 1982; Doms et al., 1985). Due to this conformational change, the fusion peptide is inserted into the endosomal membrane and destabilizes it (Harter et al., 1989; Stegmann et al., 1991).

Synthetic analogues of this fusion peptide have been used in non-viral gene delivery systems to improve the

Fig. 2. Cellular uptake of TAT-liposomes was inhibited by low temperature and metabolic inhibitors. OVCAR-3 cells were incubated with TAT-liposomes for 5 h at 37 °C (A, C, D) or at 4 °C (B). Confocal (left panel) and phase contrast (right panel) images were taken of living OVCAR-3 cells, to prevent possible fixational artifacts. (A) Control, vesicular localization of TAT-liposomes; (B) incubation at 4 °C, plasma membrane binding of TAT-liposomes; (C) in the presence of iodoacetamide, plasma membrane binding of TAT-liposomes; (D) in the presence of cytochalasin D, plasma membrane binding of TAT-liposomes. Figure reproduced from the publication of Fretz MM et al. (2004) with permission of the publisher.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transfection efficiency (Wagner, 1999; Zuidam et al., 2000; Van Rossenberg et al., 2002).

We examined whether co-encapsulation of the influenza virus-derived synthetic diINF-7 could enhance the cytosolic delivery of liposome-entrapped proteins. Using circular dichroism, the pH-induced conformational change was verified. The alpha helical content increased from 15% to 31% when the pH was lowered from 7.4 to 5.2. In addition, we showed that the fusogenic behavior of the peptide was pH-dependent; diINF-7 induced leakage of liposome-encapsulated calcein was much more efficient at pH 5.2 than at pH 7.4. For application as a mediator of cytosolic delivery, the peptide was co-encapsulated with the catalytic A-domain of diphtheria toxin (DTA). DTA inhibits protein synthesis when delivered in the cytoplasm, resulting in cell death. Liposomes targeted to the epidermal growth factor receptor (EGFR) of tumor cells showed cytotoxicity only when both the diINF-7 peptide and DTA were encapsulated, whereas targeted liposomes containing either DTA or diINF-7 did not have any cytotoxic effect (Mastrobattista et al., 2002).

3. Surface coupling of cell-penetrating peptides

The coupling of the so-called cell-penetrating peptides (CPP) to cargoes of different sizes would enable the cargo to directly enter the cytoplasm (Lindgren et al., 2000). Recently, this concept was questioned since redistribution of fluorescently labeled CPP was observed in fixed cells due to the fixation procedure (Lundberg and Johansson, 2002; Vives, 2003).

We studied the uptake mechanism of TAT-peptide modified liposomes in living cells. In this study, it was clear that the coupling of TAT-peptide to the liposomes greatly enhanced the cellular binding and subsequent uptake of the liposomes. However, when the cellular distribution of fluorescent TAT-modified liposomes in fixed cells was compared to the distribution in living cells, significant differences were observed. Instead of a diffuse cytosolic fluorescence seen in fixed cells, living cells displayed punctuate intracellular spots, indicating endocytosis. This was supported by the observation that the liposomal labels co-localized with Lysotracker Red, a marker for endosomes and lysosomes. In addition, when endocytosis was inhibited by lowering the temperature to 4 °C, by iodoacetamide or by cytochalasin D, only plasma membrane binding was observed while intracellular fluorescence was absent, as shown in Fig. 2.

We concluded that TAT-peptide modified liposomes are taken up by endocytosis rather than plasma membrane translocation (Fretz et al., 2004).

4. Photochemical internalization

Recently, a novel photochemical technique, named photochemical internalization (PCI), was developed for inducing release of molecules from endocytic vesicles (Hogset et al., 2004). In this technique, photosensitizing compounds (the so-called photosensitizers) are applied for endosomal escape. Upon illumination of these photosensitizers, highly reactive oxygen species are formed. Depending on their physicochemical properties, the photosensitizer can preferentially localize in endosomal membranes. Upon illumination and formation of the reactive oxygen species, the endosomal membrane is damaged and molecules present will be released in the cytosol. PCI has been shown to induce endosomal release of toxins (Berg et al., 1999; Selbo et al., 2000a, 2000b), immunotoxins (Selbo et al., 2000a, 2000b) and non-viral gene delivery systems (Berg et al., 1999; Hogset et al., 2002) in vitro. In the near future, we will explore the use of PCI for the cytosolic delivery of liposomal macromolecules.

5. Conclusion

Despite the ability of targeted liposomes to interact specifically with certain cell types, the cytosolic delivery of the liposomal drug contents is often inefficient, an observation which particularly holds true for macromolecular compounds like DNA and proteins. After cellular uptake via endocytosis, the liposomes will enter the acidic lysosomal compartment in which the liposomes with their entrapped drugs are degraded. Here we have described several approaches to improve the cytosolic delivery of therapeutic macromolecules entrapped in liposomes.

Co-encapsulation of the fusogenic peptide diINF-7 into immunoliposomes enables the cytosolic delivery of liposomal proteins. Although surface modification of liposomes with the cell-penetrating peptide TAT did

not lead to translocation of the liposome particles over the plasma membrane, the cellular uptake via endocytosis was greatly enhanced compared to non-modified liposomes. In combination with endosomal escape enhancers, like the diINF-7 peptide, this system could be advantageous. Furthermore, the recent developed technique photochemical internalization may also prove useful for the cytosolic delivery of liposomal macromolecules.

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