

TAT PEPTIDE-MODIFIED LIPOSOMES FOR INTRACELLULAR DELIVERY OF DRUGS AND DNA

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Intracellular delivery of drugs and DNA is hampered by the cytotoxicity of some carrier systems (such as cationic lipids) and degradation of drugs or DNA as a result of endocytic processing. It has been shown that the coupling of HIV-1 TAT protein, or shorter TAT peptides derived from this protein, to other proteins and small colloidal particles facilitates their efficient intracellular delivery [1] by direct translocation through the cell membrane, bypassing thus the endocytic pathway. Recently, we have demonstrated that it is possible to attach a large number of TAT peptide molecules (up to several hundreds) to the surface of a single liposome utilizing the new coupling method developed by us and involving the use of p-nitrophenylcarbonyl-activated polyethylene glycol-phosphatidyl ethanolamine (pNP-PEG-PE) [2]. This new amphiphilic PEG derivative readily incorporates into liposomes and micelles via its PE residue, and easily binds primary amino group-containing ligands via its water-exposed pNP groups, forming stable and non-toxic urethane (carbamate) bonds. The reaction between the pNP group and the ligand amino group proceeds easily and quantitatively at pH around 8.0, and remaining free pNP groups are promptly eliminated by spontaneous hydrolysis. Therefore, pNP-PEG-PE could serve as a very convenient tool for protein and peptide attachment to the distal ends of liposome-grafted or micelle-incorporated PEG chains.

To achieve an efficient intracellular delivery of drugs and DNA by targeting microparticulate drug carriers directly into the cytoplasm bypassing the endocytotic pathway, we have used pNP-PEG-PE-based technology to prepare TAT peptide-bearing liposomes. We demonstrated that relatively large drug carriers, such as 200 nm liposomes, could also be delivered into cells by TAT peptide attached to the liposome surface [3]. The incubation of fluorescently labeled rhodamine-PE (Rh-PE)-containing TAT liposomes with mouse Lewis lung carcinoma cells, human breast tumor BT20 cells, and rat cardiac myocytes H9C2 results in intracellular localization of certain liposomes. Steric hindrances for TAT peptide:cell interaction (attachment of TAT directly to the liposome surface without spacer or the presence of a high MW PEG on the liposome surface) abolish liposome internalization evidencing the importance of direct contact of TAT peptide with the cell surface. Low temperature or metabolic inhibitors, sodium azide or iodoacetamide, have little influence upon the translocation of TAT-liposomes into cells, confirming the energy-independent character of this process.

To additionally confirm the energy-independence of TAT peptide-mediated uptake, we have investigated the uptake by different cells of radiolabeled TAT peptide-liposomes, TAT peptide-IgG conjugates, and free TAT at various temperatures. It turned out that there is some temperature-dependence of the uptake of free TAT peptide as well as TAT peptide-IgG conjugate, especially at 37°C. This may be due to the fact that smaller molecules could also be taken by cells via a temperature-dependent spontaneous pinocytosis independent of the presence of TAT peptide, and thus their total uptake contains a temperature-dependent component. However, the uptake of TAT peptide-liposomes (which are too large to be pinocytosed) does not depend on the temperature and clearly represents an energy-independent phenomenon.

Free fluorescently labeled dextran (FITC-dextran, MW 40,000) shows only minimal intracellular accumulation, while Rh-labeled TAT peptide-liposomes loaded with FITC-dextran effectively translocate into cells. During this process and within first 4 hours of incubation, liposomes remain intact as evidenced by intracellular co-localization of Rh and FITC fluorescence. It is interesting to note that during the first hour of the intracellular residence, TAT peptide-liposomes demonstrate diffuse cytoplasmic distribution. However, after 2 and 4 hours of incubation, TAT peptide-liposomes are seen clustered in the peri-nuclear region with reduced cytoplasmic distribution. At 9 and 24 hour points, the degradation of liposomes can be seen (diffuse orange/red fluorescence in the cytoplasm and nucleus) with some liposomes still remaining in the peri-nuclear region after 9 hours. However, by this time FITC-dextran is almost totally released from liposomes into the cytoplasm.

To investigate the ability of TAT peptide-liposomes to deliver DNA into cells, we have prepared liposomes containing a sub-toxic amount of a positively charged lipid (less than 10% mol of DOTAP compared to compositions with more than 50% mol of DOTAP used for lipofection). These liposomes were loaded with a plasmid encoding for the Green Fluorescent Protein (GFP) and modified with TAT peptide. The incubation of such liposomes with various cells resulted in an efficient transfection of all cell types (the appearance of bright green fluorescence of GFP in the cell cytoplasm), while no or very little transfection was seen when cells were incubated with free plasmid or with TAT-free plasmid-loaded liposomes of similar composition. None of cell types used showed any signs of toxicity of our liposomal preparation, while under similar conditions the lipofection process has provoked a strong, though temporary, cell reaction (detachment, shape loss, etc.).

Although the exact mechanism of TAT peptide-mediated binding and uptake is still not completely understood, our experiments clearly show that the uptake of TAT peptide-liposomes does not depend on the temperature, indicating that the process is energy-independent. The temporal changes in the intracellular distribution show that the TAT-conjugation allows for peri-nuclear accumulation of liposomes, effectively bypassing the endocytic pathway. The use of TAT peptide-conjugated conventional and sterically-protected liposomes offers very

promising delivery systems for drugs and especially DNA in various protocols of local treatment or *ex vivo* cell modification.

REFERENCES

1. Schwarze, S.R., Ho, A., Vocero-Akbani, A. and Dowdy, S.F. In vivo protein transduction: delivery of a biologically active protein into the mouse. **Science** 285 (1999) 1569-1572.
2. Torchilin, V.P., Levchenko, T.S., Lukyanov, A.N., Khaw, B.A., Klibanov, A.L., Rammohan, R., Samokhin, G.P. and Whiteman, K.R. p-Nitrophenylcarbonyl-PEG-PE-liposomes: fast and simple attachment of specific ligands, including monoclonal antibodies, to distal ends of PEG chains via p-nitrophenylcarbonyl groups. **Biochim. Biophys. Acta - Biomembranes** 1511 (2001) 397-411.
3. Torchilin, V.P., Rammohan, R., Weissig, V. and Levchenko, T.S. TAT peptide on the surface of liposomes affords their efficient intracellular delivery even at low temperature and in the presence of metabolic inhibitors. **Proc. Natl. Acad. Sci. USA** 98 (2001) 8786-8791.