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Extended Abstract

Euro Biotechnology 2018: Next step in drug delivery: getting inside cells and to individual organelles- Vladimir P Torchilin- Northeastern University

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Abstract

Many pharmaceutical agents need to be delivered intracellularly to exert their therapeutic action onto specific cell organelles for maximum therapeutic outcome. It is true for preparations for gene and antisense therapy (target cell nuclei), proapoptotic drugs, (target mitochondria or lysosomes); or enzymes for therapy of storage diseases (target lysosomes). Intracellular delivery of different biologically-active molecules is one of the key problems in drug delivery in general. In some cases, intracellular drug delivery allows to overcome serious problems, such as multifrug resistance in cancer. The problem however is that the lipophilic cell membranes restrict the direct intracellular penetration of various extracellular compounds. Under certain circumstances, these molecules or even small particles can be taken from the extracellular space into cells by the receptor-mediated endocytosis, but any molecule/particle entering the cell via the endocytic pathway becomes entrapped in endosome and eventually ends in the lysosome, where active degradation takes place. As a result, only a small fraction of intact therapeutic substance appears in the cell cytoplasm and performs its function.

So far, multiple and only partially successful attempts have been made to bring various drugs and drug-loaded pharmaceutical carriers directly into the cell cytoplasm, bypassing the endocytic pathway, to protect drugs and DNA-related substances from the lysosomal degradation. Various noninvasive methods, such as the use of pH-sensitive carriers, including pH-sensitive liposomes (which under the low pH inside endosomes destabilize endosomal membrane, liberating the entrapped drug into the cytoplasm) and cell-penetrating molecules are currently applied to solve the problem.

In many cases, to increase the stability of administered drugs, improve their efficacy, and decrease side effects, various pharmaceutical nanocarriers are used. Among the most popular and well-investigated nanocarriers are liposomes, polymeric micelles, solid lipid or polymeric nanoparticles, dendrimers and some others.

Pharmaceutical nanocarriers have already a great history with quite a few of them being approved for clinical use and even more being under clinical trials.

Currently, multiple attempts are under way to make pharmaceutical nanocarriers multifunctional, i.e. capable of simultaneous or sequential performing of several functions, such as for example, a specific recognition of a target cell and endosomal escape.

Clearly, the ability to target individual cell organelles would be a highly desirable property.However, the specific subcellular delivery of bioactive molecules is still a challenging issue. One possible approach is the conjugation of a drug molecule or, better, a drug-loaded pharmaceutical nanocarrier with another compound having a specific affinity toward the organelle of interest.

Among cell organelles causing the greatest interest in terms of specific targeting, one can name lysosomes and mitochondria. Thus, the use of lysosome-targeted pharmaceutical nanocarriers may significantly improve the delivery of therapeutic enzymes and chaperones into defective lysosomes for the treatment of lysosomal storage disorders, while specific delivery of certain drugs to mitochondria may be helpful in therapy of various diseases, including neurodegenerative and neuromuscular ones, obesity, diabetes, ischemic-reperfusion injury, and cancer.

Some recently described approaches can illustrate current efforts in this direction. Thus, the aim of some of these studies was to achieve a specific intracellular targeting of lysosomes, by using liposomal nanocarriers modified with various lysosomotropic ligands, of which the octadecyl-rhodamine B (RhB) was proved to be the most efficient one.

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To follow the intracellular fate of liposomes, they were loaded with a model compound, fluorescein isothiocyanate (FITC)-dextran (FD) or with 5-dodecanoylaminofluorescein di-β-d-galactopyranoside (C(12)FDG), a Particular substrate for the intralysosomal β-galactosidase. The release of these liposomes and their content to lysosomes in HeLa cells was investigated by confocal microscopy, flow cytometry, and subcellular fractionation. Confocal microscopy displayed that RhB-liposomes co-localize well with the specific lysosomal markers, unlike plain liposomes. The comparability of the FITC fluorescence of the lysosomes isolated by subcellular fractionation also showed that the efficiency of FD delivery into lysosomes by RhB-modified liposomes was significantly higher compared to plain liposomes. These results were confirmed by the flow cytometry of the intact cells treated with C(12)FDG-loaded liposomes that alsoshowed increased lysosomal targeting by RhB-modified liposomes. Thus, the modification of the liposomal surface with a lysosomotropic ligand, such as octadecyl-RhB, can considerably increase the delivery of liposomal loads to lysosomes.

Another mitochondria-specific ligand, triphenylphosphonium (TPP), was used to target mitochondria with dendrimers. Dendrimers have emerged as promising carriers for the delivery of a wide variety of pay-loads including therapeutic drugs, imaging agents and nucleic acid materials into biological systems. The authors aimed to develop a novel mitochondria-targeted generation 5 poly(amidoamine) (PAMAM) dendrimer (G(5)-D). To achieve this goal, the TPP was conjugated onto the surface of the dendrimer. A fraction of the cationic surface charge of G(5)-D was neutralized by partial acetylation of the primary amine groups. Next, the mitochondria-targeted dendrimer was synthesized via the acid-amine-coupling conjugation reaction between the acid group of (3-carboxypropyl)triphenyl-phosphonium bromide and the primary amines of the acetylated dendrimer (G(5)-D-Ac). These dendrimers were fluorescently labeled with FITC to quantify cell association by flow cytometry and for visualization under the confocal laser scanning microscopy to assess the mitochondrial targeting in vitro. The newly developed TPP-anchored dendrimer (G(5)-D-Ac-TPP) was efficiently taken up by the cells and demonstrated good mitochondrial targeting. This mitochondria-targeted dendrimer-based nanocarrier could be useful for imaging as well as for selective delivery of bio-actives to the mitochondria for the treatment of diseases associated with mitochondrial dysfunction.

The interest towards the intracellular organelle-specific targeting resulted also in the development of new methods to follow the intracellular fate of organelle-specific nanocarriers. The control over intracellular distribution of pharmaceutical nanocarriers requires effective and noninvasive methods of their visualization inside cells. Thus, Raman microspectroscopy was applied to follow the mitochondrial association of three different types of cationic liposomes and it was shown the Raman microscopy allows the evaluation of the extent of mitochondrial association depending on the liposome composition.

Summing up, the organelle-specific targeting of pharmaceutical nanocarriers is becoming an important area in drug delivery and organelle-targeted preparations can significantly enhance many of current therapies.

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