Next step in drug delivery: getting to individual organelles

Vladimir P. Torchilin

Published online: 11 October 2012
© Controlled Release Society 2012

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 (targets cell nuclei), proapoptotic drugs (target mitochondria or lysosomes), or enzymes for therapy of storage diseases (target lysosomes) [1]. 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 multidrug 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 [2], 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 [3] (which under the low pH inside endosomes destabilize endosomal membrane, liberating the entrapped drug into

the cytoplasm) and cell-penetrating molecules [4], are currently applied to solve the problem.

Another important issue is that even after being delivered into the cell cytoplasm, active drugs still have to find their way to specific organelles (nuclei, lysosomes, and mitochondria), where they are expected to utilize their therapeutic potential in full.

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 already have a great history with quite a few of them being approved for clinical use and even more being under clinical trials [5].

Currently, multiple attempts are under way to make pharmaceutical nanocarriers multifunctional, i.e., capable of simultaneous or sequential performing of several functions, such as a specific recognition of a target cell and endosomal escape [6].

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 [7], while specific delivery of certain drugs to the mitochondria may be helpful in therapy of various diseases, including

V. P. Torchilin (✉)
Center for Pharmaceutical Biotechnology and Nanomedicine,
Department of Pharmaceutical Sciences, Northeastern University,
360 Huntington Avenue, The Fenway Room 214,
Boston, MA 02115, USA
e-mail: v.torchilin@neu.edu

neurodegenerative and neuromuscular ones, obesity, diabetes, ischemic–reperfusion injury, and cancer [8, 9].

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 [10]. To follow the intracellular fate of liposomes, they were loaded with a model compound, fluorescein isothiocyanate (FITC)-dextran (FD) or with 5-dodecanoylamino fluorescein di- β -D-galactopyranoside (C(12)FDG), a specific substrate for the intralysosomal β -galactosidase [11]. The delivery of these liposomes and their content to lysosomes in HeLa cells was investigated by confocal microscopy, flow cytometry, and subcellular fractionation. Confocal microscopy demonstrated that RhB-liposomes co-localize well with the specific lysosomal markers, unlike plain liposomes. The comparison 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 additionally confirmed by the flow cytometry of the intact cells treated with C(12)FDG-loaded liposomes that also demonstrated increased lysosomal targeting by RhB-modified liposomes. Thus, the modification of the liposomal surface with a lysosomotropic ligand, such as octadecyl-RhB, can significantly increase the delivery of liposomal loads to lysosomes.

A novel mitochondria-targeted liposomal drug delivery system was prepared by modification of the liposomal surface with a newly synthesized polymer, rhodamine-123 (Rh123)-PEG-DOPE, inserted into the liposomal lipid bilayer [12]. This novel polymer was synthesized by conjugating the mitochondriotropic dye Rh123 with the amphiphilic polyethylene glycol-phosphatidylethanolamine conjugate. The modified liposomes showed better uptake by cells (HeLa, B16F10) as was estimated by the fluorescence microscopy and FACS analysis. The co-localization study with stained mitochondria as well as with the isolation of the mitochondria of the cultured cells after their treatment with Rh123 liposomes showed a high degree of accumulation of the modified liposomes in the mitochondria. Mitochondrial-targeted paclitaxel (PCL)-loaded liposomes demonstrated enhanced cytotoxicity toward cancer cells compared with non-targeted drug-loaded liposomes or free PCL. Thus, Rh123-modified liposomes target the mitochondria efficiently and can facilitate the delivery of a therapeutic payload to the mitochondria.

Another mitochondria-specific ligand, triphenylphosphonium (TPP), was used to target mitochondria with dendrimers [13]. Dendrimers have emerged as promising carriers for the delivery of a wide variety of payloads 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)triphenylphosphonium 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 bioactives 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 the cells. Thus, Raman microspectroscopy was applied to follow the mitochondrial association of three different types of cationic liposomes [14] and it was shown that 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 the current therapies.

References

1. Torchilin VP. Recent approaches to intracellular delivery of drugs and DNA and organelle targeting. *Annu Rev Biomed Eng.* 2006;8:343–75.
2. Hymel D, Peterson BR. Synthetic cell surface receptors for delivery of therapeutics and probes. *Adv Drug Deliv Rev.* 2012;64(9):797–810. doi:10.1016/j.addr.2012.02.007.
3. Karanth H, Murth RS. pH-sensitive liposomes—principle and application in cancer therapy. *J Pharm Pharmacol.* 2007;59(4):469–83.
4. Koren E, Torchilin VP. Cell-penetrating peptides: breaking through to the other side. *Trends Mol Med.* 2012;18(7):385–93. doi:10.1016/j.molmed.2012.04.012.
5. Raj B. Nanoparticle-based therapeutics in humans: a survey. *Nanotechnol Law Bus.* 2008;5(2):135–55.
6. El-Sayed A, Masuda T, Akita H, Harashima H. Stearylated INF7 peptide enhances endosomal escape and gene expression of

- PEGylated nanoparticles both in vitro and in vivo. *J Pharm Sci.* 2012;101(2):879–82. doi:[10.1002/jps.22807](https://doi.org/10.1002/jps.22807).
7. Lachmann RH. Enzyme replacement therapy for lysosomal storage diseases. *Curr Opin Pediatr.* 2011;23(6):588–93. doi:[10.1097/MOP.0b013e32834c20d9](https://doi.org/10.1097/MOP.0b013e32834c20d9).
 8. Serviddio G, Romano AD, Cassano T, Bellanti F, Altomare E, Vendemiale G. Principles and therapeutic relevance for targeting mitochondria in aging and neurodegenerative diseases. *Curr Pharm Des.* 2011;17(20):2036–55.
 9. Gogvadze V. Targeting mitochondria in fighting cancer. *Curr Pharm Des.* 2011;17(36):4034–46.
 10. Meerovich I, Koshkaryev A, Thekkedath R, Torchilin VP. Screening and optimization of ligand conjugates for lysosomal targeting. *Bioconjug Chem.* 2011;22(11):2271–82. doi:[10.1021/bc200336j](https://doi.org/10.1021/bc200336j).
 11. Koshkaryev A, Thekkedath R, Pagano C, Meerovich I, Torchilin VP. Targeting of lysosomes by liposomes modified with octadecyl-rhodamine B. *J Drug Target.* 2011;19(8):606–14. doi:[10.3109/1061186X.2010.550921](https://doi.org/10.3109/1061186X.2010.550921).
 12. Biswas S, Dodwadkar NS, Sawant RR, Koshkaryev A, Torchilin VP. Surface modification of liposomes with rhodamine-123-conjugated polymer results in enhanced mitochondrial targeting. *J Drug Target.* 2011;19(7):552–61. doi:[10.3109/1061186X.2010.536983](https://doi.org/10.3109/1061186X.2010.536983).
 13. Biswas S, Dodwadkar NS, Piroyan A, Torchilin VP. Surface conjugation of triphenylphosphonium to target poly(amidoamine) dendrimers to mitochondria. *Biomaterials.* 2012;33(18):4773–82. doi:[10.1016/j.biomaterials.2012.03.032](https://doi.org/10.1016/j.biomaterials.2012.03.032).
 14. Chernenko T, Sawant RR, Miljkovic M, Quintero L, Diem M, Torchilin V. Raman microscopy for noninvasive imaging of pharmaceutical nanocarriers: intracellular distribution of cationic liposomes of different composition. *Mol Pharm.* 2012;9(4):930–6. doi:[10.1021/mp200519y](https://doi.org/10.1021/mp200519y).