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Review

Drug Delivery by TAT-technology

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TAT technology is a biochemical technique for introduction of full-length peptides or proteins into cells. This process occurs in a rapid, concentration-dependent fashion that appears to be independent of receptors and transporters. It has broad implications in experimental systems for regulating intracellular processes and has the potential to be used in the development of new therapeutic strategies for cancer, infectious diseases, and development of vaccines. It has been shown that different forms of TAT-p27 protein can modulate the cell cycle of cultured cell lines, depending on the concentration and type of cells. Transfer of TAT-proteins/peptides use from cell culture systems to animal disease models has been slow, but the ability of TAT conjugates to protect mice against ischemia, inhibit tumor growth, and enhance gene delivery suggests that they offer wide ranging pharmaceutical applications for treating a whole range of diseases.

Keywords
TAT-vector
protein transduction
drug delivery

INTRODUCTION

Different non-biological and biological carrier systems have been recently developed for delivery of drugs into cells. Liposomes are excellent potential carriers for some drugs, antisense oligonucleotides, ribozymes and therapeutic genes.[1] Nanoparticles and low-density lipoproteins (LDLs) are cell-specific transporters of drugs against macrofage-specific infections (HIV1).[2] The process of protein transduction, using the TAT-technology, allows the delivery of drugs and genetic materials into cells.[3,4]

A drug development challenge is how to overcome the unfavourable physicochemical nature of molecules such as proteins and oligonucleotides, which are unable to penetrate the cells membrane in order to reach their intracellular targets. Cell-penetrating peptides have been developed as a solution to this challenge; once conjugated to therapeutic molecules, these peptidic sequences facilitate intracellular delivery. Proteins and peptides are useful research and therapeutic tools; however, their applications are limited because delivery to the desired location is not easily achiveable.[5] Penetration into intracellular space can be achieved by adjusting hydrophilicity. Using this approach, small-molecule-based pharmacological agents have been successfully developed. But, it is difficult to modify hydrophilicity with proteins and peptides without influencing biological functions. TAT-protein from the human immunodeficiency virus contains a conserved cationic peptide sequence necessary for transduction across the cell membrane.[6] The transduction domain from the TAT protein is a good candidate for intracellular delivery of therapeutic macromolecules such as DNA and proteins. This technique may represent the next paradigm in the ability to modulate the cell function and offers a way for better treatment of diseases.

The importance of drug delivery is pivotal in the broad area of pharmacological research but this issue has not

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been solved to date. The main goal of every drug delivery system is the delivery of a precise amount of a drug to the desired location in order to achieve the drug concentration in the targeted organ required for effective treatment.[7] The key problem is still how to achieve curative doses in a pharmacologically active state in the desired target while avoiding side effect. The currently applied mechanisms for the transport of therapeutic molecules across biological membranes are still far from being efficient.

Cell penetrating peptides or proteins (CPP) offer a good opportunity for improving intracellular drug delivery. They have the ability to cross the plasma membranes of mammalian cells in an apparently energy- and receptor-independent fashion.[8] There is much debate over the mechanism by which this »protein transduction« occurs. CPPs can be rapidly translocated into cells, being used to deliver a broad range of therapeutics-including proteins, DNA, antibodies, oligonucleotides in a variety of situations and biological systems.[8] Moreover, structure-activity studies indicate that the internalization of CPPs does not depend on their specific primary sequence, which implies independence of receptor recognition.[9,10] Based on these results, it has been commonly accepted that the internalization of CPPs does not involve endocytosis or specific protein transporters. Instead, direct transport through the lipid bilayer of membranes has been suggested as a possible mechanism of translocation.[11]

Intracellular Delivery by TAT Vector

It is important for intracellular protein delivery to conjugate the cargo proteins with a delivery vector (Figure 1). The HIV1 transcriptional activator TAT is a multifunctional protein that is transported in and out of the cells.[12] This cell penetrating property relies on the integrity of a highly basic arginine-rich sequence (aa 49–58). After conjugation of TAT peptides with a range of macromolecules, it can facilitate cellular entry of the conjugate under *in vitro* (fluorochromes, enzymes, antibodies and liposomes)[13–18] and *in vivo* conditions.[19] The procedure of intracellular delivery is very simple. The conjugates or fusion proteins bearing a peptide vector are added to the culture medium to give a final concentration between 0.1 and 10 μM, in dependence on the cargo. Internalization is established within 5–30 min in almost all cells. It has been reported that TAT peptide does not produce any apparent cytotoxicity.[20] Using this methodology, various cellular functions have been modulated by introduction of bioactive proteins into cultured cells. These include modulation of signal transduction, cell cycles, apoptosis induction, cell migration and proliferation, cytoskeletal reorganization, *etc*.[20–22] Also, application of this methodology in *in vivo* therapy has been reported. Intraperitoneal injection of a fusion protein of TAT peptides and β-galactosidase into mice resulted in the delivery of the fusion protein to various organs (liver, kidney, lung, brain) while exerting its enzymatic activity.[23] Similarly, suppression of cancer by the delivery of the p53 (Ref. 24) protein and prevention of ischemic brain injury by administration of an antiapoptotic Bcl-X_L-related protein have been reported.[25] Injection of the TAT-Bcl-X_L protein decreased cerebral infarction in a dose dependent manner and altered ischemia-induced caspase-3 activation in ischemic neurons.[26] It was shown on the same model that brain tissue was progressively transduced with TAT-Bcl-X_L within 3–4 h after intravenous delivery and reduced infarct volume and neurological deficits after long ischemic insults. Further, TAT-Bcl-X_L decreased the number of caspase-3-reactive and DNA fragmentized cells and increased the number of viable neurons in the striatum.[27] The same TAT-Bcl-X_L construct, when injected into the eye, prevented 24 % of mouse retinal ganglion cells from undergoing retrograde neuronal apoptosis caused by an optic nerve lesion.[28] Thus, TAT-fusion proteins could facilitate protective therapy strategies for neurological disorders. TAT-technology is also applicable in vaccination strategies. Viehl *et al.*[29] transduced dendritic cells with TAT-Her2/*neu* fusion protein. After intraperitoneal injection of mice, those cells migrated in all secondary lymphoid organs. Immunized mice developed palpable tumors much later than control mice. Similarly, immunized mice had smaller resulting tumors than the control mice.

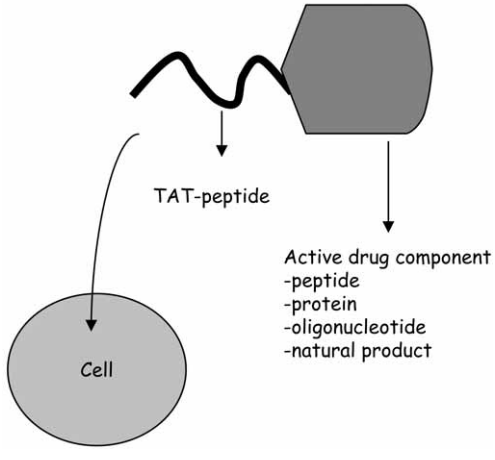

Figure 1. Scheme of intracellular delivery of TAT fusion proteins.

Mechanism of Cellular Uptake

Facile and efficient delivery of drugs into cells has aroused considerable interest in the internalization mechanism. Internalization of the TAT and related peptides does not appear to be dependent on classical endocytosis, cell type, receptors or active transporters.[30] It seems that macropinocytosis is involved in the process of internalization of TAT fusion proteins.[31-35] Macropinocytosis is a pathway of cellular uptake in which polimerization of actin filaments makes the plasma membrane thrust out of the cell

surface, resulting in the uptake of extracellular fluid.[31] This procedure is contrary to that of classical clathrin endocytosis, which is initiated by the formation of concave structures on the plasma membranes. Diameters of clathrin endosomes are estimated to be less than 120 nm whereas those of the macropinosomes often exceed 1 μm. This large size seems a plausible explanation of the cellular uptake of TAT-fusion proteins/peptides. Cellular uptake of TAT-fusion proteins is also inhibited in the presence of the macropinocytosis inhibitor amyloride as well as cytochalasin D, which prevent actin polymerization.[31,32] Cell surface glycosaminoglycans such as heparan sulfate proteoglycan are another factor important for cellular uptake of arginine peptides (positively charged).[35] Electrostatic interaction of the positively charged peptides and negatively charged proteoglycan will make these peptides condense on the cell surface, which could eventually accelerate the cellular uptake of these peptides.

TAT Technology in Modulation of Signal Transduction Pathways

To modulate signal transduction pathways responsible for proliferation and apoptosis, three forms of protein p27 were transduced into different cell lines.[36] Transduction of TAT-p27 fusion proteins affected the proliferation of human tumor cell lines, depending on the type of protein and cell line. It was also shown that some proteins responsible for the cell cycle regulation were affected. P27 protein as a key regulator of cell cycle progression induced apoptosis in tumor cell lines, in dependence on the type of cells and the form of p27 protein. The obtained results seem promising for the use of TAT technology in treatments of tumors and similar diseases.

CONCLUSIONS

TAT technology has broad implications for experimental systems and a potential to be used as a delivery tool in cancer therapy, therapy of infectious diseases, *etc*. However, application of peptide and protein based therapeutics still requires a lot of work to be done, specifically in the fight against cancer and infectious diseases.

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SAŽETAK

Primjena lijeka pomoću TAT-tehnologije

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TAT tehnologija je biokemijska metoda za unošenje cijelih peptida ili proteina u stanice. Proces ulaska je vrlo brz, ovisan o koncentraciji, a čini se neovisnim o receptorima i transporterima. Ima veliku primjenu u eksperimentalnim sustavima za regulaciju staničnih procesa, a može se također primijeniti u razvoju novih terapijskih pristupa liječenju tumora, infektivnih bolesti te u razvoju vakcina. Pokazalo se da različiti oblici fuzijskog proteina TAT-p27 mogu modulirati stanični ciklus humanih tumorskih stanica u kulturi, ovisno o koncentraciji i tipu stanica. Primjenom TAT-proteina/peptida kod bolesti na životinjskim modelima pokazala se sposobnost TAT konjugata za zaštitu miševa od ishemije, inhibiciju rasta tumora i povećani unos gena, što ukazuje da ta metoda omogućuje široku farmaceutsku primjenu u liječenju raznih oboljenja.