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Review Article

NANOTECHNOLOGY: A PROMISING CARRIER FOR INTRACELLULAR DRUG DELIVERY SYSTEM

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ABSRACT
Nanotechnology is on its way to make a big impact in Biotech, Pharmaceutical and Medical diagnostics sciences. Nanotechnology holds a tremendous potential when it applied in the fields of drug delivery. In this review it has been discussed how nanotechnology can implemented to design formulations which can effectively carry drug molecule to the targeted cell organelles. Introduction of certain functional groups or addition of surface active agents may alter the characteristics of the carrier molecule, thus increasing the sensitivity to site selection of the carrier. It has been predicted that in the near future, nanoparticles with the ability of carrying multiple drug molecules, will be designed. They can maintain the delivery of drugs at specific time interval.

Key-words: Nanocarriers, nanocrystals, intra cellular, liposome, peptides.

INTRODUCTION

The term "nanotechnology" is now commonly used to refer to the creation of new objects with nanoscale dimensions between 1.0 and 100.0 nm. Functionalities can be added to nanomaterials by interfacing them with biological molecules or structures. The size of nanomaterials is similar to that of most biological molecules and structures. Therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications. It can be predicted that over the next five years, Nanotechnology-enabled drug delivery systems (DDS) will dramatically reshape the way of existing drugs delivery. The growing range of nanotechnology enabled drug delivery methods is destined to change the way new compounds are formulated, and to extend the life cycle of existing compounds. The benefits of nanotech-based drug DDS will be dramatic. Nanotechnology can help to reproduce or to repair damaged tissue. This so called "tissue engineering" makes use of artificially stimulated cell proliferation by using suitable nano-material based scaffolds and growth factors. By the implementation of nanotechnology, much cheaper and safer formulation can be developed. Diseases, which are cured by the treatment with antibiotics for several weeks, can be cured in only few minutes. The toxicity or adverse effects of chemotherapy are due to the fact that the administered drugs are non-target specific, and damage many healthy body cells along with the diseases ones. Efforts are being made to develop nanotechnology based delivery vehicles that can enhance the circulatory persistence of drugs and/or target drugs to specific cells. Much of the cell's surface machinery and intracellular organelles operate at the nanolevel. These are regulation the actions of messenger molecules such as hormones and mediators; maintaining ionic stability; and manufacturing a wide variety of crucial building blocks for the body. Small nanosized molecules such as sugars and peptides (1–10 nm) 'dock' into larger nanosized molecules (10–100 nm) to mediate specific functions, or are processed further through the active sites of receptors or enzymes. Recently, researchers have been designing nanoparticles in the size of molecules that can be loaded with drug and targeted to the diseased or specific cells or even individual cellular organelles (such as lysosomes, mitochondria, or endoplasmic reticulum).[1, 2, 6]

Role of nanotechnology in intracellular drug delivery

Many pharmaceutical agents or biologically active molecules with therapeutic properties need to be delivered intracellularly to exert their therapeutic action inside cytoplasm or onto the nucleus or other organelles. Among such agents, there are preparations for gene and anti-sense therapy which target the nuclei; pro-apoptotic drugs, which ought to reach the mitochondria; lysosomal enzymes, for the lysosomal compartment; bacteria and virus as vaccines, and some others (figure 1). Intracellular drug delivery systems are designed to overcome certain important limitations for drug actions, such as multidrug resistance in cancer chemotherapy. So far, multiple and only partially successful attempts have been made to bring various low molecular weight and macromolecular drugs and drug loaded pharmaceutical carriers directly into the cell cytoplasm. These systems bypass the endocytic pathway to protect the durgs, or DNA from lysosomal degradation, thus enhancing drug efficacy or DNA incorporation into cell genome .

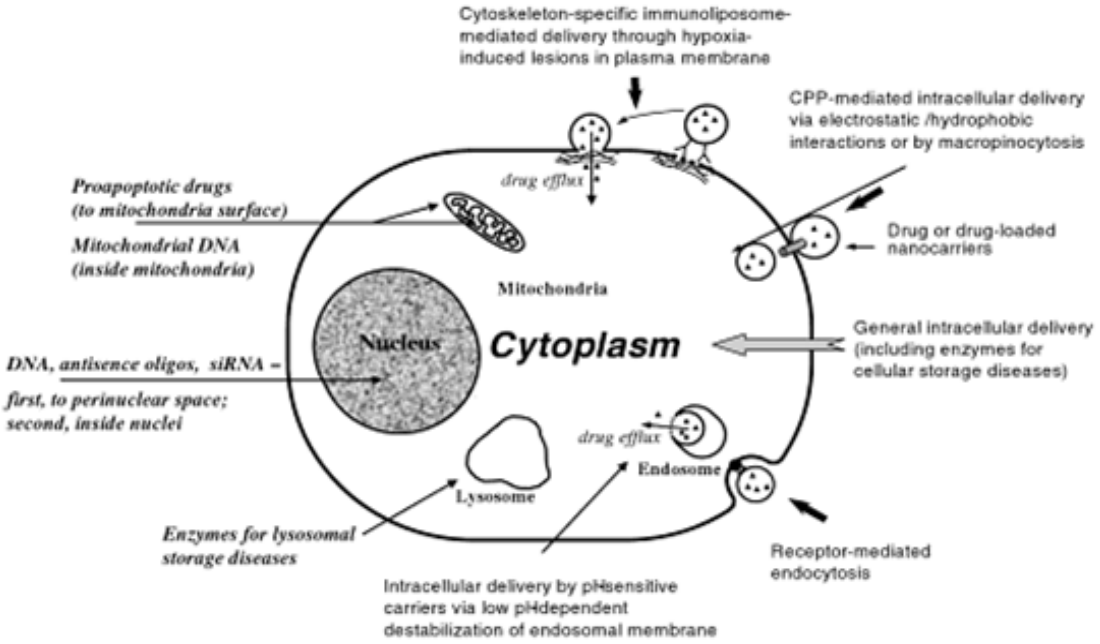

Fig.1: General scheme of intracellular drug delivery: types of diseases, organelles involved, and required drugs are shown together with possible delivery routes and protocols.

Even after being introduction in the cytoplasm, the drug carriers have to find their way in the specified cell organelles in order to deliver the drugs to achieve the desired therapeutic action. Various vector molecules promote the delivery of associated drugs and drug carriers inside the cells via receptor-mediated endocytosis. The steps involve the complexing of vector molecules, an associated drug and drug carrier to specific ligand on the surface of target cell membranes, followed by the energy –dependent formation of endosomes. The challenge faced is that any molecules being introduced in a cell via the endocytic pathway and becoming entrapped into endosome, later in the lysosome, resulting in the degradation of the drug under the action of numerous lysosomal enzymes. Thus, only a small concentration of the active drug appears in the cell cytoplasm. As a result, even if an efficient cellular uptake via endocytosis is observed, the discovery of intact peptides and proteins is compromised by an insufficient endosomal escape and subsequent lysosomal degradation.

Non-invasive methods for delivery of membrane-impermeable molecules are highly efficient and allow the endosomal escape of certain preparations. Enhanced endosomal escape can be achieved through the use of lytic peptides, pH sensitive polymers, or swell dendrites polymers. Such agents have yielded appreciable results in overcoming limitations of endocytosis-based cytoplasmic delivery, but there is still need for further improvement or consideration of alteration delivery strategies. Thus, the only way to deliver drug to individual cell organelles is to modify them with certain molecules demonstrating somewhat increased ability to associate with these organelles. In many cases, various pharmaceutical nanocarriers are used to increase the drug stability as well as improve their efficacy and bioavailability and also decrease adverse effects. Several attempts have been made to design the set of nanoparticulate drug carrier systems capable of specific delivery of various pharmaceuticals including pro-aptoptic anti-cancer drugs and DNA to intracellular targets, such as nuclei, lysosomes and mitochondria.[3,4,23]

Examples of such carrier systems are:

Nanoparticles

Nanoparticles can be classified under three categories:

i> Inorganic nanoparticles
ii> Solid-lipid nanoparticles
iii> Polymeric nanoparticles

Inorganic nanoparticles is the generic term for several nanoparticles including for example metal oxide and non-oxide ceramics, metals, calcium phosphate, gold, silicate and magnetic nanoparticles. So called "nanoshells" combine various inorganic elements or materials. They typically have a silicon core, which is sealed in an outer metallic cover.

Polymer nanoparticles involve various natural or biocompatible synthetic polymers. They include rationally designed macromolecular drugs, polymer-drug and polymer-protein conjugates, polymeric micelles containing covalently bounded drugs, and polyplexes for DNA delivery.

Solid lipid nanoparticles combine the advantages but avoiding the disadvantages of other colloidal carriers have attracted increasing attention in recent years, and are regarded as an alternative carrier system to traditional colloidal systems, such as emulsions, liposomes and polymeric microparticles and nanoparticles.

Nanoparticles are widely used in drug delivery where they can increase drug solubility and, also can lead to controlled release and/or drug targeting. They are used in anti-cancer treatment, gene delivery, asthma inhalers, hormone delivery through the skin, drug delivery through the eye and in oral and vaccine delivery systems.

Nanocrystals

Increasing the active surface area is the key to many applications of nanotechnologies, from improving automotive and industrial catalysts to improve the uptake of poorly soluble drugs in the human body. Nanocrystals are ground in special mills and the resulting drugs can be applied

intravenously as nanosuspensions or bronchially through an inhaler. This small size enhances the surface/volume-ratio and bioavailability of almost insoluble pharmaceuticals. Nanocrystals are small particles of drug substance, typically less than 1000 nanometers (nm) in diameter, which are produced by milling the drug substance using a proprietary, wet-milling technique. The resulting particles of the drug are stabilized against agglomeration by surface adsorption of selected stabilizers. This results is an aqueous dispersion of the drug substance
that behaves like a solution, which can be processed into finished dosage forms for all routes of administration. The size of the particles allows for safe and effective passage through capillaries. Nano-Crystal technology represents both an enabling technology for evaluating new chemical entities that exhibit poor water solubility and also a valuable tool for optimizing the performance of established drugs. Nano-Crystal technology is of particular benefit for drugs with poor solubility in water.

Polymer therapeutics

Polymer therapeutics differs from particle shaped drug delivery systems in their dimensions. They are molecular units with diameters of a few nanometres and can be subdivided into four groups:

• Polymer drugs
• Polymer drug conjugates
• Polymer micelles
• Dendrimers

A drug can be covalently bound to the four groups above. They differ from other drug-delivery systems in which a drug is encapsulated or solubilised and are more akin to new chemical entities since chemical conjugation occurs (i.e the combined polymer and drug behave as a compound different from either component). Early designs for a polymer therapeutic system involved attaching a water-soluble polymer to a drug through a selected linker molecule. Trapping low molecular weight drugs as polymer not only temporarily inactivates the drug, but also restricts their uptake by cells to endocytosis (the process whereby cells absorb material such as proteins from the outside by engulfing it with their cell membrane). As high molecular weight macromolecules of the drugs are unable to diffuse passively into cells, they are 'engulfed' as membrane-encircled sacs called vesicles, in which intracellular enzymes then set to work to release the drug. This means that the polymer-drug conjugate should be able to circulate longer in the body, potentially without the toxic side-effects associated with many drugs. With the appropriate biodegradable linker, and/or a cell-specific targeting group, it should be possible to deliver the drug directly to the target site.

Liposomes

Liposomes are small spheres that have a lipid layer that surrounds an active pharmaceutical ingredient. Their basic components are amphiphilic molecules, which spontaneously form liposomes in aqueous ambiance. Hydrophilic ends of the globular bilayers point to the waterside, hydrophobic ends are oriented bilateral to the centre of the layer.

Because a liposome can encapsulate an aqueous solution with a hydrophobic outer membrane, hydrophilic solutes cannot pass through the lipids. So, lipsomes can carry both hydrophobic molecules (its outer membrane) and hydrophilic molecules (the inner aqueous core). The liposome delivers its contents to the appropriate site by having its lipid bilayer fuse with bilayers of the cell membrane. By making liposomes in a solution of DNA or drugs (which would normally be unable to diffuse through the membrane) they can be delivered past the lipid bilayer. Liposomes can also be designed to deliver drugs in other ways. Liposomes that contain low (or high) pH can be constructed such that dissolved aqueous drugs will be charged in solution. As the pH naturally neutralizes within the liposome the drug will also be neutralized, allowing it to freely pass through a membrane.[2, 6, 8]

Various approaches for intracellular drug delivery

pH Sensitive pharmaceutical Nanocarriers for Cystoloic delivery

For cystolic delivery of drugs and drug products and pharmaceutical nanocarriers such as liposomes & micelles have been developed.

a) pH sensitive liposomes

Liposome is made up of pH sensitive substance. After being endocytosed in the intact form, it fuses with the endovaculor membrane due to the lowered pH inside the endosome (below 6) and destabilizes the latter, releasing its contents directly into the cytoplasm .

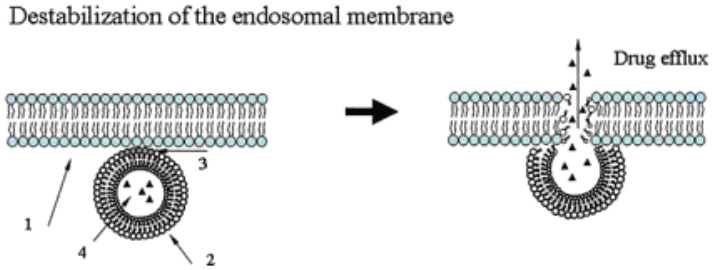

Fig. 2: Schematics of endosomal drug escape with the use of pH-sensitive liposomes. (1) endosomal membrane; (2) endocytosed liposome; (3) pH-sensitive liposomal component interating with the endosomal membrane and destabilizing it; (4) liposomal drug

The presence of fusogenic lipids in the liposomal composition, such as unsaturated DOPE, is required to make the liposome pH sensitive.[3, 8, 9, 10]

b) pH sensitive Micelles:

Targetting of intracellular organelles can be accomplished by loading the drug in micelles, which can demonstrate pH sensitivity and ability to escape from endosome. Thus, micelles is prepared from PEG-poly (aspartate hydrazone adriamycin) that can easily release an active drug at lowered pH value typical for endosomes and facilitate its cytoplasmic delivery and toxicity against cancer cells.[3, 11]

Cytoskeletal Antigen Specific Immunoliposomes for Intra-cytoplasmic Delivery: It is observed that sarcolemma lesion typical of hypoxic myocyte damage and the exposure of intracellular proteins, such as myosin, could be specially identified with the use of the antimyosin antibody. Moreover,

cytoskeletal antigen (myosin) specific immunoliposomes (CSIL) were shown to seal membrane lesions in hypoxis cardiocytes by anchoring CSIL to the exposed cytoskeletal antigen. The property was the basis of the fact that artificially imposed hypoxia could be applied for the promotion of intracellular delivery of various drugs. If target cells for drug and/ or gene delivery are exposed to artificially impose hypoxic stress, stress-induced small membrane lesions will allow for the specific attachment to these lesions of loaded liposome rendered specific for an intracellular antigen. [3, 12, 13, 14]

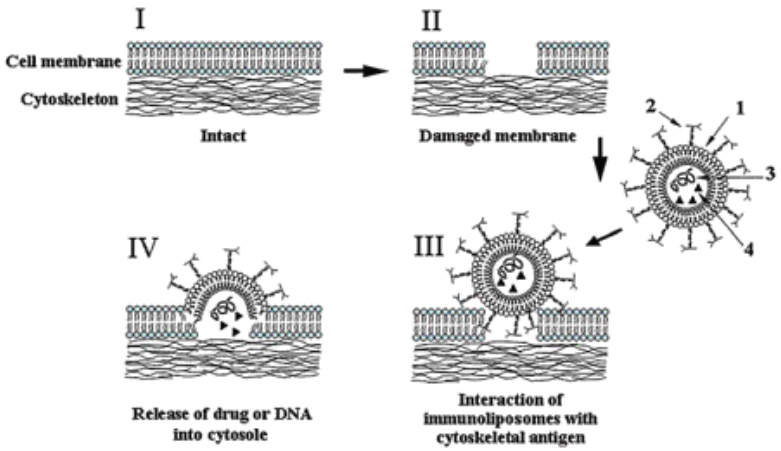

Fig. 3: Schematics of intracellular drug or DNA delivery with the use of cytoskeleton-specific immunoliposomes. When normal cell membrane (I) becomes compromised and "perforated" under the action of ischemia (II), drug/DNA-loaded immunoliposome attaches to the appearing "holes" via exposed cytoskeletal antigens (III), and, when fusing with the cell membrane, releases its contents into the cell cytoplasm. (1) Liposome; (2) cytoskeleton-specific antibody; (3) drug; (4) DNA

Intracellular delivery of Nanocarrier by Cell Penetrating Peptides: Certain protein or peptides can be attached to the hydrophilic drug of interest and together the complex possesses the ability to translocate across the plasma membrane and delivery the payload intracellularly. This process of translocation is called 'protein transduction". Such proteins or peptides contain domain of less than 20 amino acids, termed as cell-penetrating peptides (CPPs). These peptides have been used for intracellular delivery of various cargoes with molecular weights several times greater than their own. CPPs have been widely exploited for the intracellular delivery of different size cargoes in a range of cell types both in vitro and in vivo. The cargoes delivered range from peptides, proteins, genetic material, antibiotics, imaging agents and toxin to nanoparticles and liposomes. CPPs have also been used to enhance the delivery of genes via solid-lipid nanoparticles (SLN). [3, 15, 16, 17]

Lipoplexes / Polyplexes for cytosolic delivery of DNA: Plasmid DNA can be covered with lipids in an organized structure like a micelle or a liposome. When the organized structure is complexed with DNA, it is called a lipoplex. There are three types of lipids, anionic (negatively charged), neutral, or cationic (positively charged). Initially, anionic and neutral lipids were used for the construction of lipoplexes for synthetic vectors. However, in spite of the facts that there is little toxicity associated with them, that they are compatible with body fluids and that there was a possibility of adapting them to be tissue specific; they are complicated and time consuming to produce so attention was turned to the cationic versions.

The positively charged cationic lipids form complex with the negatively charged DNA. Also as a result of their charge they interact with the cell membrane, endocytosis of the lipoplex occurs and the DNA is released into the cytoplasm. The cationic lipids also protect against degradation of the DNA by the cell.

The most common use of lipoplexes has been in gene transfer into cancer cells, where the supplied genes have activated tumor suppressor control genes in the cell and decrease the activity of oncogenes. Recent studies have shown lipoplexes to be useful in transfecting respiratory epithelial cells, so they may be used for treatment of genetic respiratory diseases such as cystic fibrosis.

Complexes of polymers with DNA are called polyplexes. Most polyplexes consist of cationic polymers and their production is regulated by ionic interactions. One large difference between the methods of action of polyplexes and lipoplexes is that polyplexes cannot release their DNA load into the cytoplasm, so to this end, co-transfection with endosome-lytic agents (to lyse the endosome that is made during endocytosis, the process by which the polyplex enters the cell) such as inactivated adenovirus must occur. However polymers such as polyethylenimine have their own method of endosome disruption such as chitosan and trimethylchitosan.

New approaches in using nanocarriers for DNA delivery include the application of bimetallic nanorods that can simultaneously bind compacted DNA plasmid and targeting ligands in a spatial defined manner.[18, 19]

Nanocarrier for Targetting Lysosomes: Liposomes are suggested as pharmaceutical nanocarriers for replacement enzymes, which could protect them from the inactivation in vivo. Therefore, they enhance their intracellular delivery and transport into lysosomes. The potential ability of liposome-encapsulated enzymes to enter the cytoplasm or lysosomes of liver cells is of primary importance for the treatment of inherited diseaseas which are caused by the abnormal functioning of some intracellular enzymes. Enzymes for the therapy of lysosomal storage diseases include glucocerebrosidase, various glucosidases, phenylalanine ammonia lyase and some others.[20, 21]

Drug and DNA Delivery to / into mitochondria with Mitochondria Specific Nanocarriers: The membrane potential of mitochondria in vitro is between 180 and 200 mV, which is the maximum a lipid bilayer can sustain while maintaining its integrity. Therefore, positively charged molecules are attracted by mitochondria in response to the highly negative membrane potential. Although majority of charged molecules could not enter the mitochondrial matrix, certain amphiphile compounds are able to cross both mitochondrial membranes. They tend to accumulate in the mitochondrial matrix. It has long been known that amphiphile compounds with delocalized cationic charges accumulated in mitochondria. Mitochondria-specific drug delivery have yield good results in case of neuro-degenerative diseases, obesity, diabetes, and cancer. [3, 22]

CONCLUSION

Nanotechnology is fast developing area and has a great deal of future prospect, particularly in drug delivery. It has many challenges to overcome from technological and regulatory standpoints. However the potential benefits to improving human health, across a wide range of applications, are enormous. Though Nanotechnology is still in its early stages, it is greatly observed that nanoparticles are promising tools for the advancement of targeted drug delivery. The safety of many of the proposed nano-technological systems is yet to be determined. Key factors that may influence the outcome of human safety studies with nanotechnology-based drug delivery systems will include factors such as biodegradability and effective clearance by the body as well as evasion of immunological recognition. Many of these challenges should be tackled with care as nanotechnology products continue to be trialed and assessed in humans.

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