4. ROLE OF NANOTECHNOLOGY IN DEVELOPING ANTIVIRAL AGENTS

Introduction

Nanotechnology is the creation and utilization of materials, devices, and systems through the control of matter on the nanometer-length scale, i.e., at the level of atoms, molecules, and supramolecular structures. Nanotechnology, as defined by the National Nanotechnology Initiative (http://www.nano.gov/), is the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications. Encompassing nanoscale science, engineering and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale. It is the popular term for the construction and utilization of functional structures with at least one characteristic dimension measured in nanometers – a nanometer is one billionth of a meter (10^{-9} m). Nanobiotechnology is described in more detail in a special report on this topic (Jain 2007b).

Given the inherent nanoscale functional components of living cells, it was inevitable that nanotechnology will be applied in biotechnology giving rise to the term nanobiotechnology. Nanotechnology is applied to the study of viruses as a virus is ~100 nm in size. Role of nanobiotechnology in virology is shown in Table 4-1. Role of nanobiotechnology in improving delivery of vaccines is described in the following chapter 5.

Table 4-1: Role of nanobiotechnology in virology

Refinement of viral diagnostics (detection of single viral particles)
Monitoring of antiviral therapy
Integration of therapeutics with diagnostics (personalized medicine)
Study of interaction of nanoparticles with viruses
Nanocoating for local viricidal effect
Improvement of antiviral agents delivery
Fullerenes as antiviral agents
Nanoviricide approach for destruction of viruses

Research on virus nanoparticles has provided cues to the regulation of cytoplasmic transport. Viruses that replicate their genomes in the nucleus make use of the microtubule and the actin cytoskeleton as molecular motors for trafficking toward the nuclear membrane during entry and the periphery during egress after replication. Analyzing the underlying principles of viral cytosolic transport will be helpful in the design of viral vectors to be used in research as well as human gene therapy, and in the identification of new antiviral target molecules (Dohner and Sodeik 2005).

Study of interaction of nanoparticles with viruses

Scanning surface confocal microscopy, simultaneous recording of high-resolution topography and cell surface fluorescence in a single scan enables imaging of individual fluorescent particles in the nanometer range on fixed or live cells. This technique has been used to record the interaction of single virus-like particles with the cell surface and demonstrated that single particles sink into the membrane in invaginations reminiscent of pinocytic vesicles (Gorelik et al 2002). This method provides a technique for elucidating the interaction of individual viruses and other nanoparticles, such as gene therapy vectors, with target cells.

Silver nanoparticles undergo a size-dependent interaction with HIV-1 and particles in the range of 1–10 nm attached to the virus (Elechiguerra et al 2005). The regular spatial arrangement of the attached nanoparticles, the center-to-center distance between nanoparticles, and the fact that the exposed sulfur-bearing residues of the glycoprotein knobs would be attractive sites for nanoparticle interaction suggest that silver nanoparticles interact with the HIV-1 virus via preferential binding to the gp120 glycoprotein knobs. Due to this interaction, silver nanoparticles inhibit the virus from binding to host cells, as demonstrated in vitro.

Nanoparticle antiviral agents

Fullerenes

Fullerene technology derives from the discovery carbon-60, a molecule of 60 carbon atoms that form a hollow sphere one nanometer in diameter. The molecule was named buckyball or fullerene. Fullerenes are entirely insoluble in water, but suitable functionalization makes the molecules soluble. Upon contact with water, under a variety of conditions, C60 spontaneously forms a stable aggregate with nanoscale dimensions (25-500 nm), termed nano-C60 that are both soluble and toxic to microorganisms. Initial studies on water-soluble fullerene derivatives led to the discovery of the interaction of organic fullerenes with DNA, proteins, and living cells. Subsequent studies have revealed interesting biological activity aspects of organic fullerenes owing to their photochemistry, radical quenching, and hydrophobicity to form one- to three-dimensional supramolecular complexes.

A series of bis-fulleropyrrolidines bearing two ammonium groups have been synthesized and their activities against HIV-1 and HIV-2 have been evaluated (Marchesan et al 2005). Two trans isomers were found to have interesting antiviral properties, confirming the importance of the relative positions of the substituent on the C60 cage. None of the compounds showed any inhibitory activity against a variety of DNA and RNA viruses other than HIV.

Cationic, anionic and amino acid-type fullerene derivatives have shown inhibitory effect against HIV-reverse transcriptase and HCV (Mashino et al 2005). Out of all derivatives of fullerenes, anionic fullerenes, were found to be the most active. All the tried fullerene derivatives were more active than the non-nucleoside analog of HIV-RT inhibitor. The effect of long alkyl chains on fullerenes was not significant; rather it depressed the inhibition strength. The two important targets for anti-HIV characteristics are the HIV-protease and HIV-reverse transcriptase. The molecular modeling experimental designs exhibit that C60-core could penetrate into hydrophobic binding site of HIV protease. However, the mechanism of this anti-HIV activity is through HIV-protease inhibition, which has not been experimentally demonstrated.

Nanoviricides

Nanoviricides (NanoViricides Inc) are polymeric micelles, which act as nanomedicines to destroy viruses. As defined by Nanoviricides Inc, "a nanoviricide is a polymeric single chemical chain with covalently attached ligands that specify the virus target. The antiviral spectrum of the drug is determined by the specificity of the set of ligands attached to the chain, in addition to other functionally important aspects inherent in the chemistries". Nanoviricide is designed to seek a specific virus type, attach to the virus particle, engulf or coat the virus particle, thereby neutralizing the virus's infectivity, destabilize and possibly dismantle the virus particle, and optionally it may also be made capable of attacking the viral genome thereby destroying the virus completely. Active pharmaceutical ingredients are optional and can be hidden in the core of the nanoviricide missile.

Role of micelles in nanopharmaceuticals

Micelles, biocompatible and flexible nanoparticles varying in size from about 20 to 200 nm in which poorly soluble drugs can be encapsulated, represent a possible solution to the delivery problems associated with such compounds and could be exploited to target the drugs to particular sites in the body, potentially alleviating toxicity problems. Cell membranes are one example of a micelle, a strong bilayer covering that is made of two sheets of lipid-based amphiphiles, molecules that have a hydrophilic, end and a hydrophobic end. Like two pieces of cellophane tape being brought together, the hydrophobic sides of the amphiphilic sheets stick to one another, forming the bilayered micelle. Polymeric micelles, sometimes referred to as "soft nanoparticles" in contrast to metal nanoparticles, can be synthesized and take any of the three basic shapes: globules, cylinders and sack-like vesicles. Traditionally, various metal nanoparticles have been attached to micelles to facilitate intracellular drug delivery, e.g. in cancer. Several academic institutions and companies are working on micelles for drug delivery.

pH-sensitive drug delivery systems can be engineered to release their contents or change their physicochemical properties in response to variations in the acidity of the surroundings. One example of this is the preparation and characterization of novel polymeric micelles (PM) composed of amphiphilic pH-responsive poly(N-isopropylacrylamide) (PNIPAM) or poly(alkyl(meth)acrylate) derivatives (Dufresne et al 2004). Maelor Pharmaceuticals (Newbridge, UK) is using micelle nanotechnology to entrap drugs inside polymer nanoparticles for delivery of effective concentrations of otherwise insoluble drugs to tissues. Micelles have also been used as non-viral vectors for delivery of DNA in gene therapy.

PreserveX™ Polymeric Micelles (QBI Life Sciences), with average diameter of 21 nanometers, are useful in working with difficult to handle proteins, such as the membrane proteins. These proteins reside on, near or embedded in cellular membranes and represent 70% of all known drug targets. In the presence of native cell membrane fractions, these micelles self-assemble and embed pieces of cellular membranes in the complex creating multiple particles each providing an environment similar to that of the native membrane. Solubilization of membrane proteins and associated lipids from membrane fractions result in stabilized micelle/protein/lipid complexes. Another QBI product, PreserveX™-QML-B Polymeric Micelles, contains a biotin label enabling the placement of the micelle/protein/lipid complex onto a solid support such as a protein microarray. Once immobilized with use of streptavidin, ligand binding or enzymatic activity can be determined, or the presence of the protein can be confirmed.

Some physicochemical characteristics common to polymeric micelles

Characteristics that distinguish polymeric micelles from other pharmaceuticals and biologicals are:

- They are conformationally flexible polymers, i.e. well defined non-particulate materials. The material product can be defined operationally (i.e. in terms of processes used to make it), and further can be characterized in terms of average result values of chemistries (e.g. average MW, and MWD, average number of ligands per chain, etc.).
- As a polymer, it is not possible to manufacture a single molecular weight (MW) species. However, it is generally possible to operationally define a molecular weight distribution of a production batch. The actual MW distribution can be characterized, but the result values are strongly dependent on the technique of measurement
- Single molecular chains with heterogeneous molecular sizes.
- Only polymer chemistries enable substantial attachment of ligand for blocking open sites.

Some limitations in physical characterization of polymeric micelles are:

- Amphiphilic materials with self-assembly limit the use of many standard procedures in molecular weight distribution experiment.
- They are mostly soluble in organic, aqueous as well as intermediate solvents leading to fractionation issues.
- As non-particulate materials they are difficult to characterize by optical microscopy or by SEM, TEM, and AFM.

Structure and function of nanoviricides

NanoViricides are polymeric micelles, which bind to multiple virus-surface-receptors as antiviral agents. They are different from any of the other micellar nanotechnologies as there are no metal particles attached and the micelles can penetrate the virus and bind to multiple sites for effective destruction of the virus.

Mechanism of action of NanoViricides

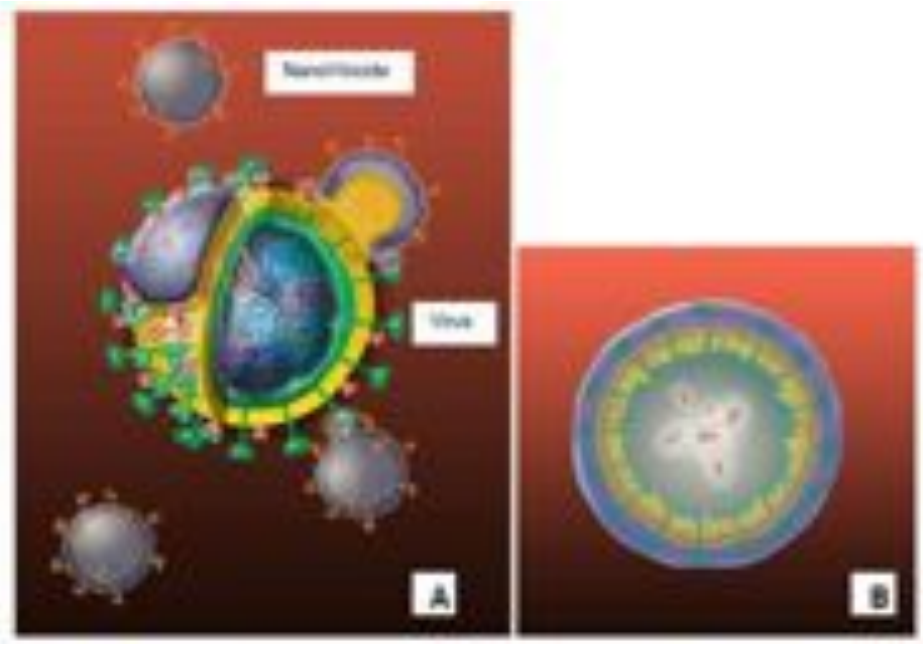
For a virus to infect a cell, it needs to bind to more than one site. For example, binding of HIV only to CD4 on T cells is insufficient to cause sustained disease; it needs HIV binding to at least two and possibly three different sites on the T cell and that too, at multiple points. For an antiviral to be effective, it should match the strategy to bind to more than one site on the virus. Ideally it should block all of these to prevent virus from infecting the cell and multiplying. Most of the current antiviral drugs have a single mechanism of action and block a single receptor. Drug combinations from different categories are required to increase the number of receptors blocked. Still this is not fully effective.

In contrast to other approaches, a NanoViricide™ micelle can recognize and bind to more than one type of binding site on the virus. The NanoViricide™ system enables design of a drug that binds to more than one type of site – currently as many as three different sites, on the virus – for a highly effective attack. NanoViricides Inc terms this as "multi-specific targeting".

A NanoViricide™ drug goes much further than just blocking all of the binding sites of the virus. The base material of a NanoViricide™ is a specially designed polymeric micelle material. It has the ability to disassemble an HIV particle by itself. Thus, after coating the virus particle, the NanoViricide™ loosens the virus particle, and weakens it. Some virus particles will even fall apart (uncoat). This provides a further therapeutic benefit. NanoViricides plans to enhance the viral disassembly capabilities of the NanoViricides™ by attaching specially designed "molecular chisels" to the NanoViricide™. Once the NanoViricide™ micelles coat the virus particle, the attached "molecular chisels" will go to work. They literally insert themselves into the virus coat at specific vulnerable points and pry apart the coat proteins so that the virus particle falls apart readily. The mechanism of action of NanoViricide is depicted schematically in Figure 4-1.

This description is a simplification. There is no fully adequate explanation of the observed efficacy because the mechanisms of action of nanomaterials as drugs and particularly, NanoViricides in vivo, are multiple and somewhat complex. Targets for this approach include influenzas, HIV, HCV, rabies and other viruses.

Figure 4-1: Schematic representation of NanoViricide attacking a virus particle



A: NanoViricide micelles attach to the virus at multiple points with nanovelcro effect and start engulfing the virus.
B. Flexible micelle coats and engulfs the virus particle, dismantles and neutralizes it, and fuses with viral lipid coat.
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Advantages of NanoViricides

NanoViricides have been compared to current approaches to viral diseases, which are seldom curative and some of the advantages include the following:

- Specific targeting of the virus with no metabolic adverse effects on the host.
- The biological efficacy of NanoViricides drugs may be several orders of magnitude better than that of usual chemical drugs. This in itself may limit the potential for mutant generation.
- There are also other key aspects of the design of NanoViricides that are expected to lead to minimizing mutant generation.
- Nanoviricides are safe because of their unique design and the fact that they are designed to be biodegradable within the body.
- The new technology enables rapid drug development against an emerging virus, which would be important for global biosecurity against natural as well as man-made (bioterrorism) situations. It is possible to develop a research drug against a novel life-threatening viral disease within 3-6 weeks after the infection is found, i.e. as soon as an antibody from any animal source is available.
- It is possible to make a single NanoViricide drug that responds to a large number of viral threats by using targeting ligands against the desired set of viruses in the construction of the drug. It is possible to "tune" the specificity and range (spectrum) of a NanoViricide drug within a virus type, subtype, or strain, by appropriate choices of the targeting ligand(s).

- The safety of NanoViricide drugs is proven now as they specifically attack the virus and not the host.
- A variety of formulations, release profiles and routes of administration are possible.
- Low cost of drug development, manufacture, distribution.

NanoViricide drug candidates are currently in preclinical studies. Clinical trials are planned. Initially injectable products are considered to be most effective but alternative routes of administrations such as nasal sprays and bronchial aerosols can also be developed. Various Nanoviricide products will be described further along with relevant viral diseases.

Advantages of Nanoviricides over vaccines are:

- Nanoviricides work where vaccines fail and are effective even when the immune system is impaired such as in AIDS.
- Nanoviricides work where effective vaccines are unavailable
- Sufficient short term protection for an individual outbreak cluster-
- Treatment can be started after infection
- No need to vaccinate whole world population for control of a viral epidemic

Advantages of Nanoviricides over immunoglobulin therapies are:

- Fully chemical, room-temperature stable NanoViricides can be made against many diseases
- Nanoviricides based on antibody fragment conjugates do not require humanized antibodies. Antibodies from virtually any source can be used for developing NanoViricides, thus significantly reducing time and cost of development.

Immunoglobulin therapies require the patient's immune system (complement system) to function well, which is often not the case in advanced disease states. NanoViricides function completely independently of the human immune system while accomplishing the same goal of reduction in viremia.

5. DELIVERY OF ANTIVIRALS

Introduction

Drug delivery is an important aspect of treatment of viral infections. For example, in case of new therapies, as well as for improving existing treatments, selective delivery of medications into liver cells would be desirable to enhance antiviral activity and avoid systemic side effects.

Methods of delivery of antiviral agents

Nucleoside analogues, together with nucleobases and nucleotide analogues, are commonly used in the treatment of viral infections. They act as antimetabolite agents and interfere with the synthesis of viral nucleic acids. However, the need of high doses due to the rapid elimination of these compounds, to their poor activation, and/or to their non-specific distribution, often leads to side effects and resistances. There is need for improving delivery of antiviral agents and to reduce side effects from excessive doses required to reach the affected area, which also damage healthy cells of the body. Methods of delivery of viral agents are shown in Table 5-1.

Table 5-1: Methods of delivery of antiviral agents

Local application
Skin, mucous membranes
Cornea
Systemic delivery
Oral
Injectables: subcutaneous, intramuscular, intravenous
Transdermal delivery: vaccines
Formulations for modifying pharmacokinetic properties of antiviral agents
Sustained release
Use of nanoparticles
Formulations to facilitate crossing of various barriers, e.g., blood-brain barrier
Organ specific targeted delivery
Liver-targeted drug delivery using prodrugs
Regional delivery
Portal circulation for hepatitis
Intrathecal, intraventricular or intracerebral for meningoencephalitis
Inhalation for viral infections of the respiratory system
Targeted systemic delivery for intracellular effect and binding to viruses
Nanoviricides

Local application of antivirals

Microbicidals for local application are used widely but they are usually antibacterial. There are few antiviral preparations for local application. The most commonly used are lotions against HSV-1 for application on lesions on lips.

Topical microbicides, as self-administered agents designed for vaginal use, which block transmission of HIV-1/AIDS, HSV-2/genital herpes and other sexually transmitted

infections at the mucosal surface, may provide a realistic method of intervention that could be distributed worldwide. An optimal microbicide should protect against infection but must also be safe, without adversely affecting the mucosal environment, including mediators of host defense. Thus, a critical component in microbicides development is to identify optimal assays that could serve as surrogate markers to predict safety of microbicides prior to embarking on large-scale clinical trials. This will require a greater understanding of the mediators of mucosal immunity in the female genital tract.

Local microbicidal agents for vaginal application in HIV/AIDS are described in Chapter 8. A local microbicidal agent against HSV-2 with immunomodulatory action for vaginal application is described in Chapter 10.

Controlled delivery of antivirals

Like other pharmaceuticals, controlled delivery of antivirals is also important. One example of this is PolyActive™ drug delivery technology, which is used in OctoPlus NV's lead product Locteron™, a controlled release formulation of IFN-α for the treatment of chronic hepatitis C. PolyActive™ is a biodegradable polymeric drug delivery system. Locteron combines PolyActive microspheres with BLX-883, a recombinant IFN-α. Key attributes of the PolyActive™ drug delivery system relevant to antivirals are:

- Controllable, including linear, release profiles
- Low initial release: no burst
- Well-preserved compound stability and activity
- Biodegradable and biocompatible

Targeted delivery of antivirals

Targeting drugs to specific organs, tissues, or cells is an attractive strategy for enhancing drug efficacy and reducing side effects. Drug carriers such as antibodies, natural and manmade polymers, and labeled liposomes are capable of targeting drugs to blood vessels of individual tissues but often fail to deliver drugs to extravascular sites. An alternative strategy is to use low molecular weight prodrugs that distribute throughout the body but cleave intracellularly to the active drug by an organ-specific enzyme. An example of this approach is a series of phosphate and phosphonate prodrugs, called HepDirect prodrugs, which enable liver-targeted drug delivery following a cytochrome P450-catalyzed oxidative cleavage reaction inside hepatocytes (Erion et al 2005). Glutathione within the hepatocytes rapidly reacted with the byproduct to form a glutathione conjugate. No byproduct-related toxicity has been observed in hepatocytes or animals treated with HepDirect prodrugs. HepDirect prodrugs represent a potential strategy for targeting drugs to the liver and achieving more effective therapies against chronic liver diseases such as hepatitis B, and hepatitis C. One of the most important barriers for drug delivery to the brain, the blood-brain barrier (BBB), will be considered here.

Delivery of antivirals to the brain across the blood-brain barrier

Several viruses affect the brain and treatment requires delivery of antiviral agents to the brain across the BBB. There is another complicating factor, i.e., some virus infections damage the BBB and its permeability may already be increased, both to the viruses as well as the therapeutics. Evaluation of the BBB permeability is a sensitive indicator of disease outcome and the antiviral efficacy (Olsen et al 2007).

If the BBB is intact, it is important to open it for delivery of antiviral therapeutics. General strategies for delivery of therapeutics to the CNS across the BBB are described in a special report on this topic (Jain 2007e). Failure to open the BBB and deliver immune effectors to CNS tissues can lead to the lethal outcome of rabies virus infection (Roy et al 2007).