CHAPTER

14

Nanoparticles in Antiviral Therapy

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OUTLINE

1 INTRODUCTION

Vaccines against serious viral infections such as measles, mumps, hepatitis A, and hepatitis B significantly reduced the incidence of the diseases. However, there are viruses with high rates of mutations that make vaccination a difficult task. Since the viral infections have an overall impact, easily develop resistance to current drugs, and new viruses still appear, there is the permanent need for the discovery of new drugs and also the improvement of the formulation of the current drugs. Several factors hinder the rapid development of antiviral drugs.

Viruses are strictly intracellular parasites composed of either DNA or RNA and a protein coat (capsid). Some viruses also have an outer lipid bilayer membrane external to the coat, called an envelope. The nucleic acid codes for enzymes involved in replication and for several structural proteins and their replication depends on the host-cell biosynthetic machinery. Thus, one of the challenges in the development of antiviral agents is the identification of the steps in viral replication that are unique to the virus and not used by the normal cell. Among the unique viral events are attachment, penetration, uncoating, RNA-directed DNA synthesis (reverse transcription) or RNA-directed RNA synthesis (RNA viruses), and assembly and release of the intact virion. For example, assembly of some virus particles requires a unique viral enzyme, protease, and this has led to the development of protease inhibitors. Moreover, each virus has specific functions, making the development of broad-spectrum antiviral drugs difficult. In some cases, antiviral agents do not selectively inhibit a unique replicative event but inhibit DNA polymerase. Inhibitors of this enzyme take advantage of the fact that the virus is synthesizing nucleic acids more rapidly than the cell; therefore, there is relatively greater inhibition of viral than cellular DNA. Further, antiviral agents developed against the viruses that cause permanent infections such as HSV and HIV, exert their antiviral effect in the acute disease but have no effects on viruses in latency resulting in chronic infections with periods of virus reactivation and recurrence of the disease requiring long-time treatment.

Limited solubility of antiviral drugs in aqueous media and their short half-life time hinder their usage and challenge the development of new drug formulations. There have been many attempts to improve the physicochemical properties of existing antiviral drugs in order to modify its bioavailability and pharmacokinetics. Another approach to improve the formulation and delivery of antiviral drugs in the human organism is the use of controlled-release delivery systems, for example, nanoparticulate carriers.

The unique properties of nanomaterials derive from their high surface-to-volume ratios, small sizes, and modifiable surfaces. Another important characteristic of nanoparticulate carriers is that multiple antiviral agents can be incorporated onto the same nanoparticle. Also due to nanometric size and controllable hydrophobicity/lipophilicity, nanoparticles can be used to provide the targeting the specific biological sites. Thus, systematic delivery of specially formulated antiviral therapeutics results in reduction of side effects on normal, uninfected cells. The nanoparticles administrated by intravenous route can circulate through the bloodstream without being retained in the lung capillaries or engulfment by the mononuclear-phagocytic cells.

Here, nanoparticulate delivery systems and their use as carriers for the transport of the most frequently used antiviral therapeutics will be discussed. Before reviewing the applications of nanomaterials as antiviral agents, a general overview of the viral replication and mechanism of actions of antiviral agents will be presented. Finally the nanoparticulate carriers used in antiviral therapy for the viruses that induce chronic infections with severe clinical manifestations and complications—human immunodeficiency virus (HIV), hepatitis C virus (HCV), and

Herpes simplex virus type 1 and type-2 (HSV-1 and HSV-2)—will be summarized.

2 VIRUS REPLICATION CYCLE

The life cycle of viruses can be divided into following steps: (1) adsorption and attachment, (2) penetration or entry, (3) uncoating, (4) production of virion components—replication, transcription, and translation, (5) assembly of naked capsid viruses and nucleocapsids, and (6) release of viral particles. These steps of virus replication cycle are shown in Fig. 14.1.

2.1 Adsorption or Attachment

The attachment of the viral particle to the surface of the host cell is the first step in every viral infection. This interaction requires an adequate contact between the virus and the cell. However, only a small percentage of the direct contact between a virus and a host cell lead to a successful infection because adsorption is a highly specific reaction that involves attachment of the surface viral proteins, or spikes, and corresponding receptors on the surface of the host cell. Receptors for human viruses are mostly glycoproteins present in the plasma membrane that have the role in cell signaling and in many reactions of the immune system. Thus, any attempts to design antiviral agents that block the binding to the receptors for a long time must consider the possibility that the loss of the normal cellular function associated with the receptors would have serious consequences for the host organism. Some viruses for adsorption to the cells use different surface molecules, called coreceptors. It was originally thought that CD4 is the unique receptor for human immunodeficiency virus type 1 (HIV-1), but
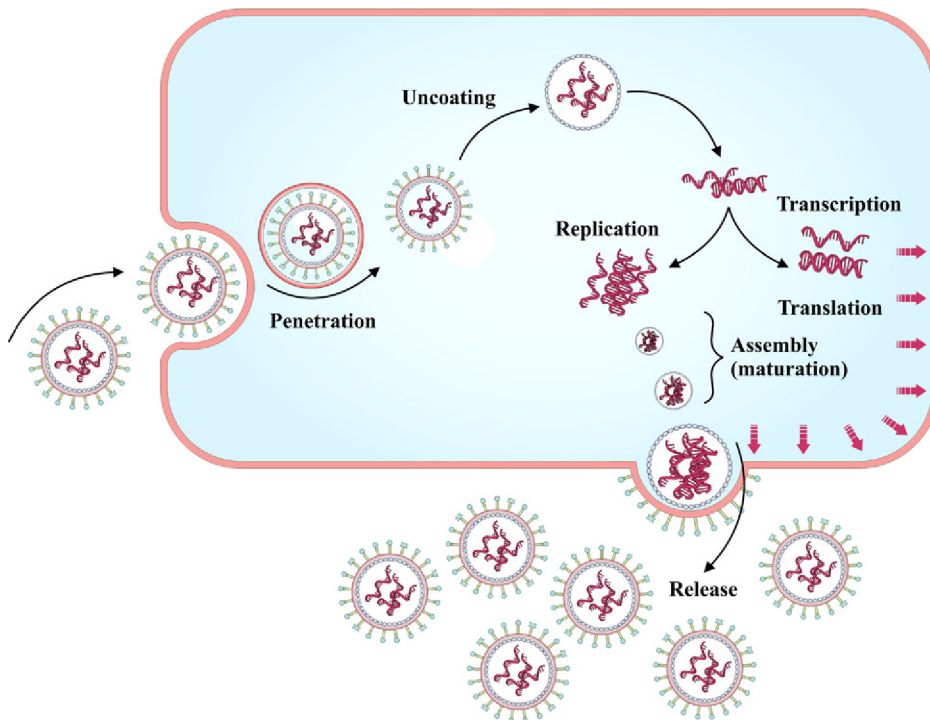

FIGURE 14.1   Viral replication cycle.

later coreceptors that normally function as che-mokine receptors (CCR5 and CXCR4) were dis-covered. In some cases, capsid protein have the role in the adhesion to the cells.

2.2 Penetration, Entry, and Uncoating

2.2.1 Enveloped Viruses

Enveloped human viruses use two basic mechanisms for entry into the cell. Both mecha-nisms involve fusion of the viral envelope with a cellular membrane, resulting in both cases in the release of the free nucleocapsid into the cy-toplasm. Paramyxoviruses, some of the retrovi-ruses (HIV-1), and herpesviruses enter the cell by the process called direct fusion. In direct fu-sion, the spikes present in the envelopes of the viruses promote fusion of the viral membrane with the plasma membrane of the cell, resulting in the release of the nucleocapsid directly into the cytoplasm. Due to the fact that the viral en-velope becomes incorporated into the plasma membrane of the infected cell and still expresses its fusion proteins, infected cells have a tenden-cy to fuse with other uninfected cells. Most of the remaining enveloped animal viruses, such as orthomyxoviruses (influenza viruses), togavi-ruses (rubella virus), rhabdoviruses (rabies), and coronaviruses enter the cell by a cellular mecha-nism called receptor-mediated endocytosis, or viropexis. In viropexis, viruses adsorb the cell through multiple interactions of virus attach-ment proteins and host cell molecules and thus facilitate the plasma membrane surrounding the host cell molecules. Pinching off of the cellular membrane by fusion encloses the virion in a cy-toplasmic (endosomal vesicle) that is acidified by a normal cellular process. In the endosomal vesicle, the nucleocapsid is surrounded by two membranes: the viral envelope and the newly acquired endosomal membrane. The low pH of the endosome leads to a conformational change in a viral spike protein, resulting in the fusion of these two membranes and release of the nucleo-capsid into the cytoplasm.

2.2.2 Nonenveloped Viruses

Nonenveloped viruses enter the cell by viro-pexis. At the low-pH of endosomal vesicle the viral capsid proteins of nonenveloped viruses expose hydrophobic domains resulting in the binding of the virions to the membrane and re-lease of the nucleic acid genome into the cyto-plasm. In some cases, the virions may escape the endosomal vesicles by simple stimulation of the lysis of the vesicle. This step is a potential target of antiviral therapy, and some drugs have been developed that bind to the capsids of picorna-viruses and prevent the release of the virus par-ticles from the endosome.

2.3 Production of Virion Components

With the exception of influenza viruses and the retroviruses, all RNA viruses replicate in the cytoplasm. Retroviruses, influenza viruses, and all the DNA viruses, except the poxviruses replicate in the nucleus and need movement from the cytoplasm to the nucleus. The larger DNA viruses, herpes viruses, and adenoviruses have to uncoat to the level of cores before entry into the nucleus, whereas smaller DNA viruses (parvoviruses and the papovaviruses) enter the nucleus intact through the nuclear pores and subsequently uncoat inside. The largest of the human viruses, the poxviruses, accomplish their entire replicative cycle in the cytoplasm of the infected cell.

2.4 Transcription

The key step in every viral infection is the production of virus-specific mRNAs that direct the synthesis of the viral proteins, structural proteins of the virion and enzymes and other specialized proteins required for genome repli-cation, gene expression, and virus assembly and release. Viral mRNAs of most DNA viruses are synthesized by the host DNA-dependent RNA polymerase (RNA polymerase II). The (single)-strand RNA viruses, (the picornaviruses, the

togaviruses, and the coronaviruses) possess positive single-stranded RNA that can be used directly as mRNAs in the process of translation immediately after their entry into the cytoplasm of the cell. One of these viral proteins is RNA dependent RNA polymerase required for synthesis of the new mRNA, since there is no cellular machinery that can use either single- or double-stranded RNA as a template to synthesize mRNA. Therefore, poxviruses and viruses that use an RNA template to make mRNAs must provide their own transcription machinery to produce the viral mRNAs at the beginning of the infection process. At later times in the course of the infection, any special enzymatic machinery that the virus requires but is not present initially in the cell can be obtained among the proteins translated from the mRNA molecules. The parvoviruses have single-stranded DNA genomes, and RNA polymerase of the cell requires double-stranded DNA as a template, but these viruses do not need to carry special enzymes in their virions because host cell DNA polymerases can convert the genomes into double-stranded DNA. However, the production of more mRNA of the picornaviruses requires the synthesis of an intermediate negative-strand RNA template that requires the translation and production of the enzyme called RNA-dependent RNA polymerase early in the course of infection. The retroviruses are a special form of positive single-stranded RNA viruses. Although the genomes of retroviruses are the same polarity as mRNA and could serve as mRNAs early after infection, the RNA genomes of these viruses are copied into negative DNA strands by reverse transcriptase, an enzyme that is always present inside the virion. The newly synthesized negative DNA strands are subsequently converted by the same enzyme to double-stranded DNA, which is accompanied with the degradation of the original genomic RNA by the RNase H activity of the reverse transcriptase. The DNA made by the activity of reverse transcriptase is integrated into the host cell DNA and transcribed by the host RNA polymerase in order to complete the replication cycle and to produce viral mRNA. The replication of the hepatitis B DNA genome is mechanistically similar to the replication process of a retrovirus. In the case of the hepatitis B virus, viral DNA is the template for the process of transcription and production of a single-stranded RNA, which in turn is reverse transcribed to produce the progeny viral DNA that becomes a part of the new virions.

2.5 Genome Replication

2.5.1 DNA Viruses

Enzymes and accessory proteins required for the replication of DNA, are in the eukaryotic cells present only during the S phase of the cell cycle, and are located only in the nucleus. The extent to which viruses use the cell replication machinery depends on their protein-coding potential and, thus, on the size of their genome. The smallest of the DNA viruses, the parvoviruses, are completely dependent on host machinery, meaning that they can replicate only in the dividing cells. When the normal S phase of the cell cycle occurs, viral DNA replicates together with the cellular DNA. Large DNA viruses are relatively independent of cellular functions, they replicate in the cytoplasm and code for almost all the enzymes and other proteins required for replicating their DNA. The remainder of the DNA viruses is only partially dependent on host machinery. The small DNA human viruses, such as the polyomaviruses and papillomaviruses code for a protein that is involved in the initiation of synthesis at the origin, but the host machinery accomplishes the remainder of the replication process. To some extent, more complex adenoviruses and herpesviruses, in addition to providing origin-specific proteins, also encode for their own DNA polymerases and other accessory proteins required for DNA replication. The fact that the herpesviruses encode for their own DNA polymerase is the key point exploited for

the creation of antiviral therapeutics. Certain antiviral drugs such as acyclovir (acycloguanosine) preferentially kills cells infected with herpes viruses because the viral thymidine kinase, unlike the cellular counterpart, phosphorylate the nucleoside analog. Phosphorylated nucleoside analogue inhibits further DNA synthesis when DNA polymerases incorporate it into DNA. The host cell enzyme discriminates better host nucleosides and the acyclovir analog fails to phosphorylate acyclovir analogue and inhibition of synthesis of cellular DNA fails. Accordingly, acyclovir does not kill uninfected cells. A similar principle applies to the chain-terminating drugs such as zidovudine and dideoxyinosine that are phosphorylated by cellular kinase and target not only the HIV-1 reverse transcriptase but also inhibit cellular DNA polymerase to some extent.

2.5.2 RNA Viruses

The replication of RNA viruses mostly takes place in the cytoplasm. Moreover, cells do not have RNA polymerases, enzymes necessary for the copy of RNA templates (RNA-based RNA transcription or replication). Accordingly, RNA viruses not only need to encode for transcriptases or polymerases (required for transcription), but also must provide the polymerases required to duplicate the RNA genome into daughter RNA genomes. Furthermore, except in the cases of the picornaviruses, in which transcription and replication are synonymous, the RNA viruses must temporally and functionally separate transcription from replication. After replication begins, these same templates are used to synthesize full-length strands for replication. There are two mechanisms that separate the process of transcription from replication: (1) in some cases, transcription is restricted to subviral particles and utilizes a transcriptase transported into the cell within the virion; (2) in other cases, the replication process either involves a functionally distinct RNA polymerase or depends on the presence of some other accessory protein specific for the virus that directs the synthesis of full-length copies of the template.

2.6 Assembly of Naked Capsid Viruses and Nucleocapsids

The process of inserting the viral genome in a capsid protein is called assembly or capsidation. Four general principles govern the construction of capsids and nucleocapsids: (1) the process generally involves self-assembly of the components, (2) assembly is stepwise and ordered, (3) individual protein structural subunits, protomers, are usually preformed into capsomeres in preparation for the final assembly process, (4) assembly often initiates at a particular locus on the genome called a packaging site.

2.7 Release of Viral Particles

Viruses may leave the cell by several mechanisms: via budding, apoptosis, exocytosis, and through induction of the cell lysis. Budding through the cell membrane is the mechanism most effective for the viruses that need an envelope. Prior to budding, the virus put its own receptor onto the surface of the cell, forming an envelope with the viral receptors already on it. This process will slowly use up the cell membrane and eventually lead to the demise of the cell. This is also how antiviral responses are able to detect virus-infected cells. Virus infected animal cells are programmed to autodestruction. Besides, viruses could also have the role in forcing the cell to undergo apoptosis, and thus release of progeny into the extracellular space is possible. Viruses, mostly nonenveloped, leave cells through exocytosis. Newly synthesized viral particles use the host cell's transport system; the vesicles containing the virus particles are carried to the cell membrane and then released into the extracellular space. Cell lysis is also accompanied with release of viral particles.
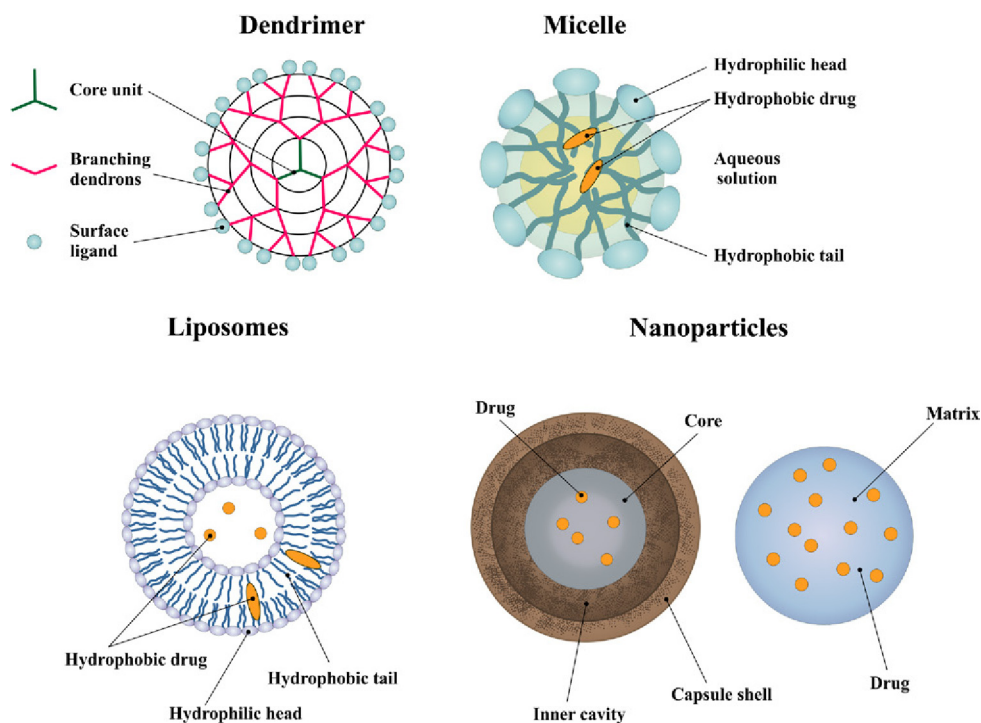
FIGURE 14.2 Proposed nanoparticles as carriers for antiviral agents.

3 SELECTED ANTIVIRAL AGENTS

The steps in viral replication that are unique for the viruses are the potential target points for antiviral agents. Among these unique processes are attachment, penetration, uncoating, RNA-directed DNA synthesis (reverse transcription) or RNA-directed RNA synthesis, and assembly and release of the virions. A general overview for the points of action of antiviral agents is shown in Fig. 14.2, list of currently used antiviral agents is in Table 14.1.

3.1 Inhibitors of Attachment

Attachment of the virus to the host cell is mediated by specific interactions of certain molecules on viruses and host cells. Antibodies can bind to the virus in the extracellular space and thus prevent this attachment to the cell. However, although therapy with antibodies is useful in prophylaxis, it has been minimally effective in treatment.

3.2 Inhibitors of Cell Penetration and Uncoating

Amantadine and rimantadine, symmetric amines, inhibit viral uncoating and show their activity against only influenza A. They have been used either as prophylaxis or for therapy, but since 2001, the rates of resistance to amantadine/rimantadine have extremely increased so that they are no longer routinely recommended. Both amantadine and rimantadine are available only as oral preparations. The major toxicity exert in the CNS.

TABLE 14.1   Summary of Antiviral Agents

Mechanism	Antiviral agent	Virus	Administration
Inhibition of viral uncoating	Amantadine	Virus influenzae A	Oral
	Rimantadine	Virus influenzae A	Oral
Neuraminidase inhibition	Oseltamivir	Virus influenzae A, B	Oral
	Zanamivir	Virus influenzae A, B	Oral inhalation
Inhibition of viral DNA polimerase	Acyclovir	HSV, VZV	Oarl, topical, intravenous
	Idoxuridine	HSV	Intravenous
	Famciclovir	HSV, VZV	Oral
	Penciclovir	HSV	Topical
	Valacyclovir	HSV, VZV	Oral
	Ganciclovir	HSV, VZV, CMV	Oral, intravenous
	Foscarnet	CMV, resistant HSV	Intravenous
	Cidofovir	CMV	Intravenous
	Trifluridin	HSV, VZV	Eye drops
Inhibition of viral RNA polimerase	Ribavirin	RSV, HCV	Oral
Antisense inhibition of viral mRNA synthesis	Fomivirsen	CMV	Eye drops
Inhibition of viral reverse transcriptase	Zidovudine	HIV	Oral, intravenous
	Dideoxyinosine	HIV	Oral
	Dideoxycytidine	HIV	Oral
	Stavudine	HIV	Oral
	Lamivudine	HIV, HBV	Oral
	Nevirapine	HIV	Oral
	Delaviridine	HIV	Oral
	Efavirenz	HIV	Oral
	Etraverine	HBV	Oral
	Adefovir	HBV	Oral
	Entecavir	HBV	Oral
	Telbivudine	HBV	Oral
	Tenofovir	HBV	Oral
Inhibition of viral integration	Raltegravir	HIV	Oral
Inhibition of viral protease	Saquinavir	HIV	Oral
	Elvitegravir	HIV	Oral
	Indinavir	HIV	Oral
	Ritonavir	HIV	Oral
	Nelfinavir	HIV	Oral
	Lopinavir	HIV	Oral
	Darunavir	HIV	Oral
	Atazanavir	HIV	Oral
	Tipranavir	HIV	Oral
	Boceprevir	HCV	Oral
	Telaprevir	HCV	Oral
Inhibition of synthesis of viral proteins	Interferon-α	HBV, HCV, HPV	Subcutaneous, intramuscular, intravenous

3.3 Neuraminidase Inhibitors

The selective inhibitors of the neuraminidase of influenza A and B viruses are oseltamivir and zanamivir. The role of the neuraminidase is cleavage of the terminal sialic acid from the glycoconjugates, which allows the release of the virus from infected cells. Zanamivir is given by inhalation. Oseltamivir phosphate is the oral prodrug of oseltamivir, a drug comparable to zanamivir in antineuraminidase activity.

3.4 Inhibitors of Nucleic Acid Synthesis

The most antiviral agents currently used are nucleoside analogs that are active against virus specific nucleic acid polymerases or reverse transcriptases and have much less activity against analogous host enzymes. Some of these agents have the role to terminate nucleic acid chain after incorporation into nucleic acids.

3.4.1 Idoxuridine and Trifluorothymidine

Idoxuridine (5-iodo-2'-deoxyuridine) is a halogenated pyrimidine that incorporates into DNA in place of thymidine. This reaction blocks nucleic acid synthesis and produces a nonfunctional molecule. Cellular thymidine kinase phosphorylate Idoxuridine making the active compound, which inhibits both viral and cellular DNA polymerase. The resulting host toxicity excludes systemic administration in humans. Idoxuridine can be used topically as effective treatment of herpetic infection of the cornea, keratitis. Trifluorothymidine, a related pyrimidine analog, is effective in treating herpetic corneal infections, including those that fail to respond to Idoxuridine.

3.4.2 Acyclovir

Acyclovir differs from the nucleoside guanosine by having an acyclic (hydroxyethoxymethyl) side chain. Acyclovir for its activation needs the phosphorylation by the viral thymidine kinase. Since acyclovir cannot be phosphorylated

and activated in an uninfected host cell, this drug is essentially nontoxic. Viral thymidine kinase catalyzes the phosphorylation of acyclovir only to a monophosphate, whereas host-cell enzymes continues the phosphorilation to the diphosphate and, finally, the triphosphate. Acyclovir triphosphate competes with guanosine triphosphate, inhibits the function of the virally encoded DNA polymerase, and finally inhibits viral replication. The selectivity and minimal toxicity of acyclovir is due to its 100-fold or greater affinity for viral DNA polymerase than for cellular DNA polymerase. A second mechanism of viral inhibition comes from incorporation of acyclovir triphosphate into the growing viral DNA chain. There is no 3'-hydroxy group on the acyclovir to serve as the attachment sites for additional nucleotides, thus the result of acyclovir incorporation is the termination of chain growth. Activity of acyclovir against herpes viruses directly correlates with the capacity of the virus to induce a thymidine kinase. Susceptible strains of herpes simplex virus types 1 and 2 (HSV-1 and -2) contain the most active thymidine kinases and are the most readily inhibited by acyclovir. Cytomegalovirus (CMV) induces little or no phosphorilation of acyclovir and is not inhibited by this drug. Varicella-Zoster and Epstein-Barr viruses are between these two extremes in terms of both thymidine kinase induction and susceptibility to acyclovir. Resistant strains of HSV have been recovered from immunodeficient patients, including patients with acquired immunodeficiency syndrome (AIDS) but rarely from immunocompetent patients, even after years of drug exposure. In most cases, resistance was the result of mutations in the viral thymidine kinase gene, that make it inactive in phosphorylation. Resistance may also result from mutations in the viral DNA polymerase. Acyclovir is available in three application forms: topical, oral, and parenteral. The oral form has low bioavailability, but achieves concentrations in blood that inhibit HSV and, to a lesser extent, Varicella-Zoster virus (VZV). Intravenous acyclovir is used for serious HSV infection (congenital

infections, encephalitis) as well as for VZV infection in immunocompromised patients. The dosage of acyclovir must be reduced in patients with renal failure, given the fact that acyclovir is excreted by the kidney. Central nervous system toxicity and renal toxicity have been reported in patients treated with prolonged high intravenous doses. Acyclovir does not induce bone marrow toxicity, even in patients with hematopoietic disorders. Acyclovir is effective in the treatment of primary HSV mucocutaneous infections or for severe recurrences in immunocompromised patients. The agent is useful in neonatal herpes and encephalitis, infection in immunocompromised patients and for varicella in older children or adults. Acyclovir is beneficial against herpes zoster in elderly patients or any patient with eye infections. In patients with frequent severe genital herpes, the oral form is effective in preventing recurrences, but it has minimal efficiency in the treatment of recurrent genital or labial herpes in otherwise healthy persons.

3.4.3 Valacyclovir, Famciclovir, and Penciclovir

Valacyclovir is a prodrug of acyclovir that shows better absorption and, thus can be used in lower and less frequent dosage. It is currently approved for therapy of HSV and VZV infections. The adjustment of valacyclovir dosage is necessary in patients with insufficient renal function. Famciclovir has similar structure as acyclovir and also requires phosphorylation, but differs slightly in its mode of action. After absorption, the famciclovir is converted to penciclovir, the active moiety, which inhibits viral DNA polymerase, but does not irreversibly terminate DNA replication. Famciclovir is currently approved for treatment of HSV and VZV infections. Penciclovir is approved for topical treatment of recurrent herpes labialis.

3.4.4 Ganciclovir

Ganciclovir is a nucleoside analog of guanosine and differs from acyclovir by a single carboxyl side chain, which make it approximately 50 times more active against CMV compared with acyclovir. Acyclovir has low activity against CMV because CMV does not carry the gene for thymidine kinase and adequate phosphorylation of acyclovir in CMV-infected cells lacks. However, ganciclovir is active against CMV because it does not require thymidine kinase for phosphorylation. Rather, another phosphorylating enzyme (UL97) encoded by the viral genome is present in CMV-infected cells that is capable of phosphorylating ganciclovir and converting it to the monophosphate. Later, cellular enzymes convert it to the active compound ganciclovir triphosphate, which inhibits the viral DNA polymerase. Oral ganciclovir is available but is inferior to the intravenous form. Oral valganciclovir, a prodrug of ganciclovir, has improved bioavailability and is equivalent to the intravenous form. Toxicity, especially neutropenia, frequently limits therapy. Thrombocytopenia occurs in approximately 15% of patients. Ganciclovir or valganciclovir are indicated for the prevention or treatment of active CMV infection in immunodeficient patients, but other herpesviruses (particularly HSV-1, HSV-2, and VZV) are also susceptible. After several months of continuous ganciclovir therapy used for control of CMV infection, between 5% and 10% of patients with AIDS excrete resistant strains of CMV. In almost all isolates, a mutation is found in the phosphorylating gene (UL97), and in a lesser number a mutation may also be found in the viral DNA polymerase. Ganciclovir resistance has been noted in transplant recipients, especially those requiring prolonged prophylaxis or treatment. Resistance is most common in patients with lung or liver transplants.

3.5 Nucleotide Analogs; Cidofovir

The most known nucleotide analog is cidofovir. Cidofovir has a phosphonate group attached to the molecule and appears to the cell as a nucleoside monophosphate, in fact as a nucleotide.

The active compound is produced after cellular enzymes add two phosphate groups to the monophosphate form. In the form of triphosphate, the drug inhibits both viral and cellular nucleic acid polymerases. However, greater selectivity toward cidofovir exerts viral enzyme, so the drug has a selective effect. Nucleotide analogs do not require phosphorylation or activation by a viral-encoded enzyme and remain active against viruses that are resistant due to mutations in codons for these enzymes, for example, a UL97 mutant CMV. Resistance to cidofovir can develop with mutations in the viral DNA polymerase. An additional feature of cidofovir is a very prolonged half-life as a result of slow clearance by the kidneys. Cidofovir is approved for intravenous therapy of CMV retinitis, and given frequently, every 2 weeks as a maintenance treatment. Nephrotoxicity is a serious complication of cidofovir treatment, and patients must be monitored carefully for evidence of renal impairment.

3.6 Nucleoside/Nucleotide Analog Inhibitors

Lamivudine was the first clinically employed inhibitor of hepatitis B DNA polymerase. It has been followed and usually replaced with similar molecules, such as adefovir, entecavir, telbivudine, and tenofovir, the last of which develops less resistance. Of these, entecavir and tenofovir have become the preferred agents for monotherapy due to their potency and very low rates of resistance development. The other polymerase inhibitors cannot be used as monotherapy due to the ease with which resistance may develop.

3.7 Other Inhibitors of Viral DNA Synthesis

3.7.1 Foscarnet

Foscarnet is a pyrophosphate analog that inhibits viral DNA polymerase by blocking the pyrophosphate-binding site of the viral DNA polymerase and preventing cleavage of pyrophosphate from deoxyadenosine triphosphate. In contrast to nucleosides such as acyclovir and ganciclovir, foscarnet does not require phosphorylation to be an active inhibitor of viral DNA polymerases. This biochemical characteristic of foscarnet is especially important with regard to viral resistance, because the viral resistance to nucleoside analogs is caused by the mutations that reduces or eliminates the phosphorylation of the drug in virus-infected cells. Thus, foscarnet can usually be used to treat patients with ganciclovir-resistant CMV and acyclovir-resistant HSV. Multiple metabolic abnormalities occur as evidence of toxicity. Excretion is entirely renal without a hepatic component, and dosage must be decreased in patients with impaired renal function.

3.7.2 Interferons

Interferons are proteins synthesized in various host cells, encoded by the host genes, in response to double-stranded RNA (dsRNA). Interferon circulates and has the role to protect uninfected cells by inhibiting viral protein synthesis. Recombinant DNA techniques now allow relatively inexpensive large-scale production of interferons by bacteria and yeasts. Interferon is beneficial in the treatment of chronic active hepatitis B and C infection, although its efficacy is often transient. Combinations of interferon-α with lamivudine, famciclovir, and certain nucleotides are being evaluated for treatment of hepatitis B. Pegylated interferon-α (Peg-IFα) is in therapeutical procedure for treatment of chronic hepatitis C disease. Peg-IFα treatment of chronic C hepatitis lasts for 6–12 months and in combination with ribavirin usually produces improved results. Topical or intralesional interferon application is beneficial in the treatment of human papilloma virus infections. Parenteral use is accompanied with severe systemic toxicity (e.g., fever, malaise), probably due to its effect on host-cell protein synthesis.

3.7.3 Ribavirin

Ribavirin is an analog of the nucleoside guanosine, which differs from guanosine in that the base ring is incomplete and open. Like the other nucleoside analogs, ribavirin must be phosphorylated to mono-, di-, and triphosphate forms, to reach the active form. Since each step of phosphorylation of ribavirin mediates cellular enzymes, the risk of toxicity is very increased. Ribavirin is active against a broad range of viruses in vitro, but its in vivo activity is limited. The mechanism of the antiviral effect of ribavirin is not clear as it is in the case of acyclovir. Ribavirin inhibits RNA polymerase, and also inhibits inosine monophosphate dehydrogenase, an enzyme important in the synthetic pathway of guanosine. Further, because of interference with both guanylation and methylation of the nucleic acid, ribavirin decrease synthesis of the mRNA 5′ cap. Administration via aerosol enables ribavirin to reach up to 10 times greater concentrations in respiratory secretions than necessary to inhibit respiratory syncytial virus (RSV) replication and significantly higher than those achieved with oral administration. Problems associated with the usage of aerosolized ribavirin include precipitation of the drug in tubing used for administration and exposure of healthcare personnel. Thus, its use for RSV infection is not generally recommended. Oral and intravenous forms have been used for patients with Lassa fever and infections with other arenaviruses. The oral form of ribavirin has activity against hepatitis C virus in combination with Peg-IFN. This combination is the main use at present. In preclinical studies, ribavirin showed teratogenic, mutagenic, and gonadotoxic effects, whereas in a reversible anemia it has been associated with its oral administration.

3.8 Inhibitor of Viral RNA Synthesis

3.8.1 Fomivirsen

Fomivirsen, the first antisense compound approved for use in human infection, is a synthetic oligonucleotide. It is complementary to sequence in CMV messenger RNA (mRNA) and thereby inhibits its coding. The major immediate early transcriptional unit of CMV encodes several proteins responsible for regulation of viral gene expression, and fomivirsen probably inhibits production of these proteins. Oligonucleotide phosphorothioate linkages replace the usual nucleases in formivirsen. Fomivirsen exerts greater antiviral activity than ganciclovir on a molar basis and is approved for the local (intravitreal) therapy of CMV retinitis in patients who have failed all other therapies.

3.9 Therapeutics for HIV Infections

3.9.1 Fusion Inhibitors of HIV

Enfuvirtide is a synthetic peptide that inhibits the fusion of HIV-1 with CD4 cells. There is no oral form, and it is usually reserved for patients failing other therapies. Maraviroc interferes with the attachment of HIV gp120 with the CCR5 receptor and thus blocks the predominant route of HIV entry into the cells. Resistance may develop by the virus adapting to another receptor, CXCR4.

3.9.2 Nucleoside Reverse Transcriptase Inhibitors

3.9.2.1 ZIDOVUDINE

Zidovudine is nucleoside analog of thymidine that inhibits the reverse transcriptase of HIV. As it was mentioned for the other nucleosides, zidovudine must be phosphorylated in order to be active. Phosphorylation of zidovudine is carried by the host cell enzymes. The basis for the relatively selective therapeutic effect of zidovudine is that HIV reverse transcriptase is more than 100 times more sensitive to zidovudine than is host cell DNA polymerase. Nonetheless, toxicity frequently occurs. Zidovudine was the first useful treatment for HIV infection, but now it is recommended for use only in combination with other inhibitors of HIV replication (lamivudine

and protease inhibitors). Toxicity of zidovudine includes malaise, nausea, and bone marrow toxicity. The toxicity to all hematopoietic components may be noticed but it is usually reversible with discontinuation of the drug or dose reduction. Resistance is associated with one or more mutations in the HIV reverse transcriptase gene.

3.9.2.2 DIDANOSINE AND ZALCITABINE

A series of oral compounds similar to zidovudine have been developed and are used in combination with other HIV antivirals. Although they have similar mechanisms of action, their side effects may differ. These compounds include didanosine and zalcitabine. Serious adverse reactions to treatment include peripheral neuropathy with either didanosine or zalcitabine, and pancreatitis with didanosine. Both side effects are dose related.

3.9.2.3 STAVUDINE

Another nucleoside analog that inhibits HIV replication is stavudine. Stavudine also terminates the growth of the viral nucleic acid chain. Stavudine is well absorbed and has a high bioavailability. Adverse effects of this therapeutic include headache, nausea and vomiting, asthenia, confusion, and elevated serum transaminase and creatinine kinase. A painful sensory peripheral neuropathy that appears to be dose-related may occur. Stavudine should be used only in combination with other anti-HIV agents.

3.9.2.4 LAMIVUDINE

Lamivudine is another oral nucleoside reverse transcriptase inhibitor. This therapeutic is a safe and usually well-tolerated agent. Lamivudine is used in combination with zidovudine or other nucleoside analogs because lamivudine suppresses the development and persistence of mutations that lead to zidovudine resistance. The newer oral nucleoside reverse transcriptase inhibitors are abacavir and emtricitabine, which, like those discussed earlier, should only be used in combination with other classes of HIV antivirals.

3.9.3 *Nonnucleoside Reverse Transcriptase Inhibitors*

Oral compounds that are not nucleoside analogs also inhibit HIV reverse transcriptase. Several compounds, such as nevirapine, delavirdine, efavirenz, and etravirine, have been evaluated alone or in combination with other nucleosides. They are collectively referred to as nonnucleoside reverse transcriptase inhibitors (NNRTIs). These compounds are very active against HIV, do not require cellular enzymes to be phosphorylated, and bind to, essentially, the same site on reverse transcriptase. Cross-resistance does not occur between nucleoside RT inhibitors and NNRTIs, but does occur between one NNRTI and another. Most of these compounds do not inhibit human DNA polymerase and are not cytotoxic at concentrations required for effective antiviral activity. Therefore, they are relatively nontoxic. Unfortunately, drug resistance readily emerges with even a single passage of virus in the presence of drug in vitro and in vivo. Thus, NNRTIs should be used only in combination regimens with other drugs active against HIV.

3.9.4 *Protease Inhibitors*

Protease inhibitors block the action of the viral encoded enzyme protease, which cleaves polyproteins to produce final viral proteins. Inhibition of this enzyme leads to blockage of viral assembly and release. The protease inhibitors are potent inhibitors of HIV replication in vitro and in vivo, mostly when combined with other antiretroviral agents. These drugs do not require intracellular phosphorylation for activation. In late 1995, saquinavir was the first protease inhibitor to receive approval. Ritonavir, indinavir, nelfinavir, darunavir, fosamprenavir, and tipranavir are other potent protease inhibitors that have since been released. These drugs may cause hepatotoxicity, as all agents inhibit P450, resulting in important drug interactions. Because drug resistance to all protease inhibitors develops, these agents should not be used alone

without other anti-HIV drugs. Lopinavir is a protease inhibitor that is marketed in combination with ritonavir. Atazanavir, another protease inhibitor, is usually prescribed with ritonavir to increase serum concentration of atazanavir.

3.9.5 *Integrase Inhibitors*

HIV integrase aids the insertion of viral DNA into host-cell DNA. This occurs after the viral reverse transcriptase (RNA/DNA-dependent DNA polymerase) produces double-stranded viral DNA. This step is the key to the cell becoming a permanent carrier. Two integrase inhibitors, raltegravir and elvitegravir, are approved for use and are usually used for treatment of experienced patients and in combination with other classes of antiretrovirals.

4 ANTIVIRAL RESISTANCE

Since viral genomes, viral replication cycle, and mechanisms of action of the available antiviral agents have been extensively studied, understanding of resistance to antiviral drugs has evolved. It has become clear that the central role in resistance to antiviral agents is gene mutations. For example, mutations in the viral-induced enzyme that catalyzes the phosphorylating of the nucleoside is the common mechanism of resistance to nucleosides (acyclovir and ganciclovir) by herpes viruses. The probability of the occurrence of resistant mutants results from at least four factors: (1) the rate of viral replication—higher rates of replication are associated with higher rates of spontaneous mutations; (2) the selective pressure of the drug—the greater the drug exposure, the more rapid the emergence of resistant mutants up to a point; (3) the rate of viral mutations—the rate of mutations differs among different viruses so that, in general, single-stranded RNA viruses (HIV and influenza) have more rapid rates of mutation than double-stranded DNA viruses (HSV); and (4) rates of mutation in differing viral genes.

5 OVERVIEW OF PARTICULATE CARRIERS FOR DRUG DELIVERY

Nanoparticles have emerged in the last few years as an alternative material for advanced diagnostic and therapeutic applications in medicine. Compared to molecular medicine, nanoparticles offer many advantages such as bioavailability and biodistribution of therapeutic agents. The first remarkable property of nanoparticles is their superior in vivo retention by decreasing enzymatic degradation and sequestration by phagocytes of the reticuloendothelial systems. This is mostly attributed to their immunochemically inert surfaces in contact with the biological environment. Increased deposition to the diseased sites via compromised vasculatures in the phenomenon called enhanced permeability and retention effect also contributes to their improved deposit to diseased sites and efficacy. Different nanoparticles have been proposed over the years as carriers for antiviral agents. Some of the mostly considered nanoparticles are shown in Fig. 14.3.

5.1 Liposomes

The first commercialized nanostructures, liposomes, which can be used as drug delivery systems, were first introduced in 1965. Liposomes are spherical vesicular systems consisting of at least one lipid bilayer. Lipid layers are mostly natural or synthetic phospholipids, most often, phosphatidylcholine. Inside the lipid layer is an aqueous core. The main types of liposomes are small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Depending on the method used for their preparation, the liposomes vary in size from 40 nm to 10 μm. Hydrophilic therapeutics can be incorporated in liposomes inside the aqueous core, whereas lipophilic therapeutics can be inserted in a lipid. The efficiency of incorporation of therapeutics in liposomes depends on the size of the liposome, the hydrophobicity of the charge of
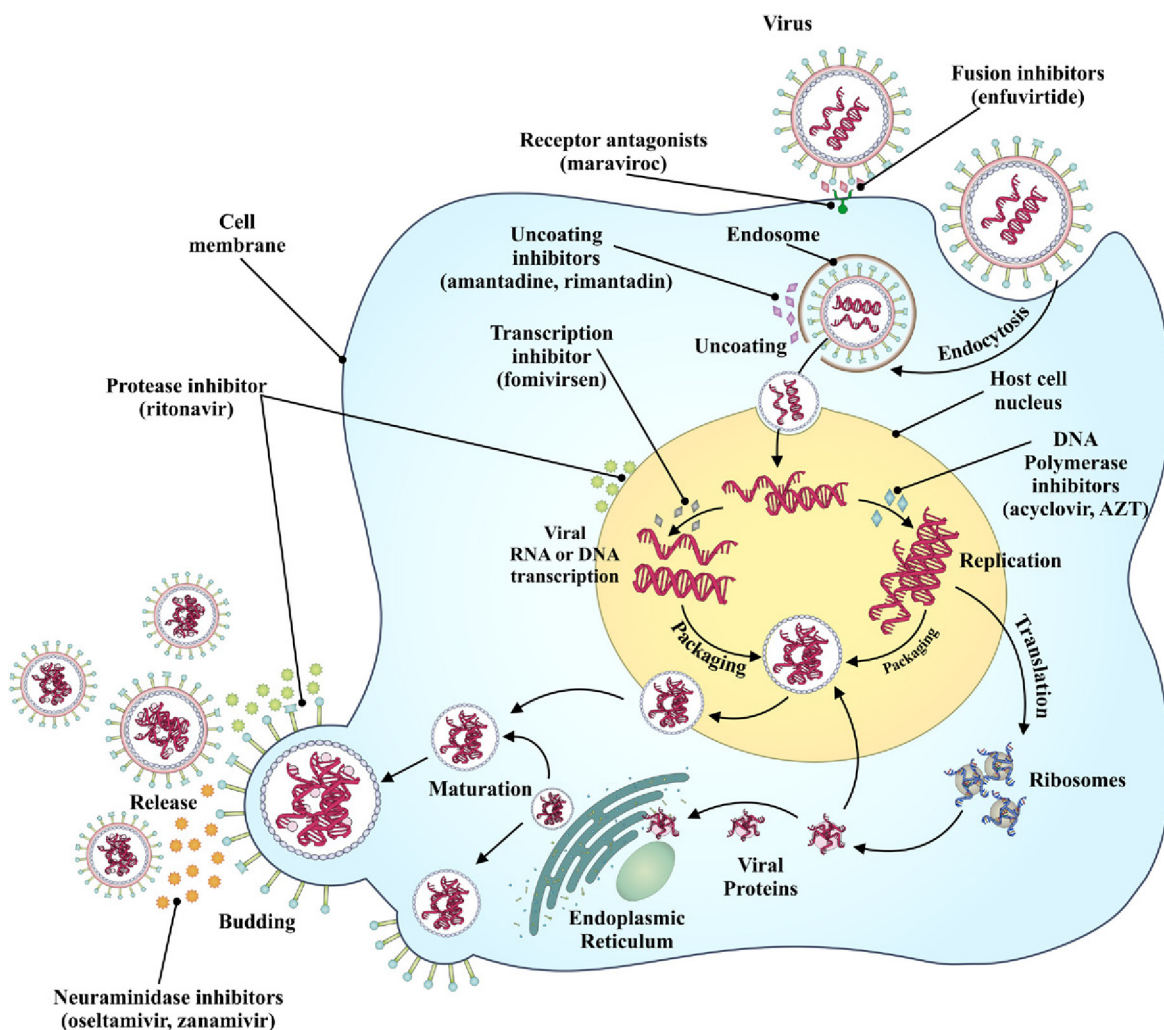
FIGURE 14.3 Points of action of antiviral agents.

its surface, and membrane fluidity. These characteristics of liposomes also influence in vitro stability, and biodistribution of applied therapeutics. The phagocitic cells in the spleen rapidly recognize liposomes after their intravenous application and internalize them. The reduction of engulfment of liposomes by the phagocytes is achieved by chemical modification of liposomes with poly(ethylene glycol) (PEG) units. However the surface of liposomes can be modified

in such a way as to increase their recognition, and subsequently the endocytosis by macrophages and other cells of the innate immune system. Since HIV resides in macrophages, liposomes have been mainly studied as carriers for anti-HIV drugs, deliveries of HIV vaccines, as well as siRNA. There are also certain disadvantages of these carriers of viral agents, such as poor stability in vitro, as well as in vivo, low efficiency of their incorporation in lipid bilayers

or aqueous core, and very high cost of their production.

The liposomal delivery system has been constructed for acyclovir for the local treatment of genital herpes and recurrent genital herpes. Liposomes were also used as the carrier for the topical application of acyclovir and also for the antisense oligonucleotides for the intravitreal administration for the treatment of ocular CMV infections. Necessary corneal penetration of acyclovir, in vitro and its good corneal absorption in vivo was achieved with constructs of acyclovir loaded on positively charged liposomes. Importantly, liposomes have also been used as the carrier for interferon-α for the oral administration.

Another form of vesicle, noisome has been designed for the drug delivery. Niosome vesicle is similar to a liposome, but instead the lipids niosome is formed of nonionic surfactant. It appears that niosomes are the better carriers for acyclovir compared to liposomes due to their superior loading and slower release of the drug. Niosomes were also found to improve the oral bioavailability of the acyclovir compared to liposomes.

5.2 Micelles

Micelles are amphiphilic colloidal structures, with particle diameters from 5 to 100 nm range. Micelles consist of molecules containing two completely different regions that have opposite affinities against water. These amphiphilic molecules, which form the micelles, associate at certain temperatures and in appropriate concentrations. The core of the micelle is formed by the hydrophobic fragments of amphiphilic molecules, whereas micelle's shell consists of hydrophilic fragments of micellar molecules. Micellar amphiphilic molecules at low concentrations exist separately in aqueous medium. The aggregation of micellar molecules takes place if their concentration is increased. But aggregation of micellar molecules happens only within a limited concentration interval. The critical

micelle concentration is the concentration of a monomeric micellar amphiphile at which aggregation begins and micelles appear. The critical micellization temperature is the temperature at which aggregates appear and below which micellar molecules exist as monomers. The aggregation of amphiphilic molecules and formation of micelles happens after the removal of hydrophobic fragments of the micellar molecules from the aqueous environment and reconstitution of hydrogen bonds in water, leading to a decrease of free energy in the system. Micelles used as carriers for therapeutics in aqueous media can carry lipophilic drugs within its core while micelle's surface binds polar molecules. Improved aqueous solubility and thus better intestinal permeability of micelles is achieved by formation of polymeric micelles. Polymeric micelles are formed of amphiphilic block copolymers and compared to conventional micelles show greater stability in vivo. Viruses use lectin receptors on host cells for their entry into the cells; the infected cells also express these lectins. In order to target viral reservoirs micelles consisted of PEG-polylactide copolymer surface modified with galactose are constructed, since galactose residues can interact with lectins.

5.3 Microspheres

Microspheres are spherical microparticles that can be used as drug carriers. The size of microspheres is in the micron range. Microspheres are mostly formed of biodegradable polymers. There are two types of microspheres: monolithic-type (matrix-type) and reservoir-type (capsular). The capsular type of microspheres is also called microcapsule. Microspheres are able to incorporate a wide range of different drugs, they are biocompatible and can be prepared from biodegradable particles. Microspheres formulated from biodegradable particles such as poly-D,L-lactide and poly(D,L-lactide-*co*-glycolide) with encapsulated antiviral drug acyclovir have been proposed for intraocular administration.

Prolonged release of acyclovir was achieved by its encapsulation within poly(D,L-lactide-*co*-glycolide) enabling reduction of the dose of acyclovir. Addition of vitamin A palmitate to poly(D,L-lactide-*co*-glycolide) microspheres with acyclovir increased loading of acyclovir, leading to prolongation of in vitro release of acyclovir for 50 days. There were many attempts to increase the bioavailability of acyclovir after oral administration. Mucoadhesive microspheres made from thiolated chitosan enhanced retention of the drug in the upper gastrointestinal tract and thus increased the oral bioavailability of acyclovir. Another form of mucoadhesive microspheres with encapsulated acyclovir made from ethylcellulose as matrix and Carpobol 947 (Lubrizol, Wicklife, OH, USA) as the mucoadhesive polymer also prolonged the residence of acyclovir in gastrointestinal tract of rats and improved its oral bioavailability. Microspheres with encapsulated interferon alpha have been designed for oral delivery. Microspheres containing cross-linked malonylchitosan with encapsulated acyclovir as topical preparation were designed in order to increase the concentration of the drug in the basal layer of epidermis. Also there are attempts to use microspheres as delivery systems for vaccines in order to sustain immunological challenge.

5.4 Dendrimers

Dendrimers are polymeric branched nanostructures with inimitable, perfect structural features. Dendrimers have central cores from which branches emanates thus forming the three-dimensional tree-like structures. They have three main portions, core, inner, and outer shell, and all these portions can have different properties as solubility, or binding the ligands. The size of the dendrimers is relatively small, diameter is lower than 100 nm, and they can bind different ligands, which make them attractive for the drug delivery. The precise biological and physicochemical characteristics of dendrimers can be achieved with usage of different types of polymer and functional groups. Also, single dendrimer can be conjugated with different drugs or other ligands. Dendrimers can be used for delivery of DNA, siRNA and antiviral therapeutics. The most widely known dendrimer used for antiviral therapy is VivaGel, formulated as mucoadhesive gel. VivaGel is the first topical nanomicrobicide formed from the divalent benzhydylamine amide of L-lysine and contains 32 sodium 1-(carboxymethoxy)naphthalene-3,6-disulfonate as terminal anionic functional groups that enable the prolonged duration of antiviral activity.

5.5 Nanoparticles

Nanoparticles are small solid, colloidal, polymeric particles with diameter lower than 1 μm. The structure of nanoparticles is similar to microspheres and can be matrix-like (nanoparticles) and capsule-like (nanocapseles). Different materials, proteins, lipids, inorganic materials can be used for creation of nanoparticles. However, polymeric nanoparticles are mostly made of natural or synthetic biocompatible and/or biodegradable polymers such as poly(lactic-*co*-glycolic acid), poly(lactic acid), alginate, cyclodextrin, hyaluronic acid, poly(glycolic acid), and polycaprolactone. Usage of these polymers maximizes tissue compatibility and reduces cytotoxic effects. Within the nanoparticles, the active molecules can be dissolved, encapsulated, adsorbed or conjugated. Since their size is very small, nanoparticles can be applicated intravenously. As it was described for liposomes, the phagocytosis of nanoparticles can be avoided by the addition of hydrophilic components, like PEG chains, on their surface. These PEGylated nanoparticles are often labeled as stealth nanoparticles.

The different polymeric nanoparticles (made from polylactide, poly(isobutyl cyanoacrylate), poly(ethylacrylate, methacrylate, and chlorotrimethyl methacrylate)) with incorporated acyclovir increased the activity and oral bioavailability of acyclovir.

Lipid nanoparticles, made from lipids that are solid at body temperature can also be used for the delivery of therapeutic agents. There are two types of lipid nanoparticles: solid lipid nanoparticles and nanostructured lipid carriers. Solid lipid nanoparticles are in stable solid state, have the ability to protect the ingredients with labile chemical properties, and can modulate the release of the drug. Nanostructured lipid carriers contain the solid lipid matrix and partly a liquid lipid phase content. Lipid nanoparticles have an advantage in comparison with liposomes and conventional emulsions due to their ability to overcome the problems of stability of the membrane that is associated with drug leaching. Lipid nanoparticles containing acyclovir in vitro showed greater activity than the free drug. Also, lipid nanoparticles with incorporated adefovir dipivoxil has been prepared for the therapy of hepatitis B infection.

Protein nanoparticles prepared from albumin polymers have also been developed as delivery system for ganciclovir and for antisense oligonucleotides for therapy of Cytomegalovirus infections. Cationic gelatin nanoparticles increased the immunostimulatory effects of CpG oligonucleotides.

Several studies showed antiviral potential of the silver nanoparticles. Ag^+ ions released from silver nanoparticles interact directly with biomolecules that contain phosphorus or sulfur, including proteins, DNAs, and RNAs. It seems that silver nanoparticles interfere with several stages of the viral replication cycle including the attachment of the viruses to the cell membrane and their entry into the cells, DNA and RNA replication, and protein synthesis.

6 TARGETED DELIVERY OF ANTIVIRAL AGENTS

Paul Ehrlich postulated the magic bullet theory in 1906. Progress on the synthesis of novel nanomaterials enabled site-specific drug delivery. There are three different strategies for drug targeting of precise localization: direct injection of the drug to a precise site, passive targeting, and active targeting.

In passive targeting, drugs can reach a specific organ or tissue carried by the nanoparticulate with specific intrinsic properties. Different sizes of nanoparticles, or lipophilicity, surface nanoparticulate carrier with incorporated drug to its target tissue. Small sizes and surface characteristics of nanoparticles enable their taking up by the lymphatic tissue in the gut, more specifically by the Peyer's patches containing M cells, after oral administration. After M cells take up nanoparticles in a size-dependent manner, lymph vessels transport them to the lymphocytes. This kind of absorption of a drug after oral administration provides the bypass of the systemic metabolism of the drug in the liver, further accumulation of nanoparticles in the lymph nodes permit targeting the lymphatic system. Since these nanoparticles accumulate in lymphatic tissue, they could be used to target viral reservoirs retained within this compartment. Lymphatic targeting with nanoparticles in injectable systems can be achieved with the particles of appropriate size, enough small to disperse through the interstitium around the injection site, but also enough large to drain through the lymph vessels. The optimal size of nanoparticles is in the range of 10–100 nm. Besides, following interstitial injection, hydrophilic nanoparticles can be cleared more rapidly than hydrophobic nanoparticles.

Active targeting can be achieved by different strategies, mostly by surface modifications, in particular via a specific ligand-receptor-like mechanism. The first strategy is based on usage of monoclonal antibodies specific for antigens expressed on certain cells or tissues. The other strategy used to target precise tissues or intracellular compartments is the usage of stimuli-sensitive nanocarriers. These stimuli-sensitive nanocarriers can be sensitive to inherent stimuli of the pathological site or intracellular organelles, such as abnormal temperature values, or

pH value, or can be sensitive to externally applied stimuli, for example, magnetic field, temperature, or ultrasound. All these internal and external stimuli affect nanoparticles, dissolve or modify them, or guide the sensitive nanocarriers, and the final result is the release of the carried drug in a precise region. The great interest in the field of therapeutic applications is for pH-sensitive nanocarriers due to the fact that enveloped viruses in the acidic environment of the endosomal lumen reject their envelope and thus infect the cell. Liposomes, nanogels, and polymeric micelles are some of the pH-sensitive carriers that can promote the intracellular release of the encapsulated drug when the pH changes. These pH-sensitive carriers are stable at physiological pH level, but in an acidic environment, such as a lysosomal environment, become unstable and release their aqueous content in the intracellular space. Labeled nanocarriers can be guided by the external stimuli, for example, by a magnetic field, or by the application of a certain temperature or ultrasound. In the case of magnetic drug delivery, the external magnetic stimulus guides the microparticles with encapsulated drug to the specified tissue, inducing the accumulation of the carrier in the target organ. However, the release of the drug from its carrier is the passive process and depends on the properties of the microparticle system. On the other hand, usage of ultrasound as external stimuli allows the activation of the release of the drug at the site of action.

Drug targeting using surface-modified nanocarriers is a strategy that permits delivery at the organ or even cell level. Targeting of macrophage can be achieved by usage of polyhexylcyanoacrylate nanoparticles with a diameter of approximately 200 nm. Further, enhancement of uptake of nanoparticles by macrophages can be enhanced by addition of targeting moieties to nanoparticles. The example is mannan-coated nanoparticles that target macrophages by exploiting the presence of receptors for mannose on macrophages and enter the macrophages by mannose receptor-mediated endocytosis. Further, coupling TAT peptide to the surface of the nanoparticles enables endosomal escape of the nanoparticulate allowing the control of intracellular distribution of the drug, which is important for the therapeutics that must reach cytosol or the nucleus.

7 TREATMENT OF SEVERAL ENVELOPED VIRUSES WITH NANOCARRIERS

7.1 Human Immunodeficiency Virus (HIV)

HIV-1 infects various human cell types, such as T lymphocytes, monocytes/macrophages, dendritic cells, Langerhans cells, and microglia. However, the most drastic effect of HIV infection is the result of the destruction of the CD4$^+$ T lymphocytes, which play a central role in the capacity of the host to mount effective and protective immune responses to a wide range of infective agents. HIV infection is characterized with an acute phase of intense viral replication and dissemination to lymphoid tissues manifested with flu- or mononucleosis-like illness. That is followed with activation of innate and adaptive immune response unable to clear the highly replicating and mutating virus; slow viral replication and immune activation is continued (chronic [persistent] asymptomatic phase, clinical latency) and final stage of the infection, immunodeficiency (AIDS) with opportunistic infections is the result of marked depletion of CD4$^+$ T lymphocytes. HIV is an enveloped virus, 100 nm in diameter, contains two copies of RNA coated with the nucleocapsid protein, the RNA–protein complexes are enclosed in a capsid (called p24), which is covered by a membrane-associated matrix (called p17) protein. The viral envelope contains two, virally encoded, glycoproteins gp120 and gp41. The virion core also contains three virus-specific

nonstructural proteins (enzymes) that are essential for viral replication: reverse transcriptase, protease, and integrase. Gp120 is needed to attach to the host cell, while gp41 is critical for the cell fusion process. HIV adheres to the cells that express the CD4 molecule, and the gp120 molecule binds CD4 molecules resulting in the conformational change of the gp120. After conformational change, gp120 binds the second cellular molecule, chemokine receptors CCR5 or CXCR4, known as coreceptors. The next event is the conformational change of gp41 that enables fusion of the membranes and delivery of the capside to the cytoplasm. Virion-associated RNA-dependent DNA polymerase, reverse transcriptase, mediates the reverse transcription of RNA into complementary DNA. DNA-dependent DNA polymerase activity of the same reverse transcriptase enzyme mediates the conversion of this complementary DNA to a double-stranded DNA. The viral RNA template is removed from the RNA–DNA hybrid by RNAase H activity of the same reverse transcriptase enzyme. The newly synthesized DNA integrates into the cellular genome and later is transcribed into the full-length viral RNA and also into the number of spliced mRNA transcripts. The process of the transcription of the viral DNA and translation is mediated by the cellular enzymes. The synthesized viral proteins together with the newly transcribed viral RNA are transported to the cell membrane, where assembly of the virus particle takes place and the virus buds from the cellular surface and exits into the extracellular space.

The life expectancy of individuals infected with HIV is significantly improved after the introduction of highly active antiretroviral therapy (HAART). HAART is a therapeutic approach that includes several antiretroviral drugs, with different mechanism of action. Currently, there are six classes of antiretroviral agents, including nucleoside analog reverse transcriptase inhibitors (NRTIs), nonnucleoside analog reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), the gp41 fusion inhibitor (enfuvirtide), CCR5 antagonists (maraviroc), and integrase inhibitors (raltegravir, elvitegravir, doluyegravir). However, the constant emergence of drug-resistant viral strains caused the need for development of new therapeutic approaches.

To overcome the therapeutic selectivity and low efficacy of therapeutics used for the treatment of HIV infected individuals, different nanostructures have been synthesized over the years and proposed as prophylactic agents against HIV. The first attempt to use nanoparticles in antiviral therapy was a synthesis of silver nanoparticles with the potential to control HIV infection. Silver nanoparticles in sizes from 1 to 10 nm establish interactions with HIV. Although other mechanisms of action of silver nanoparticles several studies indicate that their interaction with glycoproteins in HIV envelope prevents the binding and fusion with cell membrane and thus disables HIV to enter the susceptible cell. Poly(N-vinyl-2-pyrrolidone) (PVP)-coated silver nanoparticles bind the residues of the gp120 glycoprotein and thus block interaction of the virus with host cell. Since the antiviral effect of AgNPs exerts at very high concentrations, PVP-coated AgNPs have been proposed only as a potential topical vaginal microbicide to prevent HIV-1 transmission.

Further, various different delivery systems for targeting of HIV infected cells were explored—namely, liposomes, dendrimers, and biodegradable and nonbiodegradable polymers. Recently, albumin nanoparticles with incorporated antiretroviral therapeutic, efavirenz, were synthetized. The size of these particles was 250 nm and analysis showed that this carrier significantly increased the delivery of efavirenz into several tissues compared with free drug. Another approach involves viral DNA that codes gag and env viral glycoproteins adsorbed on the surface of cationic poly(lactide-*co*-glycolide) nanoparticles in order to induce adequate immune response to HIV antigens. The polimeric

nanoparticle and the viral DNA together form a pathogen-like nanoparticle, known as Derma-Vir. Sugar residues that have the role in the uptake by antigen-presenting cells are present at the surface of the DermaVir nanoparticles. The role of nanoparticule polymer inside the cell is to protect the viral DNA from endosomal degradation and to facilitate the delivery of these DNA into the nucleus, which is required for the expression of DNA-encoded antigens. It was shown that gold nanoparticles also could have the role in the inhibition HIV fusion with host cells. However, the usage of gold nanoparticles is followed with toxic side effects that are related to size, shape, charge, composition, and surface functionality of the nanoparticles. Appropriate surface coating, which also enables better selective targeting and internalization can achieve the significant reduction of gold nanoparticles toxicity. The advantage of the nanostructure constructs is the transformation of biologically inactive molecules into a multivalent conjugate enable to effectively inhibit fusion of HIV envelope and human cells. CCR5 antagonist, SDC-1721, is unable to bind the receptor with high affinity, but its conjugation with gold nanoparticles in multivalent manner transform biologically inactive SDC-1721 into therapeutically active molecule and allow effective HIV-fusion inhibition. Also, the DC-SIGN-mediated HIV transinfection of human T lymphocytes can be prevented with gold nanoparticles coated with oligomannoses from the gp120 molecule. It was shown recently that carbon nanotubes have advantages over other nanoparticles. Carbon nanotubes are able to cross cell membranes and deliver therapeutics into various types of cells. Carboxyl groups in nanotubes allow prolonged contact of therapeutic with its target molecule through promotion of electrostatic and hydrogen bond interactions with the amino acid residues of reverse transcriptase. Highly hydrophilic and dispersible carboxylated multi-walled carbon nanotubes with incorporated antiretroviral drugs and lamivudine were recently synthesized and their antiviral potential was reported.

7.2 Hepatitis C Virus (HCV)

HCV infection causes acute or chronic hepatitis. A significant percentage (50%–80%) of HCV infections have chronic courses leading to cirrhosis, hepatic insufficiency, and hepatocellular carcinoma over several years. HCV virion of 50 nm in diameter contains a very simple, positive sense, single-stranded RNA genome, enclosed in an icosahedral capsid and a lipid-bilayer envelope containing two virus-specific glycoproteins E1 (gp31) and E2 (gp70). Six groups and many different subgroups of HCV were identified based on the phylogenetic analysis of HCV strains isolated in many different regions of the world. Therapeutics used currently for therapy of HCV infections mostly target HCV protease. HCV protease inhibitors, if given alone, generate virus resistance quickly. Approved therapy for patients with HCV genotype 1 infection is HCV protease (NS3/4A) inhibitors boceprevir and telaprevir in combination with pegylated interferon-α and ribavirin. However, drug-resistant mutant strains of HCV virus continuously arise, inducing reduced efficiency of the current therapeutics and need for the development of new drugs.

It was demonstrated that the HCV has multiple strategies to escape the immune responses of the host during the entry into the cells, including the spreading of the virus from cell to cell and evasion from neutralizing antibodies. Now it is considered that well-conserved receptors and coreceptors present on the host cells are more promising targets for therapeutics that can efficiently prevent HCV infection independently on the virus genotype. The examples of these target molecules are CD81, SR-B1, and claudin 1. Usage of small compounds that target glycoproteins in the HCV envelope and thus block viral entry is genotype-specific. Usage of nanostructures could be an alternative approach in the therapy

of HCV infections. The potential of lipid nano-somes and cationic liposomes with incorporated siRNA to inhibit expression of HCV gene have been proven in vivo. It was shown that the al-ternative therapy for PEGylated IFN-α can be a target-specific hyaluronic acid interferon-α gold conjugate. The hyaluronic acid interferon-α gold conjugate significantly enhances the expression of 2,5′-oligoadenylate synthetase 1 in the liver tissue. It is known that 2,5′-oligoadenylate syn-thetase 1 has a role in the innate immunity to viral infection; it has RNAse activity. Dextran-coated magnetic iron oxide nanoparticles conjugated to a synthetic DNAzyme, induce knockdown of the HCV gene, which encodes helicase and protease essential for the virus replication. These dextran-coated magnetic iron oxide nanoparticles accu-mulate primarily in the liver and have a great potential for application of anti-HCV drugs. Recently, it has been shown that boronic-acid modified nanostructures inhibit entry of HCV into hepatocytes. Among the molecules report-ed to inhibit HCV entry in hepatocytes are vari-ous glycan-recognizing proteins, lectins. Lectins selectively interact with glycans present on the surface of the viruses. It was shown that lectins, cyanovirin-N, and griffithsin have the great po-tential to interact with high-mannose glycopro-teins on the surface of HCV envelope reducing the contact of viruses with host cells and the entry of HCV into the cells. So-called pseudo-lectins, such as boronic acid-based compounds, are much simpler structures than natural lectins and are more accessible synthetically. There are many advantages of boronic acid-based pseu-do-lectins over their natural counterparts: their interactions are more stable, they are less expen-sive to produce and purify, and they do not exert mitogenic stimulation. The affinity of the lectins for appropriate glycan significantly increases when multiple copies of these synthetic lectins (e.g., boronic acid derivative) are attached onto polymeric nanoparticle. Besides boronic acid based pseudolectins, many other pseudo-lectins have been investigated as possible therapeutics

for HCV including silica-, diamond-, and iron-, derived nanoparticles. However, these nano-structures have moderate maximal inhibition potential, which is the major obstacle for their further development as inhibitors of viral entry. More powerful inhibitors of HCV entry are am-phiphilic boronic acid moieties inserted in the lipid nanocapsules.

7.3 Herpes Simplex Virus (HSV)

Herpes simplex viruses are ubiquitous and have existed throughout human history. After the initial lytic infection, latent infection is estab-lished. Latent infection persists for the life of the host. There are two types of HSV, type 1 and type 2 (HSV-1 and HSV-2) and they both infect around 60%–90% of adults worldwide. HSV-1 is mainly associated with infections of the face and central nervous system, while HSV-2 mainly causes in-fections of the anogenital area. Infections with HSV-1 in immunodeficient persons can be life threatening. HSV is an enveloped virus that has a central core with the linear double-stranded DNA. There are 11–12 viral glycoproteins in the HSV lipid bilayer envelope. The entry of HSV entry into the host cells is the most critical step in pathogeneses of viral disease and depends on the binding at least five glycoproteins of viral envelop to three different receptors on the mem-brane of the host cell (nectin-1, nectin-2, herpes virus entry mediator, and cell surface glycos-aminoglycans, preferentially heparan sulfate). The important step in the HSV attachment to the cell is irreversible binding of two viral gly-coproteins with cellular heparan sulfate. Drastic reduction in susceptibility to HSV infections is noticed in cells deficient in heparan sulfate in-dicating that heparin can be a highly attractive target for nanoparticles conjugated with anti-heparan sulfate peptide. The viral DNA replica-tion is mediated by viral DNA polymerase. Most drugs used for therapy of HSV infections target the viral DNA polymerase. A guanosine ana-logue, acyclovir, is the most important clinical

drug for the prophylaxis and treatment of HSV infections. Although acyclovir is considered gold standard in therapy of HSV infections, its extensive use has led to the emergence of acyclovir resistant HSV strains, mostly in immunodeficient persons. This caused the intensive search for alternative drugs, and for alternative therapies that block with viral attachment to the host cells at different levels. Recently, it was shown that only a few structures have anti-HSV activity; gold and silver nanoparticles with sulfonate functions, inhibited the viral entry and prevented the viral spreading from cell to cell. Gold nanoparticles with the mercaptoethane-sulfonate 4 nm in size imitate heparan sulfate present on the host cell and thereby block HSV attachment to the cell and inhibit viral entry. It was reported that partially negatively charged ZnO micro-nanostructures (MNSs) arrest HSV-1 in vitro. Gold nanoparticles conjugated with tannic acid reduce HSV-2 infectivity in vitro and in vivo, due to the high affinity interaction of tannins with HSV glycoproteins that prevents the attachment and entry of HSV in the susceptible cells. Since the HSV glycoprotein spikes has an average space of 9–13 nm between center-to-center, tannic acid-modified gold nanoparticles with diameter of 33 nm had the best antiviral effect. Since, therapeutics used for the treatment of HSV infections—acyclovir, peniclovir, and oxyresveratrol—have the slow absorption in the gastrointestinal tract and consecutively low oral bioavailability, new approaches consider usage of polymeric nanoparticles with encapsulated conventional antiviral drugs. Polycaprolactone matrices and 400 nm spherical carboxylated cyclodextrin-based nanosponges containing acyclovir showed better efficiency against a clinical isolate of HSV. Microemulsions with introduced acyclovir could be used as more effective topical preparations. Also, microemulsion-based topical formulations with introduced penciclovir showed increased penetration of penciclovir through mouse skins. Idoxuridine with liposomal carriers also showed increased retention of idoxuridine in the skin compared with classic preparation. In mice it was shown that intravaginal administration of poly(lactic-co-glycolic acid nanoparticles with encapsulating siRNA that knock-down the host-cell protein nectin effectively prevents genital HSV-2 infection.

8 CONCLUDING COMMENTS

It is expected that usage of innovative nanomedicine will have the great positive effects in the treatment and eradication of infectious diseases. Nanoparticles could improve the efficacy of antiviral drugs and reduce the adverse side effects of the drug. These characteristics of nanoparticles are relevant in antiviral therapy in which the high dose of a drug is needed and the drugs are often very expensive. Usage of nanoparticulate carriers can reduce the frequency of the drug intake and the time of treatments, enhance the effectiveness of the approved antiviral therapeutics and overcome their limitations, such as low bioavailability.

The main objectives of the future research of antiviral therapy will be the finding the new technologies for the characterization of nanomaterials, the development of highly biocompatible and biodegradable nanocarrier systems that have no cytotoxicity, the development of nanocarriers that effectively target specific sites of viral infection and thus have reduced drug toxicity in other tissues, and the design of nanomolecules that, besides their role in delivery, possess the intrinsic antiviral therapeutic characteristics (dendrimers and metal nanoparticles).

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