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Review article

Potential targets for antiviral chemotherapy*

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Summary

Serendipity and random screening have been successful in producing effective antiviral agents. The increase in our knowledge of the basic biochemistry of viral replication and of virus–host interrelationships has revealed not only an understanding of the targets upon which existing antiviral agents exert their inhibitory effect, but also has uncovered new potential targets. The hope is that such molecular understanding will afford the synthesis of compounds with selective antiviral activity. A review of various viral targets which are potentially susceptible to attack, and a few approaches for development of antiviral agents are presented.

antiviral agents; chemotherapy; viral targets

Introduction

There are many compounds that have potent antiviral activity in cell culture and in experimental animals, but only 5 have been approved by the FDA in the United States for clinical use. These include: amantadine · HCl in 1966 for prevention or very early treatment of infections caused by the influenza A viruses; 5-iodo-2′-deoxyuridine (idoxuridine) in 1962, 5-trifluoromethyl-2′-deoxyuridine (trifluridine) in 1964, 9-β-D-arabinofuranosyladenine (vidarabine, ara-A) in 1972, and 9-(2-hydroxyethoxymethyl)guanine (acyclovir) in 1982, for various infections caused by members of the herpesvirus family. In addition to these drugs, a number of other compounds are

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undergoing either clinical or pre-clinical evaluation. Unfortunately none of the compounds either in clinical use or on the horizon are without some adverse characteristics, and hence there is a need to develop antiviral drugs with improved properties.

The ideal approach is to identify potential targets that are unique to the virus, and which, if successfully attacked would produce no detrimental effect to the individual. A number of reviews have appeared recently which address this problem [14–16,21,22,40,73,81,82,84–86,99].

What are the prospects for future antiviral drug development? Is there a rational approach? Two major problems exist: first to identify virus specific targets, and second to develop a compound that selectively interacts with the target. Today we know of a number of virus specific targets. These have been found either from elucidation of the molecular basis for antiviral activity of existing compounds, or from studies of the molecular biology of viral replication. What some of these targets are will be discussed, but how to develop antiviral drugs with this knowledge will be the critical problem.

The ideal target is one that will prevent a viral infection, and this is best approached by the use of an appropriate vaccine. The perfect example of this approach is the world-wide elimination of the virus responsible for smallpox. The target for a vaccine is the virus prior to penetration into the cell when the virus is present either in the blood stream or in a reversible phase of adsorption to the cell. Although vaccines are available to prevent a number of viral infections, vaccines are not available for all viral infections of clinical importance for a variety of reasons.

The **rhinoviruses** are a major cause of respiratory disease, but there are over 100 antigenically distinct strains and it would be difficult to prepare a vaccine effective against all.

The **influenza** viruses present another problem in that strains of this virus continuously change in antigenic composition. Although a vaccine is available, the vaccine developed this year may or may not be effective against the strain of virus that will appear next year.

The **herpesviruses** are responsible for a number of clinically important infections, but some members of this virus family (e.g. HSV-1 and HSV-2) are neurotropic and may not have a viremia state prior to establishment of an acute infection or of latency. Nonetheless a herpesvirus vaccine could be of value to prevent the spread of the virus via the blood stream to other tissues. There is considerable effort being made in the United States, Europe and Japan to develop an effective vaccine against the herpesviruses. The present status of antiviral vaccines has been reviewed recently [1,7,63,114].

In the absence of an effective vaccine for a specific virus, we are dependent upon the development of an antiviral drug. What are some of the targets that one can consider as potential sites for antiviral drug development? Various targets for which antiviral agents have been demonstrated to exert an inhibitory effect, or which are potential targets for future drug development are listed in Table 1.

Target 1: Virus adsorption to the cell surface

Reproduction of a virus requires that the virus adsorb to the cell membrane and subsequently penetrate into the cell. This involves an initial reversible interaction of

TABLE 1

Potential targets for antiviral drug attack

(1)	Adsorption of the virus to the cell surface
(2)	Transport of the virus across the cell membrane
(3)	Uncoating of the virus
(4)	Release of the viral genome into the cytoplasm or transport into the nucleus
(5)	Replication of the viral genome
(6)	Transcription
(7)	Post-transcriptional modification of viral RNA: methylation, polyadenylation, capping and splicing
(8)	Translocation of nuclear RNA
(9)	Protein synthesis and processing
(10)	Virus encoded enzymes
(11)	Assembly of macromolecules into a virion (maturation)
(12)	Release of virions from the cell

the virus with a receptor on the host cell membrane, which quickly undergoes a change, such that the virus can no longer be dissociated from the cell by mild procedures. The nature of the conversion from reversible to irreversible binding is not clear, but it may involve an increase in the fluidity of the cell membrane, which allows other binding sites on the virion to react with additional cell receptor molecules. The site of attachment may be a glycoprotein, a glycolipid or contain functional sulfhydryl groups. Presumably the initial interaction is electrostatic and serves to orient the virus–cell association for subsequent penetration or transport across the cell membrane into the cytoplasm [20,30].

Specific binding sites for some viruses have been demonstrated, and this may involve specific recognition of a virus protein by a host-cell membrane macromolecule [18,113]. With some viruses, such as the myxovirus (influenza) or the paramyxovirus (measles), a glycoprotein present on the surface of the virion is cleaved by a host cell protease to form two polypeptides which remain bonded by a disulfide linkage. This creates a new highly hydrophobic amino acid region, which is required for interaction of the virion with a receptor on the cell membrane. Those cells that do not have an activating protease are not susceptible to infection. Choppin and colleagues [17,88] have synthesized oligopeptides with amino acid sequences similar to the N-terminal region of the cleaved viral glycoprotein, and these compete with the virus for sites on the cell membrane. This results in the specific inhibition of the infection process and is an example of how an antiviral agent can be designed based on knowledge of the structure and function of a viral protein that is critical for survival of the virus.

Another approach which has not been fully exploited relates to the findings that various negatively charged polyanions, such as heparin, prevent or decrease the rate of attachment of the virus to the host cell, presumably by forming a complex with the virus (references cited by Shannon [96]).

Targets 2 and 3: Transport of the virus across the cell membrane and subsequent uncoating

Enveloped viruses enter cells by direct fusion of the viral membrane with the host cell plasma membrane, by endocytosis, or possibly by both procedures [116]. Direct fusion of the viral and host plasma membranes results in release of the viral nucleoprotein into the cell cytoplasm. Alternately, the virus, after binding to the plasma membrane of the cell, may migrate to clathrin coated pits, and enter the cell by endocytosis, into vesicles called 'endosomes'. The endosome containing the virus fuses with a lysosome and the pH within the vesicle is decreased to about pH 5 by an ATP-driven proton pump. This acidification causes the protein of influenza and possibly other enveloped virions to undergo a rearrangement, which is required for fusion of the viral membrane with the membrane of the vesicle.

Thus, one of the proposed mechanisms of action of the drugs amantadine and rimantadine, which are primary amines, is to increase the pH within such vesicles. The increased pH prevents the conformational alteration of the capsid protein which is required for the fusion process [101,102].

Another target which also would prevent the acid catalyzed conformational change could be inhibition of the H^+ pump responsible for maintaining the pH at 5.0, provided this could be achieved in the infected cell specifically.

It should be noted that the mechanism of action of amantadine is still not clear, and hence other targets have been proposed. One alternative proposed for these compounds is the inhibition of the dissociation of the influenza virus M-protein from the nucleocapsid or ribonucleoprotein transcription complex present in subviral particles. Only ribonucleoproteins free of the M protein may enter the nuclei, therefore inhibition of this dissociation which is part of the uncoating process would decrease virus infectivity [8].

Amantadine may however interact with the external plasma membrane of the cell, thereby altering the surface charge of the membrane with consequential inhibition of endocytosis or uncoating [49,52]. Some evidence has been presented which supports the prime target for amantadine to be on the external surface of the plasma membrane, since it has been found that the antiviral activity is very rapidly lost when cells are transferred into an amantadine-free medium [89]. Also in support of an effect of amantadine on the plasma membrane are the findings that this drug blocks the association of the vesicular stomatitis virus with the plasma membrane clathrin-coated pit regions [92].

Several aromatic mono- and diamidines were found to significantly retard the penetration of the respiratory syncytial virus (a paramyxovirus) into the host cell, but not its attachment to the cell membrane [31]. Two possible mechanisms were postulated: One suggests a strong affinity for a specific binding site on the cell membrane in competition with the virus. The amidines have a strong cationic as well as a hydrophobic region which may afford such an interaction. The second hypothesis suggests these compounds function as potent protease inhibitors. Protease inhibition as a target site will be discussed below. A specific compound may exert an effect at different sites which may depend on the virus. Thus De Clercq found some diarylamidines inhibit

reverse transcriptase, presumably by interaction with the nucleic acid template [25].

Arildone was reported to inhibit polio and herpesviruses by preventing transport or uncoating, and recently several second generation compounds, which are isoxazole analogs, were found to have potent activity against the rhinoviruses and other picornaviruses. These compounds may be preventing the conformational alteration of the capsid which is required for transport or uncoating [29,55,62,70].

Target 4: Integrity and transport of released viral genome

Once the viral genome is released from the virion into the cytoplasm, a vast array of events occur that are frequently unique to a specific virus. The released nucleic acid, depending on the virus, may either remain in the cytoplasm as with poxvirus DNA, or migrate into the nucleus of the cells, as with herpesvirus DNA. How is the nucleic acid protected from enzymic degradation after release? How is herpesvirus DNA transported into the nucleus? Will interference with stabilization of viral DNA transport constitute a viable target? If one can preferentially destroy the uncoated viral genome, then replication of the virus would obviously be prevented.

Target 5: Replication of viral genome

Replication of the viral genome can be interfered with through at least 5 target sites (Table 2).

A. Decreased formation of nucleoside triphosphates

For example, ribavirin after conversion to the monophosphate inhibits inosine monophosphate dehydrogenase, which is one of the key enzymes responsible for the synthesis of guanine nucleotides. The affinity of ribavirin monophosphate for this enzyme is about 70 times larger than the normal substrate IMP. This results in decreased pool sizes of GTP and dGTP and could be responsible for the decreased synthesis of nucleic acids in ribavirin treated cells [104,109].

B. Inhibition of the utilization of nucleoside triphosphates by RNA or DNA polymerase

A variety of nucleoside analogs as well as non-nucleosides such as aphidicolin [34,76] inhibit at this target site. Thus the triphosphate derivatives of IdUrd, $K_i = 0.18$ μM [69], ara-A, $K_i = 0.33$ μM [69] or acyclovir, $K_i = 0.003$–1.42 μM [38], competitively

TABLE 2

Target sites for inhibition of genome replication

A.	Formation of nucleoside triphosphates
B.	Utilization of nucleic acid precursors
C.	Elongation of the genome
D.	Nucleic acid polymerase
E.	Nucleic acid template

inhibit the utilization of dTTP, dATP and dGTP, respectively, during herpesvirus-DNA synthesis.

C. Inhibition or slowing of genome elongation

Genome replication can be affected by premature termination or slowing of genome elongation, as for example by adenine arabinoside (ara-A) or by acyclovir. DNA with araAMP incorporated at the terminus could be further elongated, but this depends on the activity of the 3′-exonuclease associated with the HSV-DNA polymerase. Thus araATP could act as a pseudo chain terminator by continuous incorporation and removal of araAMP from the terminal position, in effect slowing the elongation of the DNA polymer [28,77]. Incorporation of acyclovir into the viral DNA results in termination of DNA synthesis because it does not have a 3′-hydroxyl. Although the HSV-DNA polymerase has an associated 3′-exonuclease, the enzyme cannot remove acyclovir from the 3′-terminus of DNA [39].

D. Inhibition of DNA or RNA polymerase

Genome replication can also be affected by inhibition of the enzyme responsible for the final reaction involved in its synthesis. This is a target of several antiviral agents. Acyclovir not only is a DNA chain terminator, but also, its incorporation into the viral DNA results in the formation of a complex which binds with strong affinity to the viral DNA polymerase and thereby inactivates the enzyme. Thus acyclovir is considered to be a suicide inactivator of HSV-DNA polymerase [39].

Whereas the antiherpes drugs discussed so far require an initial phosphorylation for activation, phosphonoformate (PFA) does not require any metabolic alteration to exert its antiviral activity. PFA may be considered to be an analog of pyrophosphate, which is a product of the DNA polymerase reaction. It is not clear whether PFA binds at, or overlaps the binding site, of pyrophosphate, but both DNA polymerase activity and the associated 3′-exonuclease activity are inhibited [45,66]. It has also been postulated that PFA may form a complex with cations at the active site and this prevents the binding of dNTP or release of pyrophosphate from the enzyme [19,78].

An interesting finding is that certain crown ethers inhibit the polymerase of picornaviruses, and this has been attributed to either binding to an amino acid sequence of the enzyme or chelation of the metal coenzyme [48,50].

E. Inhibition of the RNA or DNA template

Genome replication or function can be affected by an agent that either is incorporated into the macromolecule, or binds to the template. For this reason, synthetic oligonucleotides have been studied as potential antiviral or anticancer agents [13,80,108,110). These compounds may induce interferon, bind to virally encoded polymerases, or hybridize with a complementary viral RNA or DNA. Can an oligonucleotide be synthesized that could be transported into the cell, and then strongly bind or covalently interact with the viral, but not the cellular nucleic acid template? The consequence would be interference or prevention of genome replication, transcription, or both.

Ts'o and his colleagues have synthesized several oligonucleotides with decreased

polarity, by conversion of the phosphodiester linkages between nucleosides to either phosphotriesters or to alkyl phosphonates [111]. His group recently reported that an oligodeoxyribonucleoside methyl phosphonate which is complementary to a section of HSV-1 mRNA, and has a chain length of 8 nucleotidyl units, produced a 2 log reduction in virus yield [103].

Asseline and colleagues [2] prepared an oligothymidylate which was covalently linked at the 3'-phosphate by a pentamethylene chain to the 9-amino moiety of an acridine derivative. The intercalating agent, acridine, markedly increased the interaction of the oligonucleotide with its complementary target sequence. Additional stabilization was obtained by decreasing the polarity of the phosphate moiety by linking it to a positively charged substituent such as piperidinium, a trimethyl ammonium, or a morpholinium derivative. This is a very interesting development with potential for the synthesis of molecules with strong affinity and high specificity for a target nucleic acid base sequence.

Another approach is based on the binding to specific regions of a genome by proteins which may be involved in the regulation of genome expression. Can the viral genome be made a viable target for a tight binding polypeptide with similar specifications? There are a number of examples of specific DNA-binding proteins [6,27,42,64,79,87]. A study of potentially great significance for the design and synthesis of polypeptides for interaction with specific sites of the DNA was reported by Wharton and Ptashne [115] and Frederick and coworkers [35]. Using x-ray crystallographic procedures, these investigators determined the three-dimensional structure of a co-crystal of a regulatory protein and its target DNA sequence. Studies of DNA–protein binding have been reviewed recently [61].

There are a number of synthetic compounds, as well as antibiotics, which may inhibit viral replication by binding to the viral template, such as distamycin A (cited in refs. 4,13,83,96).

In addition to direct interaction with the nucleic acid template, major physical and biological effects are produced by incorporation of nucleoside analogs into the viral genome. For example, single and double-strand breaks in the viral DNA can be produced by incorporation of the 5'-amino analog of idoxuridine (IdUrd) into HSV-1 DNA [33], or single stranded breaks by incorporation of 5-(2-bromovinyl)-2'-deoxyuridine [59].

Target 6: Transcription

There are a number of sites that one could target to prevent the formation of RNA, or to increase its rate of degradation, or to produce non-functional mRNA. Can one interfere selectively with viral RNA biosynthesis? We indicated earlier that one might be able to synthesize a small oligonucleotide or polypeptide for selective binding to the viral template, and if the dissociation of the complex were low, then little or no viral mRNA would be formed.

Another approach to decrease the availability of viral mRNA is to increase the rate of RNA degradation. This has been achieved with interferon or interferon inducers. One of the effects seen in extracts of interferon-treated cells is the activation of two

enzymes (2–5)A synthetase and (2–5)A-dependent endonuclease L. The former, in the presence of double-stranded RNA, catalyzes the synthesis from ATP of (2′–5′)oligoadenylates [(2–5)A]. The (2′–5′)oligoadenylate in turn activates an endonuclease which degrades the viral mRNA (cited in refs. 23,57,95).

Target 7: Post-transcriptional modification of viral RNA: splicing, polyadenylation, methylation, capping

If a virus encoded enzyme were involved in the splicing of primary viral transcripts then this enzyme would be a logical target.

Although the immediate-early transcripts, upon infection with herpesviruses, are produced from the input viral DNA, early and late RNA are transcribed from the newly formed DNA. Interference with the fidelity of the base sequence of the newly synthesized viral DNA will affect the formation of early and late RNA, and thus constitutes a viable target. Specificity may be achieved by the use of antiviral agents that are activated only in the virus-infected cell and are incorporated into the viral DNA or RNA genome. Thus Otto and coworkers [71,72] found early and late but not immediate early transcription was affected when HSV-1 was grown in the presence of analogs of thymidine. The amount of HSV-1 specific poly(A^+) RNA declined about 8 h post-infection, relative to that observed in the control infected cells. This may be a result of incorporation of the analog into the newly synthesized DNA, or by a direct inhibition of polyadenylation. The consequence of producing such changes in RNA were reflected in the proteins that were translated. Vidarabine (ara-A) as the triphosphate was previously reported to inhibit the formation of polyadenylated RNA in chromatin and nuclear sap derived from rat liver nuclei [90].

In addition to polyadenylation of the 3′-end of mRNA, the 5′-terminus undergoes a capping reaction. The initial step in the capping of mRNA involves the transfer of GMP from GTP to the 5′-terminus by mRNA guanyltransferase with the formation of a 5′-5′ linkage via a triphosphate bridge. Ribavirin, after conversion to the triphosphate, inhibits this reaction [104]. Within the cap structure, the guanylate at the 5′-terminus is methylated in the N-7 position by the enzyme guanine-7-methyltransferase. The methyl group is derived from *S*-adenosylmethionine which, after donation of the methyl group, forms *S*-adenosylhomocysteine which is then cleaved by *S*-adenosylhomocysteine hydrolase. Several antiviral compounds such as 9-β-D-arabinosyladenine (ara-A), (*S*)-9-(2,3-dihydroxy)propyladenine, 3-deazaadenosine and the carbocyclic analog of 3-deazaadenosine [24,51] inhibit this hydrolytic enzyme. The consequence is an accumulation of *S*-adenosylhomocysteine which is a potent inhibitor of *S*-adenosylmethionine-dependent methylation reactions, such as of viral mRNA. Inhibition of cap methylation has also been observed in extracts of interferon-treated cells (references cited by Sen [95]).

A unique target is presented by the influenza virus. The synthesis of mRNA by the influenza virus requires the availability of capped host cell RNA. The first step is the cleavage of 10–13 nucleotides from the cap end of a cellular mRNA by a virus encoded endonuclease. This capped oligonucleotide derived from the host cell acts as a primer. The virus transcriptase adds GMP, which is complementary to the 2nd nucleotide

from the 3'-terminus of the viral genomic RNA, to the cleaved capped fragment. The viral transcriptase can now proceed with elongation of the transcript. This unique process should be susceptible to attack. Both the endonuclease and the transcriptase are good targets.

Target 8: Translocation of nuclear RNA into cytoplasm

Whereas mRNA transcripts formed in the cytoplasm have ready access to ribosomes, those formed from viral genomes present in the nucleus must first be transported across the nuclear membrane. Translocation of polyadenylated mRNA from the nucleus to the cytoplasm is regulated by an enzyme present in the nuclear envelope. AraATP inhibits the efflux of RNA, but the mechanisms involved are not known [93]. Is there a difference in the transport of cellular and viral mRNA that one can exploit as a target?

Target 9: Protein synthesis and processing

While immediate early protein synthesis by herpesvirus depends on mRNA transcribed from the input viral genome, early and late protein synthesis is dependent upon mRNA derived from progeny nucleic acid and hence can be affected by factors which do not impact on the very early events [36,46]. For example, incorporation of various nucleoside analogs of thymidine into the DNA of HSV-1 affects protein synthesis as a consequence of 'faulty' transcription [71,72,100].

Interferon, in addition to activating an endonuclease which degrades mRNA, also activates a protein kinase which leads to an inhibition of protein synthesis. The activated protein kinase catalyzes the phosphorylation of the alpha subunit of the initiation factor, eIF_2, thereby inhibiting the initiation of protein synthesis [23,57,95].

Inhibition of protein synthesis has been reported which takes advantage of the observation that membranes of virus-infected cells are altered both structurally and functionally, and therefore allow the transport of highly ionic compounds into the infected cell. Thus the GTP analog, guanosine-5'-(β,γ-methylene)triphosphate, $GppCH_2p$, inhibits protein synthesis in the virus-infected cell but not in normal cells. Membrane 'leakiness' is produced by a number of viruses, including herpesvirus [10,12]. However, Pasternak and coworkers have not found evidence for such a generalized increase in membrane permeability, and have attributed the increased inhibition of protein synthesis by $GppCH_2p$ in the infected cell to an impaired metabolism in these cells as reflected by a lower ratio of GTP/GDP [41,75].

Another potential target is selective binding of macromolecules critically involved in protein synthesis. For example, the formation and regulation of HSV alpha-, beta- and gamma-proteins is sequential and interdependent [46]. Alpha-polypeptides are required for synthesis of beta-polypeptides, which include the virus encoded enzymes involved in DNA replication such as thymidine kinase and DNA polymerase. Can we specifically interact an inhibitor with the alpha-protein receptor thereby preventing essential viral protein synthesis? Gamma proteins shut off the synthesis of beta proteins. Can we interact at this site with a polypeptide which mimics the gamma protein involved, and hence prematurely shut off beta protein synthesis?

RNA and DNA viruses which have envelopes surrounding the viral capsid contain one or more glycoproteins in their membranes. Several compounds are known which affect glycosylation reactions essential for the formation of these viral glycoproteins, and the consequence is either an inhibition of the formation of virions, or the formation of virions with decreased infectivity. Antiviral compounds which affect the synthesis of glycoproteins include 2-deoxy-D-glucose, D-glucosamine, tunicamycin [9,37,106], and more recently 5-(2-bromovinyl)-2′-deoxyuridine (BvdUrd) [68,100].

The processing of certain viral proteins by proteolytic cleavage is a critical event for many viruses. Both cellular and viral encoded proteases are involved in this process. The target in such a system is either the substrate protein or the proteolytic enzyme. A number of compounds target the sulfhydryl group of the picornavirus protease. Korant found carbobenzoxy leucine chloromethyl ketone to be an irreversible inhibitor of the poliovirus protease: both protein cleavage and virus production were inhibited. Zinc inhibits the cleavage of rhinovirus proteins by binding to cysteine or other residues in the protein ([54] and references therein).

Several other inhibitors of proteases have been found. Derivatives of diamidine (bis(5-amidino-2-benzimidozolyl)methane) inhibit specific proteases and also inhibit cell fusion caused by respiratory syncytial virus [31]. Aprotinin, which is a polypeptide of 6000 molecular weight, was found to inhibit the replication of myxovirus by inhibition of the protease required to activate this virus by cleavage of a viral glycoprotein [118]. ε-Aminocaproic acid has been reported to inhibit the proteolytic cleavage of influenza virus haemagglutinin and to exert significant protection to mice and chickens infected with a lethal dose of this virus [117].

Target 10: Virus-encoded enzymes

Appropriate reviews are cited in which references for this section may be obtained [3,5,11,15,22,26,53,67,74,84,97,99].

A major target for the development of antiviral agents is based on the virus introducing into the infected cell enzymes that are encoded by the viral genome. These unique enzymes may be associated with the virion or induced during the infection process. The RNA transcriptase of the influenza and parainfluenza viruses, the RNA replicase of the enteroviruses and rhinoviruses, and the reverse transcriptase of the RNA oncogenic viruses are examples of unique enzymes.

In addition there are a number of virus encoded enzymes which are isozymes of those that normally occur in the uninfected cell. These enzymes may be sufficiently different from the comparable host cell enzyme that selective targeting is possible. Differences between the viral and cellular enzymes may be found in migration during gel electrophoresis, in isoelectric point, in immunological properties, in sensitivity to inhibition, in ability to utilize substrates, or in the binding affinity for the same substrate, etc. Such enzymes are exemplified by DNA polymerase and deoxyribonuclease encoded by HSV-1, HSV-2, varicella-zoster virus, cytomegalovirus and Epstein-Barr virus; thymidine kinase by HSV-1, HSV-2 and varicella-zoster virus; and ribonucleotide reductase by HSV-1, HSV-2 and Epstein-Barr virus.

The thymidine kinase specified by the herpesvirus genome is critical for the activa-

tion of a number of antiviral agents with diverse structures. These compounds are not substrates, or are poor substrates for the cellular thymidine kinase. These unique biochemical properties make this enzyme a prime target for selective activation of antiviral agents in the HSV-1, HSV-2 or varicella-zoster virus infected cell. Compounds such as acyclovir, 5-(2-bromovinyl)-2′-deoxyuridine, 5-iodo-5′-amino-2′,5′-dideoxyuridine, 9-(3,4-dihydroxybutyl)guanine, 9-(1,3-dihydroxy-2-propoxymethyl)-guanine, 5-methoxymethyl-2′-deoxyuridine, and 1-(2-fluoro-2-deoxy-β-D-arabinosyl)-5-iodocytosine are either unique or preferential substrates for the viral thymidine kinase. Activation by phosphorylation is required before they exert their inhibition of virus replication, and thus, these compounds are highly selective antiviral agents.

DNA polymerase is induced by all the members of the herpesvirus family and this enzyme is a unique target, as production of this enzyme is an absolute requirement for virus replication. Not all antiviral agents that inhibit the viral DNA polymerase need undergo an activation process. For example, compounds like phosphonoformate inhibit this enzyme without undergoing any metabolic alteration.

Ribonucleotide reductase, as a target, is under intensive study. Shipman and coworkers [98] found 2-acetylpyridine thiosemicarbazone to be a good inhibitor of HSV-1 and HSV-2. Some have postulated the mechanism to be by chelation of a metal cofactor required for this enzyme. Recently, Spector and coworkers [105] found that a morpholinoethyl derivative of 2-acetylpyridine thiosemicarbazone potentiated the antiviral activity of acyclovir, presumably by decreasing the de novo synthesis of dGTP, and permitting an increase in the pool size of acyclovir-triphosphate. This would result in less competition for interaction of ACV-triphosphate with the viral DNA polymerase.

However, Cheng and coworkers [65] have questioned the utility of viral ribonucleotide reductase as a target, because they found that a 95% inhibition of this enzyme by hydroxyurea produced less than a 10-fold decrease in virus yield. This they attributed to the utilization of deoxyribonucleosides derived from enzymic degradation of host-cell DNA.

Targets 11 and 12: Assembly and release of virion

The macromolecules synthesized during the infection process must interact to form a mature virion. The molecular basis for this assembly process is not completely understood, but should provide a good target. The mature virion must then be released from the cell. Interferon, in addition to exerting an effect on several target sites discussed above, has been reported to also inhibit virion assembly or release in some cells infected with murine leukemia virus (references cited by Sehgal et al. [94]). Amantadine has also been suggested to prevent assembly of the fowl plague virus [44].

Conclusion

Serendipity was primarily responsible for development of those antiviral drugs which we have today. The targets for their antiviral activity were elucidated subse-

quently. Now that we know a number of appropriate targets, how can we exploit this knowledge for the development of selective antiviral drugs or to use the available drugs more effectively?

With the exception of amantadine, the clinically approved drugs are targeted against only certain herpesvirus infections. We are in desperate need to find exploitable targets for treatment or prevention of AIDS or of recurrent genital herpes. We also must find targets to attack the rhinoviruses responsible for respiratory infections and infections of the lower gastrointestinal tract. In addition, there is a great need to find exploitable targets in order to treat infections produced by cytomegalovirus, Epstein-Barr virus, and hepatitis B virus. Other viral infections for which exploitable targets would be welcome include severe enteroviral infections caused by the coxsackie and echo viruses, and acute respiratory disease in small children and the elderly caused by adenovirus, parainfluenza virus and respiratory syncytial virus.

There is a need to find target enzymes other than herpesvirus thymidine kinase for activation of drugs as well as to develop antiviral drugs that do not become incorporated into the viral genome.

As our molecular understanding of virus replication, virus–host interrelations, and the physical chemistry of drug–target interactions increases, new targets for antiviral chemotherapy will no doubt evolve. Once we have identified a target we desire to attack, the problem exists of how to develop an antiviral agent which will exert its effect at this site.

We also have the problem of how to best deliver the drug to the target sites. What is the potential of combination therapy to attack several target sites, and should this be done simultaneously or sequentially?

Some thoughts for the development of antiviral agents have been presented [81]. The question addressed was how can we exploit the fact that the virus encodes unique enzymes and regulatory proteins? How can we determine the structural and spatial requirements for a compound to interact uniquely with such targets? Several approaches to achieve this will now be discussed.

The design of specific enzyme inhibitors on a rational basis was reviewed by Stark and Bartlett [107]. They discussed multisubstrate analogs, transition state analogs, affinity labels and suicide inhibitors, as well as approaches to designing such inhibitors through use of computer modeling and energy minimization programs. Ideally one would like to know the x-ray crystal structure of the enzyme. Using these data, new computer graphics techniques allow the visualization of the three-dimensional arrangement of ligand binding sites as well as how the enzyme and ligand interact sterically and electronically.

If a protein is in the crystalline state, it is possible to combine subzero temperature studies of enzyme-substrate interaction (cryoenzymology) [32] with x-ray diffraction to provide structural information with high resolution. A major problem has been preparation of single crystals of protein large enough that the three-dimensional structure could be elucidated by x-ray diffraction. Littke and John [58] recently reported the successful formation of single crystals of β-galactosidase and lysozyme under conditions of microgravity that were 27 and 1000 times larger, respectively, than those obtained under normal gravitation.

Rossman and coworkers [91] have been reported to have determined the complete three dimensional structure of human rhinovirus-14 by use of the Cyber 205 supercomputer into which they loaded x-ray pictures of the crystallized virus. Such studies may provide the atomic requirements for not only virus interaction with the host cell, but also information for the design of compounds that can interact with the virus.

Nuclear magnetic resonance (NMR) is also being used to define the nature of the drug when it is bound to the enzyme. As stated by Markley and coworkers [60] "The two methods are complementary in that x-ray provides the spatial geometry of atoms in the crystalline solid and NMR yields data on the chemical environment, dynamics, chemical properties, and neighboring groups in solution". Markley and coworkers [60] have used a two-dimensional approach to study protein structure and function.

The application of computer-assisted molecular design for the synthesis of drugs is becoming of increasing importance [43,47,112]. An example of a successful application of these approaches is the design of analogs of trimethoprim by Kuyper and coworkers [56] following determination of the three-dimensional molecular structure of the enzyme, dihydrofolate reductase, by x-ray crystallography.

We have just scratched the surface in our attempts to find additional targets, to develop new antiviral agents as well as to modify existing agents in an attempt to decrease toxicities or other undesirable properties, but yet retain antiviral activity. It is the expectation that the application of physical approaches such as those just described will be of great value in the design and synthesis of clinically useful antiviral drugs. The future looks very promising, but will require considerable effort on our part, and, as Paul Ehrlich so aptly stated, considerable 'Geld'.

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