Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CHAPTER 3

Antiviral drugs: general considerations

3.1. Background

Since viruses, in contrast to bacteria, replicate inside cells and use part of the cellular machinery for this replication, the development of selective antiviral drugs has been difficult and slow. However, the situation improved about two decades ago when the first virus-coded enzymes were characterized and when a few selective antiviral drugs such as idoxuridine, methisazone and amantadine were discovered. These compounds were found by serendipity but today the scientific situation has changed and a number of virus specific processes are used as targets for a more rational selection and design of antiviral drugs.

Possibilities of inhibiting virus multiplication can be localized to discrete events in the virus replication, separate from normal cellular functions. These steps will be briefly discussed in this chapter. A more detailed discussion of the mechanism of action of different inhibitors will be found in later chapters and, for example, the compounds affecting herpesviruses are discussed at some length in Chapter 11. The use of interferon and immunostimulators as antiviral agents are briefly described in this chapter as well as aspects of the inhibition of myxo and paramyxoviruses.

Inhibitors of virus replication could either change a cellular function so that the virus can no longer replicate, or could interact with a viral function as indicated in Fig. 3.1. In the latter case a compound could act directly as an inhibitor of a viral enzyme (e.g. inhibition of herpesvirus DNA polymerase by foscarnet) or be activated by a viral enzyme (e.g. phosphorylation of acyclovir by a viral thymidine kinase) to form a compound which then affects virus replication and, in some cases,

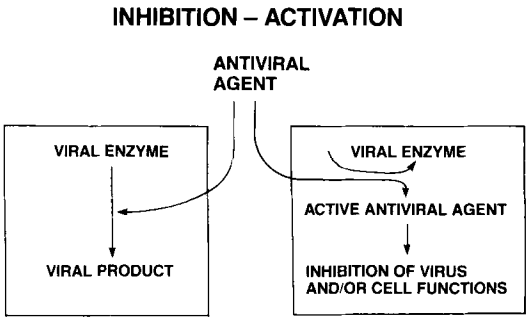

Fig. 3.1. Modes of action of antiviral agents. In the left hand situation the antiviral agent penetrates a cell and selectively blocks a viral function required for the multiplication of the virus. In the other case the antiviral drug penetrates into a cell and is selectively transformed by a viral enzyme into an inhibitor either of the viral multiplication only or of both viral and cellular functions. In the latter case this should result in a toxic effect only on virus-infected cells.

also cellular functions. It is clear from Table 3.1 that most viruses induce RNA or DNA polymerases and also other enzymes which can be used as targets for antiviral compounds.

A large number of authors have reviewed the functions of viral enzymes and their use as targets for antiviral drugs (Kit, 1979, Helgstrand and Öberg, 1980) and have

TABLE 3.1.
Virus specific enzymes which could be useful as 'targets' for antivirals

Virus	RNA or DNA polymerase	Other enzymes
Picorna	+	+
Reo	+	+
Toga	+	+
Orthomyxo	+	+
Paramyxo	+	+
Rhabdo	+	+
Retro	+	+
Arena	+	?
Corona	+	+
Bunya	+	?
Parvo	?	–
Papova	–	+
Adeno	+	+
Herpes	+	+
Irido	+	+
Pox	+	+
Hepatitis B	+	+

discussed different antiviral drugs (Becker, 1976, Swallow, 1978, Chang and Snydman, 1979, Galasso et al., 1979, Shannon and Schabel, 1980, Drach and Sidwell, 1981, Galasso, 1981, Gordon et al., 1981, Oxford, 1981, Sugar, 1981, De Clercq, 1982, Schinazi and Prusoff, 1983). Those aspects mentioned here represent a necessarily small but useful selection of what has been considered by others.

3.2. Virus specific events

The replication of a DNA virus can be schematically outlined as in Fig. 3.2 and several distinct steps can be defined. Apart from the difference due to the type of genome nucleic acid, the replication of an RNA virus is rather similar, as shown in Fig. 3.3. Figure 3.4 outlines the different strategies for replication of positive strand RNA viruses, negative strand RNA viruses and retroviruses. Some properties of these steps will be discussed here with respect to their possible use as targets for antiviral drugs.

3.2.1. Virus adsorption, penetration and uncoating

General considerations

The cellular structures responsible for adsorption of viruses are called receptors. (For reviews see Lonberg-Holm and Philipson, 1974 and Lonberg-Holm and Phi-

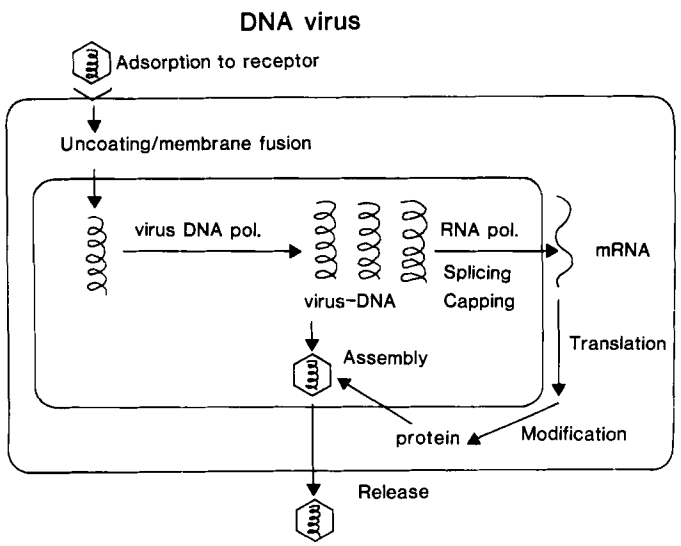

Fig. 3.2. Replication of DNA virus. The figure shows schematically different steps during the multiplication of a DNA virus in a cell.

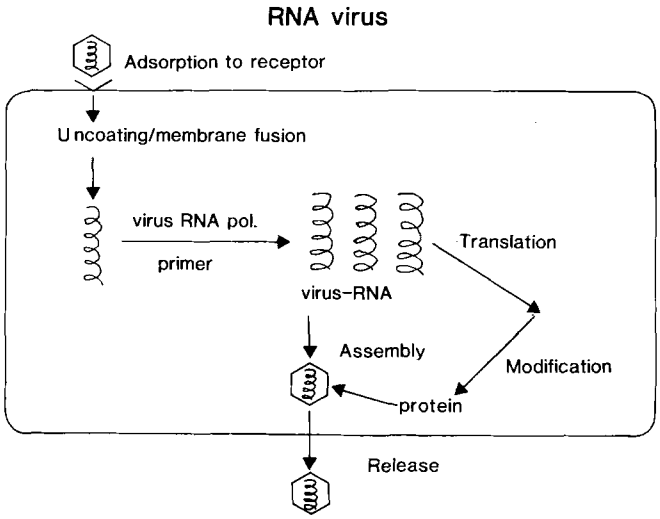

Fig. 3.3. Replication of an RNA virus. The figure shows schematically different steps during the multiplication of an RNA virus in a cell.

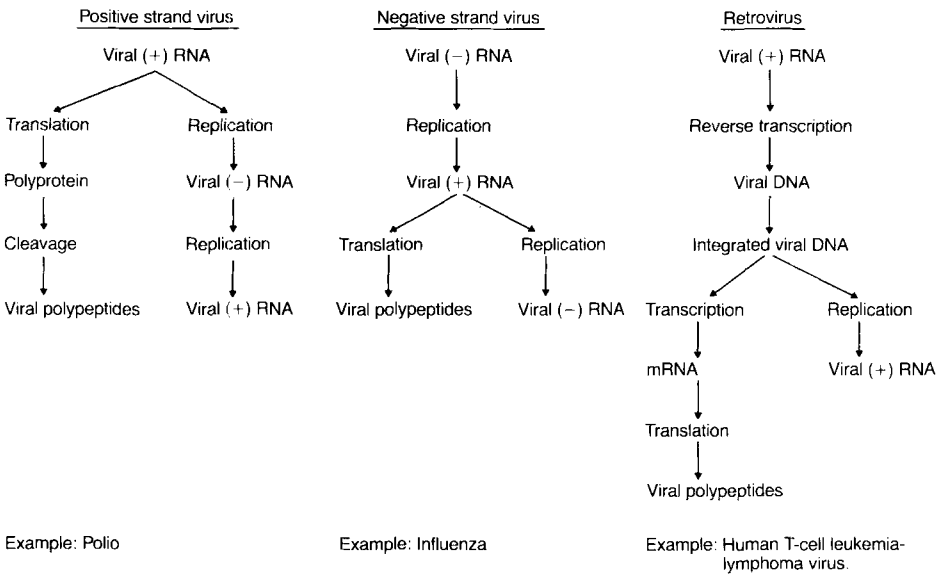

Fig. 3.4. Replication strategies for RNA viruses.

lipson, 1980, Dimmock, 1982). Their functions, apart from the binding of virus particles, are not fully known but for example histocompatibility antigen sites may also be receptors for arboviruses. The receptors seem to be composed of proteins, lipids and often polysaccharides. Different viruses can either share receptors or use differ-

ent receptors. Introduction of receptors in the cell membrane of a cell normally resistant to infection can make it susceptible. The number of receptors for a virus will depend on the type of virus and cell and has been estimated to be $10^4 - 5 \times 10^5$ per cell.

The polypeptide sequence and structure of the protein (HA) in influenza A virus responsible for binding to a receptor is now known and future work in this area will provide detailed information for other viruses about the viral peptide sequences responsible for the binding to a receptor. The possibility of preventing influenza virus adsorption has been investigated using oligopeptides with sequences similar to those of the viral proteins responsible for absorption (see Chapter 8 for details of oligopeptides versus measles virus). An advantage of this type of inhibitor is that it should act on the outside of cells and is not required to penetrate cells. This could be of advantage from a toxicity point of view. A disadvantage is the possibility that these inhibitors are likely to be competitive and might require high concentrations of drug and also that certain of the peptides may be hydrophobic, leading to problems of solubility.

Isolated receptors, or part of them, could possibly be used as antiviral agents by binding to the virus particles and thus preventing their association to receptors on cells. The binding site for vesicular stomatitis virus (VSV) on Vero cells has been characterized in some detail by Schlegel et al. (1983) and was shown to involve phosphatidylserine. The isolated phosphatidylserine binds to VSV particles, but not to HSV, and seems to be an example of a purified receptor structure functioning as an antiviral agent. The plaque formation by VSV is reduced by 80–90% at 1 μm phosphatidylserine.

Viruses such as myxoviruses and paramyxoviruses need to fuse with the cellular membrane at some stage of the life cycle and this process seems to be pH dependent and to involve haemagglutinin (HA) or fusion (F) protein, respectively. Lysosomotropic compounds such as amantadine could possibly act by increasing the lysosomal pH and thus prevent low pH fusion and subsequent infection. This is an important approach which could give inhibitors with a fairly broad range of antiviral specificity. Even though this is an effect on a cell function, the antiviral activity takes place at concentrations not prohibitively toxic to uninfected cells.

Myxoviruses and paramyxoviruses

The combination of the techniques of genetic engineering and rapid DNA sequencing has resulted in extensive new data about the amino acid sequence of influenza proteins. In an early series of experiments Porter et al. (1979) cloned gene 4 (coding for the important haemagglutinin (HA)) protein of an influenza A virus by inserting a DNA copy into an *E. coli* plasmid. Rapid DNA sequencing techniques then allowed a determination of the complete nucleotide sequence of the HA molecule and this classic study has now been extended and applied to the HA of other influenza A viruses. It is apparent by inspection of the amino acid sequences of the HA of

viruses so unrelated antigenically as A/PR/8/34 (H1N1) and an H3N2 virus isolated in the 1970s (See Chapter 7) that certain short amino acid stretches have been conserved over this 45 year period, whereas most of the rest of the molecule is completely different (reviewed by Laver et al., 1980). This might imply a common and important function of this short common sequence, and a resemblance to the amino acid sequence in the fusion (F) protein of the paramyxovirus Sendai led to the suggestion that the sequence at the N terminus of the HA2 polypeptide of influenza HA had a similar function (namely, fusion with cell membranes), allowing exit of nucleic acid from the virus and entry into the cell nucleus. Quite separate biological experiments by White et al. (1981) established that under low pH conditions influenza viruses could lyse red blood cells and cause fusion of tissue culture cells. Richardson et al. (1980) synthesized the short peptides Z-Gly-Leu-L-Phe-Gly and Z-Gly-L-Phe-L-Phe-Gly which mimicked the sequences at the N terminus of the HA[2] polypeptide described above and relatively low concentrations (20–50 μM) of the peptides inhibited influenza virus replication in tissue culture studies (See Chapter 7). The possibility of short peptides acting in vivo as antivirals in a milieu of proteolytic enzymes would seem remote, but it may be possible to synthesize analogues or add groupings to prevent rapid enzyme digestion in vivo.

These biochemical studies have also raised basic questions about the mode of action of amantadine. Since the compound acts early in infection, does it inhibit virus fusion? The interpretation of the data is even more complicated by the results of a recent study (Richman et al., 1982) which demonstrated quite clearly that although amantadine is taken up rapidly by tissue culture cells, it is less rapidly lost when the cells are incubated in amantadine-free medium. On the other hand, the antiviral action is lost almost immediately the cells are placed in the drug free medium. This suggests that intracellular amantadine has little effect on the antiviral state and that perhaps the interaction of amantadine with the external surface of the plasma membrane could explain the antiviral action. Amantadine belongs to a class of compounds termed 'lysosomotropic' which can increase intralysosomal pH (Poole and Ohkuma, 1981). If fusion is an important stage in influenza virus replication, and if such a fusion occurs only at a low pH in cytoplasmic vacuoles, then it is still possible that amantadine could act by raising the intravacuolar pH, thus stopping fusion and blocking virus replication at this stage.

Similarly, the complete sequence of the neuraminidase (NA) gene in influenza A/PR/8/34 has been determined by cloning a DNA copy of the gene into bacteriophage M13 (Fields et al., 1981). Anti-neuraminidase antibodies can inhibit viral replication as also can specific inhibitors of the NA and hence the enzyme is a suitable target for chemotherapy (reviewed by Palese and Schulman, 1977). If sequences of the NA protein are conserved independently of antigenic drift then short oligopeptides could be synthesized and any inhibitory effect investigated as described above for HA protein. The RNA coding for the NA protein is 1413 nucleotides long including 20 nucleotides 5′ to the first AUG and 31 nucleotides 3′ to the last

codon. Only one reading frame is open, beginning with AUG at position 21 and the other two reading frames are blocked by termination codons. The predicated NA protein is co-linear with the RNA and occupies 97% of the coding capacity of the gene and thus the gene codes for a single protein. A major hydrophobic region is located near the N-terminus from residues 7 to 35. In the two human subtypes (N1 and N2) total conservation of the first 12 amino acids has been described and thus some critical role such as interaction with another virus protein or NA subunit might be anticipated for this short sequence. The NA polypeptide may have an extended signal sequence responsible for transferring the NA protein across the membrane and remaining in the bilayer to anchor the NA.

An alternative and relatively new approach to the search for influenza virus inhibitors is to synthesize compounds which compete with virus for receptor binding sites on the cell. Earlier studies of cell surface receptors for paramyxoviruses indicated the importance of sialic acids of cell surface oligosaccharides, since sialidase treatment of host cells prevented virus infection. More recent studies have shown that the host cell receptors for Sendai virus are more complex and that specific gangliosides act as host cell receptors (Holmgren et al., 1980). In an experimental approach Markwell et al. (1981), removed cell receptors to Sendai virus on MDBK cells by sialidase and incubated the cells with individual highly purified gangliosides and determined if the MDBK cells regained their susceptibility to virus absorption and infection. Incubation of the cells with ganglioside containing the sequence NeuAcα2, 3 Gal B1, 3 Gal NAC fully restored susceptibility of infection to the cells. A ganglioside in which the sequence ended in two sialic acids in a NeuAcα2, 8NeuAc linkage instead of a single sialic acid was 100 times more effective as a receptor than other gangliosides. Certain polysialo gangliosides induce cell–cell fusion when incubated with cells and one could speculate that the attachment of Sendai virus to the cell surface may stabilize a clustering of receptor molecules, so facilitating virus-cell fusion (Markwell et al., 1981).

If virus cell fusion is demonstrated to be a central event in the infective process in both para and myxoviruses then a search for fusion inhibitors could result in a broad spectrum antiviral molecule. Certain diamidines have been shown recently to inhibit fusion of respiratory syncytial virus (Dubovi et al., 1980, 1981). This approach would have the added attraction with influenza that the 'fusion' sequence in HA2 is common to many antigenic subtypes, thus potentially allowing broad spectrum antiviral activity.

Comparable receptor binding site studies have been reported with influenza A virus recently and this work additionally emphasizes the phenotypic diversity of these viruses, which is discussed in more detail below (Carroll et al., 1981 and see Chapter 7). Thus, the authors examined absorption to red blood cells of two strains of influenza A virus (A/R1/5−/57 and R1/5+/57). The HAs of the two viruses were found to have totally different specificities binding to the NeuAcα2, 3 Gal and NeuAcα2, 6 Gal linkages, respectively. Examination of the crystal structure of the

HA shows that the host cell receptor cleft is distinct from the antigenic sites (Wiley et al., 1981). So variations in receptor specificity could arise from natural selective pressures by host specified sialyloligosaccharides present on cell surface glycoproteins. In this case inhibitors of this receptor function might have to be rather specific, as mutants circumventing their activity might easily arise.

3.2.2. Virus nucleic acid synthesis

Most viruses code for DNA and/or RNA polymerases as shown in Table 3.1. Some viruses also induce the formation of enzymes involved in the synthesis of nucleosides and nucleotides or are used to degrade RNA and DNA. Details about these enzymes are given in the respective chapter discussing the different viruses. A majority of the antiviral drugs found in recent years have used the viral enzymes involved in nucleic acid synthesis as targets.

Synthesis of nucleosides and nucleotides

Some viral enzymes involved in the formation of nucleosides and nucleotides are listed in Table 3.2. No useful antiviral drugs have yet been found affecting or using the ribonucleotide reductase or the deoxycytidine deaminase. These enzymes will, in the future, be explored in more detail in this respect, possibly using them for a selective activation of drugs. The HSV ribonucleotide reductase has been well characterized and might be required for virus replication. The effect of a few nucleoside analogue triphosphates on cellular and HSV ribonucleotide reductase has been studied and BVDUTP, for example, showed some selectivity, inhibiting the viral enzyme (Nakayama et al., 1982).

Nucleoside kinases, which are enzymes required for the phosphorylation of nucleosides to nucleoside monophosphates, have been found in virus infected cells, especially for members of the herpesvirus family as indicated in Table 3.2. It seems to be a general property of virus induced thymidine kinases (pyrimidine kinases)

TABLE 3.2.
Virus enzymes involved in the synthesis of nucleosides and nucleotides
(See Helgstrand and Öberg, 1980)

Virus	Enzyme
HSV-1, HSV-2	Adenosine diphosphate: thymidine-5'-phosphotransferase
HSV-1	Deoxycytidine deaminase
HSV-1, HSV-2	Ribonucleotide reductase
HSV-1, HSV-2	Thymidine kinase
VZV	Thymidine kinase
Vaccinia	Thymidine kinase
HSV-1	Thymidylate kinase

TABLE 3.3.
Relative rates of phosphorylation of nucleoside analogues

Compound	Thymidine kinase		
	Cytosol	Mitochondrial	HSV-1
dThd	100	100	100
IDU	87	—	115
ara-T	33	82	62
(E)-BVDU	⩽5	35	90
(Z)-BVDU	⩽5	15	112
(E)-BVara-U	0	177	71
FIAC	4.2	201	31
ACG	0	0	28
R-DHBG	0	0	77
S-DHBG	0	0	51

Data from Cheng et al. (1981a and b), Keller et al. (1981), Larsson et al. (1983), and A. Larsson (personal communication).

to have a less strict structural requirement for substrates than similar cell enzymes. Compounds such as acyclovir (Elion et al., 1977), DHBG (Larsson et al., 1983) and BVDU (Cheng et al., 1981a) can be phosphorylated by the viral kinases but not by the cell thymidine kinases, thus leading to a selective activation only in virus infected cells. These phosphorylations are competitive reactions influenced by the concentration of thymidine (Larsson et al., 1983). The further phosphorylation of the nucleoside monophosphates to di- and triphosphates seems to be carried out by cellular enzymes in competitive reactions that might be of importance for the final concentration of a nucleoside triphosphate.

A large number of pyrimidine analogues have been found to be phosphorylated by herpesvirus thymidine kinase and some are discussed later in the chapter on herpesvirus inhibitors. A few are shown in Table 3.3 where for example the difference in phosphorylation between cytosol and viral kinase is obvious. The mitochondrial enzyme has a substrate specificity resembling that of the viral enzyme. The phosphorylations of purine analogues are mediated by cellular enzymes. It should be noted that the acyclic guanosine analogues are recognized by the TKs as pyrimidines but after phosphorylation to monophosphates they seem to be regarded as purine monophosphates and further phosphorylated by GMP kinase.

Polynucleotide synthesis

Since most viruses induce the formation of DNA and/or RNA polymerases (see Table 3.1) a substantial amount of work has been directed towards finding selective inhibitors of these viral polymerases. In general, a polymerase inhibitor could bind to different sites on the polymerase, competing at the sugar, the base or the tri-

phosphate binding sites or affecting the site where pyrophosphate is split off during the polymerization. Some currently known inhibitors are listed in Table 3.4. Triphosphates of nucleoside analogues seem to act as competitive inhibitors/substrates as exemplified by araATP (Müller et al., 1978), acyclovir triphosphate (Furman et al., 1979, Derse et al., 1981) and BVDU triphosphate (Allaudeen et al., 1981, Ruth and Cheng, 1981) while pyrophosphate analogues such as foscarnet and PAA act as non or uncompetitive inhibitors (See Boezi, 1979, Eriksson and Öberg, 1983, Öberg, 1983a). The kinetic parameters for some nucleoside analogue triphosphates are shown in Table 3.5 together with K_m values for the competing substrates.

In earlier studies, several series of molecules reacting with the virion associated RNA transcriptase enzyme of influenza virus were synthesized on the semi-logical basis, that, in common with many DNA polymerase enzymes, the influenza enzyme might be a zinc metalloenzyme. Compounds were synthesized which could bind reversibly with zinc, which would itself be bound reversibly but more firmly to the enzyme – this is the zinc metalloenzyme hypothesis (Perrin and Stunzi, 1980). Compounds with good zinc chelating activity such as 2-acetyl pyridine thiosemicarbazone and selenocystamine had marked inhibitory effects on the virion associated RNA transcriptase enzyme of a number of influenza A and B viruses. However, these compounds had no detectable activity in cells. Subsequently, Helgstrand and Öberg (1978) used the influenza RNA transcriptase enzyme test as the basis for

TABLE 3.4.
DNA and RNA polymerase inhibitors

Substrate analogues	Product analogues	Template base-pairing	Template modification
Base modifications	Foscarnet	d(AATGGTAAAATGG)	ACG
Ribavirin			
IDU			
BVDU			
TFT			
Sugar modifications			
ara-A			
ara-C			
ACG			
DHBG			
Base and sugar modifications			
BVara-U			
FIAC			
AIU			
HPUara			

Note: Most nucleoside analogues are inhibitors as triphosphates.

more extensive in vitro screening for inhibitory molecules, and some compounds active as inhibitors of the polymerase have also shown anti-influenza activity in cells (Stridh et al., 1981).

Since influenza virus is unique in possessing cap recognizing proteins in the RNA transcriptase complex (Kroath and Shatkin, 1982), this could provide an additional target for the selection of inhibitors.

Cloning studies already in progress in several laboratories should eventually delineate RNA transcriptase enzyme binding sites on the RNA and this data may enable the synthesis of specific inhibitors. A recent study of sequence specific contacts of vesicular stomatis virus (VSV) RNA polymerase on the leader RNA gene may therefore provide a model of this approach (Keene et al., 1981). The RNA polymerase complex of VSV consists of the L, NS and N proteins and since transcription starts at the 3′ terminus of the RNA it is assumed that the promoter for the polymerase resides in the leader gene. The authors utilized methylation protection to study sequence specific interactions with VSV RNA. The N protein rendered the RNA resistant to nuclease, although it did not alter the reactivity of the RNA to methylation by dimethyl sulphate (an alkylating agent). The binding of the polymerase L and NS proteins to the nucleocapsid was examined. When the NS protein was present the reactivity of RNA with dimethyl sulphate was altered in the middle

TABLE 3.5.
Inhibition of DNA polymerases by nucleoside triphosphates

Nucleoside triphosphate	DNA polymerase, K_i or K_m (μM)						Reference
	α	β	HSV-1 (Strain)		HSV-2 (Strain)		
dATP	6.4	14.3	13.7	(Lennette)	—		Müller et al. (1978)
β-ara-ATP	7.4	5.6	0.14	(Lennette)	—		Müller et al. (1978)
α-ara-ATP	3.1	>20	>20	(Lennette)	—		Müller et al. (1978)
TTP	5.4	8.6	0.14	(KOS)	0.18	(333)	Ruth and Cheng (1981)
5-Propyl-dUTP	19.7	21	0.24	(KOS)	0.38	(333)	Ruth and Cheng (1981)
E-5-Propenyl-dUTP	5.7	8.7	0.14	(KOS)	0.12	(333)	Ruth and Cheng (1981)
BVDUTP	3.6	6.5	0.068	(KOS)	0.054	(333)	Ruth and Cheng (1981)
BVara-UTP	0.29	12	0.013	(KOS)	0.021	(333)	Ruth and Cheng (1981)
2′-Fluoro-ara-TTP	1.2	18	0.048	(KOS)	0.060	(333)	Ruth and Cheng (1981)
TTP	5.3	17.8	0.66	(HF)	—		Allaudeen et al. (1981)
BVDUTP	3.6	16.4	0.25	(HF)	—		Allaudeen et al. (1981)
dGTP	1.2	4.6	0.15	(KOS)	0.15	(333)	Derse et al. (1981)
ACGTP	0.18	No inhibition	0.0003	(KOS)	0.003	(333)	Derse et al. (1981)
dGTP	1.08	—	0.97	(KOS)	—		Furman et al. (1979)
ACGTP	2.32	—	0.55	(KOS)	—		Furman et al. (1979)
dCTP	3.4	2.4	0.092	(KOS)	0.11	(333)	Ruth and Cheng (1981)
FIACTP	1.25	5.2	0.028	(KOS)	0.041	(333)	Ruth and Cheng (1981)
ara-CTP	10	10	0.15	(KOS)	0.12	(333)	Ruth and Cheng (1981)

of the leader gene. Thus, NS may function as an initiator protein for transcription by recognizing and binding to specific sites on the genome. The NS protein binds to the sequence:

$$3'\,\mathrm{GGUA}\overset{20}{\mathrm{A}}\mathrm{UAAUAGUAA}\overset{30}{\mathrm{U}}\,5'.$$

Ribavirin triphosphate has been shown to inhibit influenza RNA polymerase in cell-free assays (Eriksson et al., 1977) but it is unclear to what extent that is important in vivo.

Attempts have been made to use oligonucleotides complementary to viral DNA or RNA as inhibitors, but the results differ for different viruses and the rather extended size of oligonucleotides capable of a stable and specific base-pairing makes the penetration into a cell very difficult and it is not clear whether the viral RNA is freely accessible to base-pairing (Zamecnik and Stephenson, 1978, Stridh et al., 1981).

Smith et al. (1980) and Stridh et al. (1981) have described the RNA transcriptase inhibitory activity of certain polynucleotides. In common with the experiments described above these molecules inhibited a wide range of influenza A and B viruses. Indeed, this is a marked feature of such inhibitors, since, as far as is known, only minimal genetic variation occurs in the virus P and NP polypeptides of influenza viruses which together are considered to constitute RNA transcriptase enzyme activity.

Smith et al. (1980) calculated that 50% inhibition of influenza RNA transcriptase activity occurred with only 4 molecules per RNA molecule and inhibition probably involved direct binding to the transcriptase enzyme. However, the polynucleotide compound has only mild antiviral activity in vivo (Potter et al., 1981).

TABLE 3.6.
Viral nucleases (see Helgstrand and Öberg, 1980)

Virus	Enzyme
Polio	Endonuclease
Reovirus	Nucleoside triphosphatase
Sindbis	Endonuclease
Influenza	Endonuclease, nucleoside triphosphatase
Sendai	Endonuclease
Newcastle disease	ATPase
Vesicular stomatitis	Endonuclease, nucleoside triphosphatase
Avian myeloblastosis	Endonuclease, nucleoside triphosphatase
HSV-1, HSV-2, CMV	DNase
Frog virus 3	DNase, RNase, ATPase
Vaccinia	DNase, nucleoside triphosphatase, polynucleotide 5'-triphosphatase

Degradation of nucleotides and polynucleotides

It is difficult to assess the presence of nucleases in purified virus preparations where contamination with cellular enzymes is often possible. In some cases virus induced nucleases have been identified and they can be part of the viral polymerases. A selection of virus induced or associated nucleases are presented in Table 3.6. The DNase activity associated with HSV-1 DNA polymerase is inhibited by foscarnet (Derse and Cheng, 1981). Interestingly, activation by cleavage of a dinucleotide containing one toxic nucleoside has been suggested as a way to utilize viral nucleases as targets for antiviral drugs (Cheng et al., 1982). Inhibition of the cap dependent endonuclease activity of influenza virus (Ulmanen et al., 1983) should constitute a logical point of attack for an antiviral drug and prevent the utilization of the cellular mRNA cap by the viral polymerase.

3.2.3. Viral protein synthesis and modification

A viral infection often results in a shut-off of cellular protein synthesis. The virus mRNA uses cellular components such as initiation factors, ribosomes and tRNA in the synthesis of viral polypeptides but no effective antiviral drugs have been described which interfere with these steps. Cleavage of viral polypeptides is a common event and in the case of picornaviruses this cleavage is mediated by virus coded proteases. In addition, cleavage of the influenza haemagglutinin polypeptide (HA_0 to HA_1 and HA_2) is necessary for viral infectivity and is a possible target to block by an antiviral drug. Very little has been published in this area but as the sequence determinations and cleavage patterns for viral polypeptides become more clear it is likely that means of inhibiting these cleavages will be found. The use of protease inhibitors against influenza replication has been reported by Zhirnov et al. (1982). Butterworth (1977) and Korant (1981) have reviewed the processing of viral polyproteins to viral proteins.

Several important post-translational events such as glycosylation and phosphorylation of viral polypeptides occur. Compounds such as 2-deoxy-D-glucose and D-glucoseamine interfere with glycosylation and inhibit the multiplication of influenza and herpesvirus, but the compounds studied so far have not resulted in any antiviral drug with therapeutic activity in vivo.

The importance of correct phosphorylation of viral proteins as a way of regulating their functions has been shown with viruses such as influenza. It has also been shown (Collett and Erikson, 1978) that the function of the src gene in avian sarcoma virus is that of a protein kinase. Several other viruses such as corona virus (Siddell et al., 1981) and polyoma virus (Smith et al., 1979) have been reported to contain protein kinases, but their function remains largely unclear. Inhibitors of viral protein phosphorylation and protein kinases have not been reported yet but are likely to come in the future. It is too early to judge the potential usefulness of compounds interfering with protein phosphorylation as possible antiviral drugs, but in

this context it should be noted that one function of interferon is to induce a protein kinase which phosphorylates and impairs the activity of peptide chain initiation factor eIF-2, thus inhibiting protein synthesis (see Lengyel, 1982).

3.2.4. Virus assembly and release

The assembly of viral nucleic acid and viral proteins is a process which appears to be distinct from known cellular functions but no clear-cut inhibitors of viral assembly have been reported. Influenza neuraminidase has been implicated in the release and dispersion of new virus particles (Palese and Schulman, 1977), and several inhibitors of influenza neuraminidase such as FANA (Palese et al., 1974) have been described and have been active in cell cultures but not in infected animals (Palese and Schulman, 1977). These could be fertile areas for future work.

3.2.5. Virucidal effects

Most virucidal compounds (having a direct destructive effect on the intact virus particle) seem to be toxic also to cells, and attempts to find any antiviral selectivity at the present time have to resort to purely trial and error. Virucidal compounds not penetrating into cells could possibly have a lower and hence selective toxicity, but their distribution to the affected organ might be restricted. Unfortunately, from the point of view of this approach, the spread of virus from one cell to another could take place without the virus being exposed extracellularly, as is the case with herpesviruses and certain paramyxoviruses. Butylated hydroxytoluene might act as a virucidal agent disrupting herpesviruses even in infected animals (Keith et al., 1982).

TABLE 3.7.
Examples of antiviral drugs affecting different steps in virus multiplication

Target function	Drug	Type of virus	Reference
Adsorption, penetration and uncoating	Amantadine, rimantadine	Influenza A	Oxford and Galbraith (1980)
	Arildone	Picorna, herpes	Diana et al. (1977)
	FANA	Influenza A	Palese and Schulman (1977)
	Oligopeptides such as Z-Gly-L-Phe-L-Phe-Gly	Influenza, measles	Richardson et al. (1980)
	Polysaccharides, heparin, hyaluronic acid	Picorna, paramyxo, herpes	Becker (1976)
	2-Acetylpyridine thiosemicarbazones	Herpes	Shipman et al. (1981)

TABLE 3.7. *(continued)*

Target function	Drug	Type of virus	Reference
Nucleic acid synthesis	Acyclovir	Herpes	Elion et al. (1977)
	AdThd	Herpes	Pavan-Langston et al. (1982)
	AldUrd	Herpes	Chen et al. (1976)
	AraA	Herpes	North and Cohen (1979)
	AraAMP	Herpes	Preiksaitis et al. (1981)
	AraC	Herpes	North and Cohen (1979)
	AraT	Herpes	Aswell et al. (1977)
	BVaraU	Herpes	Machida et al. (1982)
	BVDU	Herpes	De Clercq et al. (1979)
	Complementary oligonucleotide	Rous	Zamecnick and Stephenson (1978)
	Cyclaradine	Herpes	Vince and Deluge (1977)
	DHBG	Herpes	Larsson et al. (1983)
	DHPG, Biolf 62, 2'NDG	Herpes	Smith et al. (1982)
	Distamycin	Herpes	Hahn (1977) Grehn et al. (1983)
	EHNA	Herpes	North and Cohen (1978)
	EDU	Herpes, vaccinia	Gauri and Malorny (1967)
	FIAC	Herpes	Lopez et al. (1980)
	FMAU	Herpes	Cheng et al. (1981b)
	Foscarnet	Herpes	Öberg (1983a)
	IDU	Herpes	Prusoff and Goz (1973)
	IDC	Herpes	Schildkraut et al. (1975)
	PAA	Herpes	Boezi (1979)
	Pr-dUrd	Herpes	Ruth and Cheng (1982)
	Ribavirin	DNA and RNA viruses	Sidwell et al. (1979)
	TFT	Herpes	Heidelberger and King (1979)
Protein synthesis and modification	Protein synthesis inhibitors penetrating only virus infected cells	Picorna	Carrasco (1978)
	2-Deoxy-D-glucose	Herpes, influenza	Shannon and Schabel (1980)

TABLE 3.7. *(continued)*

Target function	Drug	Type of virus	Reference
	Bis-(5-amidino-2-benzimidazolyl) methane	Respiratory syncytial, influenza	Dubovi et al. (1981)
Virucidal	BTH	Herpes	Keith et al. (1982)
Not conclusive	Carboxylic 3-deaza-adenosine	Broad spectrum	De Clercq and Montgomery (1983)
	3-Deaza-adenosine	Retrovirus	Bader et al. (1978)
	S-DHPA	Broad spectrum	De Clercq et al. (1978)
	Dichloroflavan	Rhino	Tisdale and Selway (1983)
	2-(3,4-Dichloro phenoxy)-5-nitro benzonitrile	Picorna	Torney et al. (1982)
	4',5-Dihydroxy-3,3',7-trimethoxyflavone	Picorna	Ishitsuka et al. (1982)
	Enviroxime	Rhino, echo, coxsackie	De Long and Reed (1980)
	HBB	Picorna	Eggers and Tamm (1966)
	Methizasone	Pox	Bauer (1977)
	Sodium 5-aminosulfonyl-2,4-dichlorobenzoate	Myxo, paramyxo, picorna	Ohnishi et al. (1982)

3.2.6. Integrated genomes – latent infections

When a viral genome has been integrated into the cellular genome the replication of the viral genome is probably mediated by cellular enzymes. This severely restricts the possibilities of eliminating the virus from the host cell. Examples of this type of integration are retroviruses, probably hepatitis B virus in chronic infections, and papilloma in warts. If, however, viral enzymes are expressed these could be used for a selective activation of compounds which are then transformed into toxic entities, thus affecting only cells which possess the viral genome. However, no compounds have been reported to be active in such a situation.

In the case of latent infections, where the viral genome has not been integrated, it is possible that a slow viral multiplication takes place. This should then represent a possible target for antiviral compounds. The development in animals of latent infections caused by HSV-1 and HSV-2 have not been affected by antiherpes drugs which block viral replication. A major problem is the lack of data concerning the state in which latent herpes simplex virus exists in the ganglia or elsewhere, and how it is reactivated (Stevens, 1980, Klein, 1982).

3.3. Types of compounds showing antiviral activity

In the search for antiviral drugs a very large number of compounds have been tested in different cell and animal models. The major part of the testing seems to have utilized cell cultures infected with influenza, herpes, rhino or some other virus of interest as a target for antiviral chemotherapy. In most cases the antiviral effects observed in cell cultures have not been followed by any therapeutic effects in infected animals. To illustrate the wide range of compounds showing antiviral efficacy in cell cultures a selection of structures has been compiled in Table 3.7. Interferon and immunomodulators are discussed separately. Some of these compounds are discussed in more detail in later chapters, others have probably no future as antiviral drugs but could serve as useful starting points in the search for better compounds, and a few might require further evaluation. The mode of action is only indicated at one step, although there could be several reactions affected, and only a very limited number of references have been included. Most of these compounds have been discussed in the reviews on antiviral drugs referred to at the beginning of this chapter.

3.4. Interferons

The possibility of using interferon in the clinic, either prophylactically or therapeutically, was limited for a long time by the small amount of interferon available. Because of DNA hybrid technology the situation has now changed and large controlled trials using interferon have now become feasible. The situation has, at the same time, been complicated by the finding of several different human interferons and the need to analyze both different interferons and combinations of interferons for antiviral activity in man. Table 3.8 lists the three main types of human interferons and also indicates that there are several different species within one type of interferon and that the origin of the cell is not enough to define the type and species of interferon.

TABLE 3.8.
Human interferons

Human interferon alpha:	Hu IFN-α. Earlier name: human leukocyte interferon (HLIF) At least 18 different species: HuIFN-α_1, α_2 etc.
Human interferon beta:	Hu IFN-β. Earlier name: human fibroblast interferon (HFIF) At least 2 different species: HuIFN-β_1, β_2
Human interferon gamma:	HuIFN-γ. Earlier name: human immune interferon (HIIF) Unknown number of species.

Addition of (Ly=lymphocyte) or (Le=leukocyte) can be used to specify origin e.g. HuIFN-α(Ly) for human interferon alpha from lymphoblastoid cells.

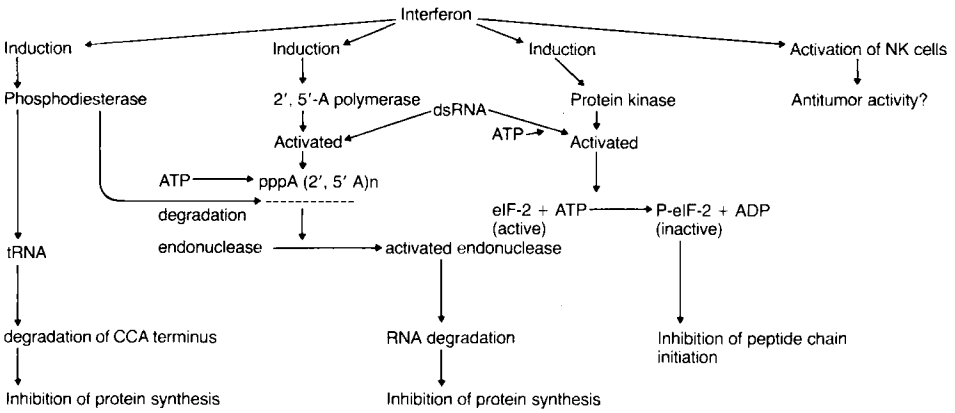

Fig. 3.5. Interferon action.

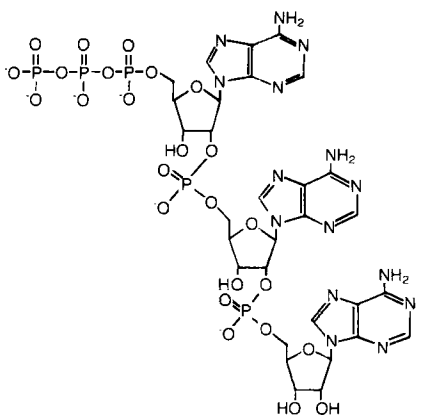

Fig. 3.6. Structure of 2',5'-A.

Interferon was first detected by Isaacs and Lindenmann (1957) by its antiviral action in cell culture. The synthesis of interferon in a cell can be induced by a number of agents such as viruses, bacteria, nucleic acids, mitogens etc. Several interferon inducers have been investigated and most of these have been polynucleotides such as poly(I) poly(C) or other polymers of rather high toxicity. A few low molecular weight interferon inducers have been developed such as N,N-dioctadecyl-N',N'-bis(2-hydroxyethyl)-propan-1,3-diamine, carboxyethylgermanium sesquioxide, tilorone, 2-amino-5-bromo-6-methyl-4 pyrimidole and 1,5-diaminoantraquinones. The use of interferon inducers and interferon in virus infected animals has been reviewed by Kern and Glasgow (1981).

When the induced interferon reaches other cells it can exert a number of functions as schematically illustrated in Fig. 3.5 (See Lengyel, 1982), but the relative

importance of the different pathways in interferon action is not known. The induction of 2′,5′-A (Fig. 3.6) seems to have a central role for antiviral activity. The synthesis of 2′,5′-A, by one or more cellular enzymes induced by interferon (Laurent et al., 1983), is balanced by degradation by an interferon induced enzyme (2′-Di) and the resulting 2′,5′-A activates a latent ribonuclease (RNase L) which can degrade both mRNA, rRNA and viral RNA. Since 2′,5′-A is rather labile, efforts have been made to synthesize analogues to 2′,5′-A intended to be more stable and to activate RNase L. Several structural analogues to 2′,5′-A have been synthesized with this intention (Epstein et al., 1982, Kwiatkowski et al., 1982, Sawai et al., 1983, Haugh et al., 1983), and this is a very interesting approach. An increased stability can be obtained but the modified 2′,5′-A is still a compound with a molecular weight too high for easy penetration into cells. This is an active area of research at present and improved compounds are to be expected. Their use would presumably be as broad range drugs against RNA viruses, but it is unclear at present if they will be sufficiently virus-specific and non-toxic to the cell.

Overall et al. (1981) have determined the interferon concentration in herpes vesicles in humans and found it to be high but, in general, interferon production in a virus infected tissue has not been quantitated. The limited knowledge of actual and optimal levels of interferon in tissue during viral infections makes it difficult to predict the therapeutic efficacy of exogenously given interferon. There is a more obvious possibility of interferon as a prophylactic drug assuming that the toxicity is acceptable. Studies on the therapeutic and prophylactic use of interferon will be presented in the following chapters on each type of virus infection and the reader can judge for him/herself. The clinical use of interferon has been reviewed by Dunnick and Galasso (1979, 1980) and Table 3.9 lists some (but not all) clinical trials using interferon. In most cases the limited number of patients preclude an evaluation of clinical efficacy but it seems likely that the hopes that interferon would turn out to be a new wonder drug were grossly exaggerated. However, most likely there will be situations where it can be used both for prophylaxis and therapy, and probably also in combination with other antiviral drugs.

3.5. Immunomodulators

The possibility of modulating the natural immune response has been an obvious way to develop antiviral agents. The rapid progress in our understanding of immunological processes makes it likely that in the future it will be possible to design compounds with therapeutic or prophylactic efficacy against viral infections. However, the compounds tested so far, levamisole and inosiplex, have shown little, if any, effect. The mechanism of action of levamisole is to increase phagocytosis, cell-mediated immune responses and polymorphonuclear leukocyte chemotaxis. The use in herpes and papilloma infections seems doubtful (Russel, 1980) but it might

TABLE 3.9.
Recent clinical trials with interferon (Modified after Dunn and Galasso, 1980)

Subject of study	Preparation, dose, method, schedule, and design (no. of patients)	Results, side effects	Reference
Rhinovirus infection			
Common cold	HuIFN-α2, 5×10^6 U twice daily intranasally	High frequency of nasal side effects, some prophylactic effects of interferon on rhinovirus associated colds	Betts et al. (1983)
	HuIFN-α2, 2×10^7 U/day for 4 days, intranasally (22) or placebo (22)	Protection against challenge infection	Scott et al. (1982)
Influenza virus infection			
Influenza	HuIFN-α2 5×10^6 U twice daily intranasally	Prophylactic effect reducing mean symptom sore and virus shedding	Dolin et al. (1983)
Rubella virus infection			
Congenital rubella	HuIFN-α, $2–7 \times 10^5$ U/kg/day for 10 days (3)	Transient decrease in pharyngeal virus excretion	Arvin et al. (1982b)
Adenovirus infection			
Adenovirus infections of the eye	HuIFN-β, $1–2 \times 10^5$ U per day, 6–8 times a day for 6–7 days (20); control patients received either dexamethasone phosphate with neomycin sulfate or sulfacetamide or 2% human albumin drops	Length of disease in treated group reduced to 6.5 days from 27 in controls; complication rate reduced from 50% to 5%	Romano et al. cited by Dunnick and Galasso (1980)
Hepatitis			
Hepatitis B	HuIFN-β, 1×10^6 U per day, i.m., for 82 days	Disappearance of HBcAg and HBeAg; reduction in HBsAg; no visible side effects	Dolen et al. (1979)

TABLE 3.9. *(continued)*

Subject of study	Preparation, dose, method, schedule, and design (no. of patients)	Results, side effects	Reference
Hepatitis B	HuIFN-α, $2–10\times10^6$ U/day or $5–20\times10^6$ U/day (16)	Decrease in HBV DNA polymerase during treatment; 4 patients remained polymerase negative and lost HBeAg	Scullard et al. (1981)
Hepatitis	HuIFN-α, 12×10^6 U/day (16)	Transient drop in HBV DNA polymerase. Leucopaenia in 6 of 8 treated	Weimar et al. (1980)
Herpesvirus infections			
Dendritic keratitis (patients virus-positive)	Debridement plus HuIFN-α, 1×10^6 U/ml, 1 drop per day (18); debridement plus HFLF, 1×10^6 U/ml; 1 drop per day (20)	Both preparations equally beneficial at increasing healing rate	Sundmacher et al. (1978a)
Dendritic keratitis	HuIFN-α, 6×10^6 U/ml, 2 drops per day, with either thermocautery (18) or minimal wiping debridement (24)	Best treatment was with debridement	Sundmacher et al. (1978b)
Dendritic keratitis	Trifluorothymidine and HuIFN-α drops, 30×10^6 U/ml	Reduced healing to 2,9 days	Sundmacher et al. (1978c)
Dendritic keratitis	Trifluorothymidine and HuIFN-α drops 100×10^6 U/ml	Better effect than 30×10^6 U/ml	Sundmacher et al. (1983)
Herpes reactivation after surgery for tic douloreaux	HuIFN-α, 7×10^4 U/kg per day, for 5 days	Reduced incidence of viral shedding from 42% to 9% and reactivation from 83% to 47%	Pazin et al. (1979)
Transplant recipients			
CMV infections after renal transplantation	HuIFN-α, 3×10^6 U, i.m., on day of transplant and on day 1, then twice a week	Reduced incidence of CMV viraemia and excretion; depression in white cell and platelet count	Cheeseman et al. (1979)

TABLE 3.9. *(continued)*

Subject of study	Preparation, dose, method, schedule, and design (no. of patients)	Results, side effects	Reference
EBV infections after renal transplantation	HuIFN-β, 3×10^6 U i.m., twice a week for 7 weeks (21) or placebo (20)	Reduced virus shedding	Cheeseman et al. (1980)
Prevention of virus infections after renal transplants	HuIFN-β, 3×10^6 U, i.m., twice a week, for 3 weeks	No effect	Weimar et al. (1979)
CMV pneumonia after marrow transplant	HuIFN-α, 2×10^4–6.4×10^5 U/kg per day (8)	All 8 patients died of pneumonia; no marrow toxicity at doses of $<1.6\times10^5$ U/kg per day	Meyers et al. (1980)
CMV infection after marrow transplant	HuIFN-α and araA	No effect on infection; neurotoxicity and decreasing neutrophil counts	Meyers et al. (1982)
Patients with cancer			
Varicella in children	HuIFN-α, 4.2×10^4–2.5×10^5 U/kg (18)	Varicella complications in 6 of 9 placebo and 2 of 9 HuIFN-α recipients	Arvin et al. (1978)
Varicella in children	HuIFN-α up to 3.5×10^5 U/kg (44)	Reduced formation of new vesicles and less visceral complication in HuIFN-α recipients	Arvin et al. (1982a)
Zoster	HuIFN-α 5.1×10^5 U/kg	No cutaneous dissemination; no effect with lower doses of HuIFN-α	Merigan et al. (1981)
Human laryngeal papilloma	HuIFN-α, 3×10^6 U, i.m., 3 times a week	Disease modified by treatment in 7 of 7 patients	Haglund et al. (1981)

be effective in, for example, children with frequent upper respiratory tract infections (Van Eygen et al., 1979).

Inosiplex (isoprinisone) seems to restore T-lymphocyte function which has been depressed by a viral infection and can possibly also augment interferon action

(Werner, 1979). The usefulness of inosiplex is at present unclear and will have to await further double-blind placebo-controlled trials (Chang and Heel, 1981, Editorial, Lancet 1982). A combination of immunomodulators and selective antiviral drugs is a possibility that has not been explored.

3.6. Drug delivery

The problem of drug delivery is not unique to antiviral drugs but because of their novel character very little data has been reported. For example, it is likely that topical application for herpetic infections will pose more penetration problems on dry skin than on mucous membranes or the cornea of the eye. It is also likely that the vehicle and method of application could be as important as the drug used. A general problem is, and will be, the need to use an antiviral drug at the very onset of the infection. Because of the often very rapid course of the disease, this will require a rapid diagnosis and preferably a direct delivery of the medication to the affected skin by a topical application or to the infected upper airways by an aerosol. A correct diagnosis by the patient and the appropriate drug at hand is a goal as far as rapidly progressing viral diseases like cold sores, genital herpes, common colds and influenza are concerned. Infections with a more prolonged course of virus replication, such as those in immunocompromised patients, might be easier targets for antiviral drugs.

3.7. Development of drug resistance

In spite of the limited use of antiviral drugs so far, concern has been expressed for the possible development of drug resistance (Field and Wildy, 1982). The problems concerning drug-induced resistance have recently been reviewed by Field (1983) and are discussed in detail in Chapter 17. It is clear that use of antiviral drugs in cell cultures results in a selection of drug resistant virus. It is equally clear that the selection of drug resistant bacteria is a more rapid process, perhaps not surprisingly since the replication time of a virus is in the order of several hours and that of bacteria is less than half an hour. This certainly implies that penicillins should not be used indiscriminately but also that the concern for virus resistance should not be exaggerated.

In protocol terms we observe that the wide-spread use of amantadine and rimantadine in the USSR has not resulted in development of resistance although a few resistant viruses have been reported in the GDR (Heider et al., 1981). In the case of recurrent disease, such as a labial and genital herpes, it is not clear whether a possible development of resistance during one episode of the disease will result in emergence of a resistant virus during the next episode. In generalized herpes infec-

tions in immunocompromised patients, long term systemic treatment has resulted in development of resistance to acyclovir (Sibrack et al., 1981, Burns et al., 1982, Crumpacker et al., 1982, Wade et al., 1982) but this situation is clearly different from the treatment of a local recurrent herpes disease in an immunologically normal person. Should development of drug resistance become a problem for herpes infections there remains the possibility of combining drugs with different modes of action and thus decreasing the risk of resistant strains becoming established.

3.8. Prediction of clinical efficacy

Most of the development of antiviral drugs has started with cell culture experiments to determine the inhibition of virus multiplication in vitro. This method has certain advantages, such as low cost and ease of use. However, it can give quite misleading results when substrate analogues, for example, are investigated, since these are often competitive inhibitors or are being activated in a reaction where they have to compete with the natural substrate. If the natural substrate has a higher concentration in the infected human organ than in a cell culture, the compound will have a lower efficacy in the infected organ than in the cell culture. It is also possible that experiments in animal cells or intact animals could be misleading if for example the phosphorylation of an antiviral drug involves cellular enzymes which could have species differences in their substrate specificity.

At present most would agree that the combined use of cell-free assay systems, cell cultures and animal models seem required to make a reasonable prediction of clinical efficacy of an antiviral. An example of this type of correlation between different levels of analysis is shown in Table 3.10 where the lack of correlation between

TABLE 3.10.
Comparison of antiviral activity at the level of enzymes, cell cultures and infected animals (after Öberg, 1983b)

Compound	HSV-1 TK		Cell TK	50% inhibition of HSV-1 in cell culture (μM)	% Reduction in HSV-1 lesion score, guinea pigs
	Relative rate of phosphorylation	K_i (μM)	K_i (μM)		
R-DHBG	77	1.4	>250	4	55 (5% ointment, 20 treatments topically, R, S)
S-DHBG	51	1.5	>250	12	
Acyclovir	28	97	>250	0.3	54 (5% ointment, 20 treatments topically)
Foscarnet	—	—	—	20	90 (2% cream, 6 treatments topically)

cell culture and animal experiments possibly could be explained by the different affinities to thymidine kinase for the nucleoside analogues and the presence of thymidine in guinea pig skin (Harmenberg, 1983). Forscarnet on the other hand is unaffected by thymidine. As more antiviral drugs appear and pass clinical tests it is fair to conclude that we will be in a better position to predict the efficacy of future drugs. The number of antiviral drugs tried so far in proper double-blind placebo-controlled clinical trials is still too small for precise correlations of preclinical and clinical efficacy.

3.9. Development of antiviral drugs

The development of an antiviral drug is, as for other drugs, a long process. The first part of this is outlined in Fig. 3.7, where a stepwise evaluation and modification of structures to optimize antiviral activity is indicated. In most programmes the screening and evaluation start using inhibition of virus multiplication in cell cultures, but in some instances the screening starts in animal models of different viral diseases. In these cases the mechanism of action has to be analyzed after the in vivo effect has been found. When viral enzymes are the targets, a screening can use these cell-free enzymes as the first step and in this way, hopefully, be more rational, defining the mechanism of action from the start.

It is not possible to specify precisely the time and resources required in a newly started project to find a compound active against a virus infection but 5–10 years is a reasonable estimation. For some viruses such as herpesviruses, where a number of active inhibitors are already known, the task is simpler than it is to find inhibitors

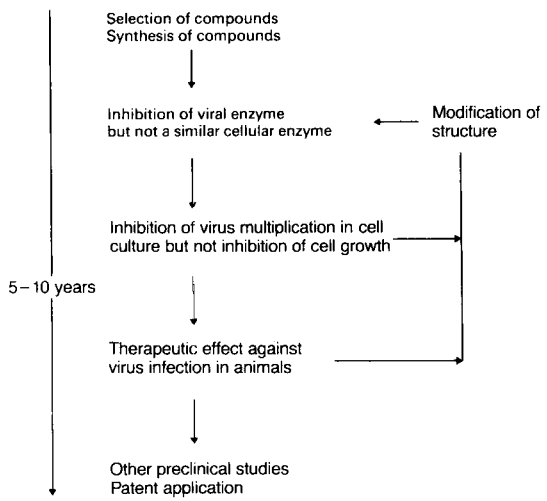

Fig. 3.7. Development of antiviral drugs I.

of a virus such as influenza against which only a few active inhibitors have been reported. Information about inactive structures is helpful in the search for active inhibitors, but unfortunately, mostly the active inhibitors are reported in the scientific literature, and not the compounds tested and found to be inactive!

In some screening programmes a thorough evaluation of the cell-toxic properties is carried out before animal experiments start (Stenberg, 1981). By this procedure a considerable number of toxic inhibitors can be eliminated at an early stage and this reduces the number of animal experiments required. This is an advantage both from an ethical and an economical point of view. It should also be pointed out that human cells should be used in these studies.

The further evaluation of an inhibitor to a registered drug is outlined in Fig. 3.8. The actual scheme for a specific drug could deviate considerably from Fig. 3.8 but the time and effort involved are probably not less. In this evaluation, as in that outlined in Fig. 3.7, toxicity or other problems can at any step involve a restart and synthesis of a new compound. The safety evaluation in animals is very expensive but it should be realized that the animal studies are models and in the first human trial a limited number of volunteers has to be carefully monitored for any adverse effects. Evaluation of clinical efficacy in humans is a large and difficult part of the development of an antiviral drug. The number of uncontrolled clinical studies

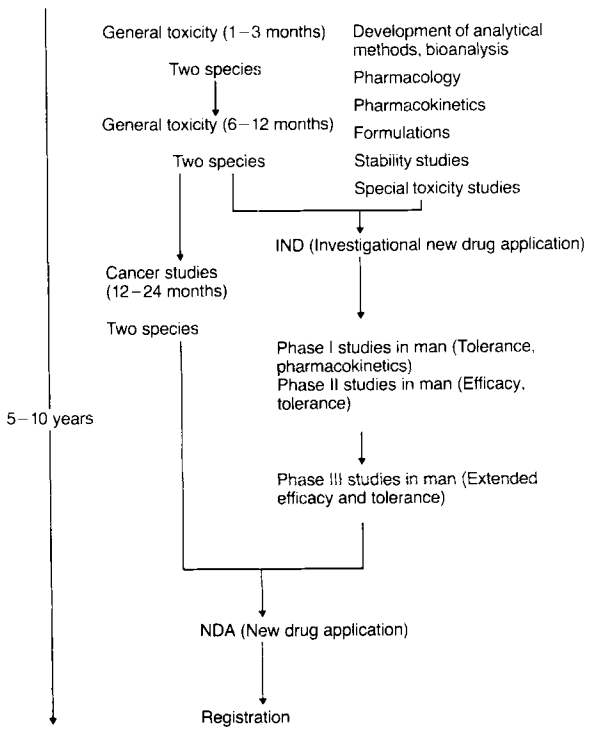

Fig. 3.8. Development of antiviral drugs II.

claiming efficacy of different drugs against viral diseases is depressingly large. It is essential to perform double-blind, placebo-controlled and statistically well evaluated studies to be able to judge the clinical efficacy of an antiviral drug. A praiseworthy high standard in these respects has been shown in the studies carried out in the US by the National Institute of Allergy and Infectious Diseases (NIAID) programme on antiviral substances. These studies could in many respects serve as guidelines for proper clinical evaluation, especially of antiherpes drugs (see Chapters 11, 12 for further details).

3.10. Prospects for the future

As the knowledge of the detailed natural history and molecular biology of viral diseases and viruses themselves increases we will obviously have better opportunities to find new drugs. Methods such as X-ray diffraction measurement and NMR determinations will probably lead to a detailed understanding of the structures and interactions taking place at the active site of viral enzymes and their cellular counterparts. This could hopefully result in a rational, computer aided design of compounds with high selectivity for the viral enzymes. We also need a more detailed insight into the natural history of some viral diseases to be able to choose the right approach for inhibition. The development of rapid diagnosis of viral infections is an important complement to the development of new antiviral drugs. Ideally the method should make a correct diagnosis possible within half an hour. Sensitive methods using immunological principles and monoclonal antibodies or nucleic acid hybridization are being developed but will require simplification to be generally accessible.

Finally, the development of antiviral drugs against uncommon viral diseases is not likely to be pursued by pharmaceutical companies for economic reasons, but could appear as spin-off products in the search of drugs against more common diseases. In this important area international agencies or countries themselves should consider a cost-benefit analysis for developing an antiviral drug. If this consideration turns out in favour of the development of a drug then the actual research work is probably most efficiently carried out by contract to an existing pharmaceutical company or institute. This type of arrangement has not been tried to date for antiviral drugs but should have obvious applications for virus infections in developing countries as well as for rare infections in the industrialized world. Ironically it is also possible that the more or less secret development of antiviral agents against viruses potentially useful in biological warfare or of other military importance could result in therapies against infections such as yellow fever, Japanese B encephalitis and other togavirus infections.

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