

Approaches to Antiviral Drug Development

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At present, only a few drugs have been approved by the FDA for therapy of viral infections in humans. There is a great need for antiviral drugs with increased potency and decreased toxicity, as well as drugs to treat viral diseases for which no drug or vaccine is currently available. Two approaches for development of antiviral drugs are described—an empirical strategy and a rational strategy—with several examples of each.

Although many compounds have potent antiviral activity in cell culture, only a small fraction of these will go on to become antiviral drugs for use in humans. At this time, only seven synthetic compounds and alpha interferon have been approved by the FDA for therapy of viral infections in humans. None of these approved drugs are without toxicities, however, and hence there is a great need for antiviral drugs with increased potency and decreased toxicity, as well as for drugs to treat viral diseases for which no drug or vaccine is currently available. Two approaches for the development of antiviral drugs—the empirical and the rational strategies—and their applications and future directions are discussed.

INTRODUCTION

Many compounds have potent antiviral activity in cell culture, but only about 1 percent of these compounds that have antiviral activity in cell culture are also active in animal systems. Of those that have good antiviral activity and acceptable toxicity in animals, only a few become antiviral drugs for use in humans. Seven synthetic compounds (Fig. 1) and alpha interferon have been approved by the FDA as antiviral agents. The specific viral infections for their use as well as the molecular basis for their antiviral activity have been well reviewed [1-9] (Table 1). Four of these compounds are for the therapy of infections caused by members of the herpesvirus family. The clinically approved antiherpetic drugs include: 5-iodo-2'-deoxyuridine (idoxuridine; IUdR; IdUrd); 5-trifluoromethyl-2'-deoxyuridine (trifluridine; F₃TdR; F₃dThd; TFT); 9-β-D-arabinofuranosyl adenine (vidarabine; ara-A); and 9-(2-hydroxyethoxymethyl)guanine (acyclovir; ACV). A fifth compound, amantadine (1-aminoadamantane; 1-adamantanamine HCl), is approved for the therapy and prophylaxis of respiratory infections caused by the influenza A virus. A sixth antiviral agent, ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide; virazole), is approved for therapy of severe respiratory infections in infants and children caused by the respiratory syncytial virus. The seventh antiviral agent, 3'-azido-3'-deoxythymidine (AZT; zidovudine), has

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Abbreviations: ACV: acyclovir AIDS: acquired immunodeficiency syndrome ara-A: vidarabine AZT: zidovudine HIV-1: human immunodeficiency virus type 1 IDU (IUdR; IdUrd): idoxuridine TFT (F₃TdR; F₃dThd) trifluridine

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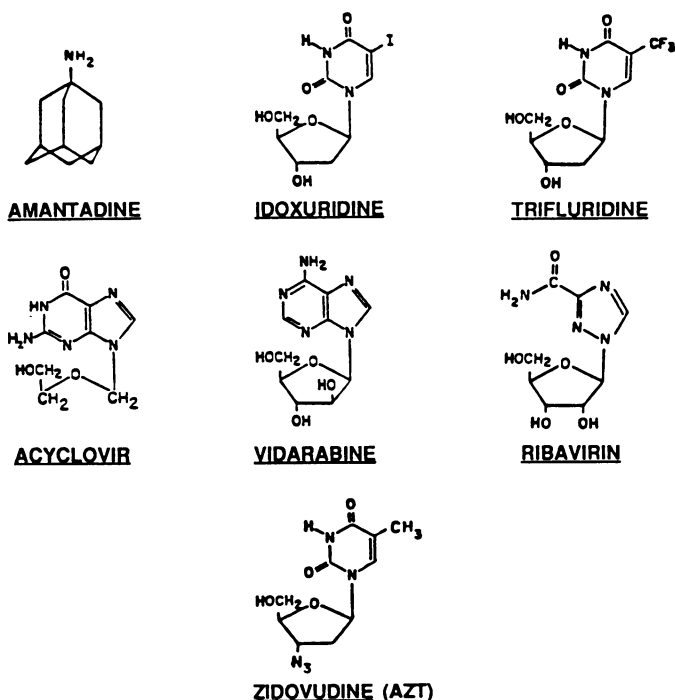


FIG. 1. Structure of anti-viral agents approved by the FDA for use in humans.

TABLE 1
Site of Inhibition of FDA-Approved Antiviral Drugs

Drug	Proposed Site of Inhibition
Amantadine	Interacts with the external surface of the cellular plasma membrane to prevent penetration of influenza A virus. High concentrations of amantadine also inhibit fusion of viral and endosome membranes by increasing the pH of the lysosome, thereby preventing the pH 5-catalyzed conformational change of the membrane protein that is required for subsequent release of the viral genome into the cytoplasm.
Idoxuridine	This compound is phosphorylated to the mono-, di-, and triphosphate derivatives with subsequent incorporation into viral DNA. A direct correlation exists between incorporation of idoxuridine into HSV-1 DNA and inhibition of the formation of infectious virus. Substituted viral DNA decreases formation of polyadenylated mRNA with subsequent inhibition of the formation of specific proteins.
Trifluridine	Like idoxuridine, it is phosphorylated to the mono-, di-, and triphosphate derivatives. As the triphosphate, it is incorporated into DNA with a subsequent adverse effect on mRNA and protein biosynthesis. In addition, the monophosphate of trifluridine inhibits thymidylate synthetase, thereby decreasing the pool sizes of competing thymidine nucleotides.
Vidarabine	There are multiple sites of inhibition, but the inhibition causally related to the antiviral activity has not yet been established: <ol style="list-style-type: none"> (1) Ara-ATP is a potent inhibitor of <i>ribonucleoside diphosphate reductase</i> from both infected and uninfected cells, but that from HSV-infected cells is more sensitive to inhibition. (2) Ara-ATP prevents post-transcriptional addition of <i>poly A</i> to viral mRNA. (3) Ara-ATP inhibits <i>RNA-dependent RNA polymerase</i> of vesicular stomatitis virus.

TABLE 1—Continued

Drug	Proposed Site of Inhibition
	<p>(4) Ara-ATP is a competitive inhibitor of dATP for both mammalian as well as viral <i>DNA-polymerases</i>; however, HSV DNA polymerase is about tenfold more sensitive with a K_i of 0.3 μM for viral DNA polymerase versus 5.0 μM for cellular DNA polymerase α.</p> <p>(5) Ara-ATP is also a <i>substrate for DNA polymerase</i> and is incorporated in internucleotide linkages in both viral and cellular DNA.</p> <p>(6) <i>Slowing of DNA elongation</i> has been reported, and this effect could be a consequence of the 3'-exonuclease activity associated with HSV DNA polymerase. Thus by continuous incorporation and removal of ara-AMP from the terminal position, ara-ATP could act as a pseudo chain terminator.</p> <p>(7) Ara-ATP inhibits <i>terminal deoxynucleotidyl transferase</i>.</p> <p>(8) Ara-A, without being phosphorylated, <i>inhibits S-adenosylhomocysteine hydrolase</i>, and this inhibition results in an accumulation of S-adenosylhomocysteine. The consequence of this effect is an inhibition of S-adenosylmethionine-dependent methylation reactions such as those involved in capping of viral mRNA.</p>
Acyclovir	<p>The molecular basis for its selective antiviral activity is the preferential phosphorylation of ACV by HSV-1 encoded thymidine kinase. Once the monophosphate of ACV is formed, it is converted by cellular enzymes to the di- and triphosphate derivatives. The triphosphate of ACV is a competitive inhibitor of the utilization of dGTP for the synthesis of viral DNA. It preferentially inhibits herpesvirus-encoded DNA polymerase, for which it has about a hundredfold greater binding affinity than for the cellular enzyme. It also is incorporated into the viral DNA and terminates elongation of the DNA. When incorporated, the ACV-substituted DNA template inhibits the viral DNA polymerase by formation of a tight complex.</p>
Zidovudine (AZT)	<p>AZT is phosphorylated by cellular enzymes to the mono-, di-, and triphosphate derivatives. The triphosphate of AZT is terminally incorporated into DNA and is responsible for the inhibition of HIV-1 replication. The toxic effects of AZT may result from its inhibition of the replication of mitochondrial DNA in bone marrow cells, which could lead to a diminishing cellular content of mitochondria until ATP or some other critical metabolite becomes limiting for cell growth or viability [10].</p>
Ribavirin	<p>It is phosphorylated to the mono-, di-, and triphosphate derivatives by cellular enzymes. As the monophosphate, it may decrease the pool size of GTP and dGTP, which are required for RNA and DNA synthesis, respectively, by inhibition of inosinate dehydrogenase. This enzyme converts inosinate to xanthylate, which is the precursor for guanosine monophosphate. The triphosphate of ribavirin inhibits the viral-specific mRNA capping enzymes, guanyl transferase and N^7 methyl transferase, the consequence being an adverse effect on viral protein formation. It also is a potent inhibitor of the influenza virus RNA polymerase.</p>
Interferon	<p>The basis for the antiviral activity is not clear, and different sites of inhibition may be involved, depending on the specific virus and/or host cell involved. The antiviral state is initiated by the binding of interferon to a specific receptor on the cell surface. Synthesis of viral proteins may be prevented by one or both of the following interferon-induced events:</p> <p>(1) Induction of 2', 5'-adenylate synthetase which converts ATP to a 2', 5'-polyadenylate, which in turn activates a latent endonuclease that hydrolyzes viral mRNA.</p> <p>(2) Induction of a protein kinase that phosphorylates an inactive initiation factor (eIF-2), which now inhibits initiation of peptide chain synthesis. Viral adsorption, penetration, uncoating, assembly, and release have also been implicated.</p>

TABLE 2
Some Toxicities of FDA-Approved Antiviral Drugs

Drug	Toxicity
Amantadine	Nervousness, difficulty in concentrating, drowsiness, anxiety, insomnia, dizziness, headache High levels of drug or when administered to patients with renal impairment: acute delirium, visual and auditory hallucinations, convulsions, coma
Idoxuridine	<i>Systemic administration:</i> Bone marrow depression, alopecia, stomatitis <i>Topical administration:</i> Contact dermatitis, follicular conjunctivitis, lid changes, lacrimation, punctate epithelial keratopathy in therapy of herpetic keratitis
Trifluridine	Toxicities similar to idoxuridine. Most frequent is mild transient stinging upon therapy of herpetic keratitis, punctate epithelial damage, eyelid edema
Vidarabine	<i>Topical administration:</i> Therapy of herpes keratitis can produce lacrimation, foreign body sensation, burning, irritation, photophobia. <i>Systemic administration:</i> Nausea, vomiting, and diarrhea. High doses may cause dizziness, hallucinations, psychosis, hepatotoxicity, and bone marrow depression. In experimental animals, it is oncogenic and teratogenic and therefore a concern in use during pregnancy or in infants.
Acyclovir	<i>Topical administration:</i> Mild transient stinging upon instillation in eye, punctate keratitis, follicular conjunctivitis, hypersensitivity <i>Systemic administration:</i> Intravenous bolus may produce reversible renal damage due to deposition of drug crystals in renal tubules; lethargy, obtundation, tremors, confusion, hallucinations, agitation, seizures, coma, phlebitis at injection site, rash, hives
Zidovudine (AZT)	Macrocytic anemia, granulocytopenia, fever, rash, edema, back pain, chest pain, arthralgia, muscle spasm, anxiety, depression, reversible confusion
Ribavirin	<i>Aerosol administration:</i> Deterioration of pulmonary function in patients with chronic obstructive lung disease or asthma; dyspnea, chest soreness, cardiac arrest, hypotension digitalis toxicity, pulmonary edema, reversible anemia, mild headache, mild abdominal cramps, diarrhea. Precipitation of drug when used with a ventilatory apparatus may produce serious problems during therapy of RSV.
Interferon	Fever, bone marrow depression, gastrointestinal symptoms, alopecia, nose bleeds by nasal administration

been approved for therapy of acquired immune deficiency syndrome (AIDS) caused by human immunodeficiency virus type 1 (HIV-1). Alpha interferon has been approved recently for treatment of genital warts caused by the papilloma virus and applied by direct injection into the lesion. Interferon is also approved for therapy of hairy cell leukemia and Kaposi sarcoma.

None of these FDA-approved drugs, however, is without toxicities [9,10] (Table 2) and hence there is a strong need for improved drugs not only to improve efficiency but also to circumvent these problems of toxicity. There is also a need to find an effective therapy of viral infections for which we do not at present have clinically useful drugs or vaccines [11].

Among the human viruses or the diseases they produce for which we need useful or improved drugs or vaccines, we may list the following: HIV-1 (AIDS), cytomegalovirus, Epstein-Barr virus, HSV-1, HSV-2, varicella virus, influenza A, influenza B, enterovirus, hepatitis A, hepatitis B, adenovirus, common cold, bronchiolitis, croup, dengue, poliomyelitis, measles, rabies, warts, chickenpox, mumps, and rubella.

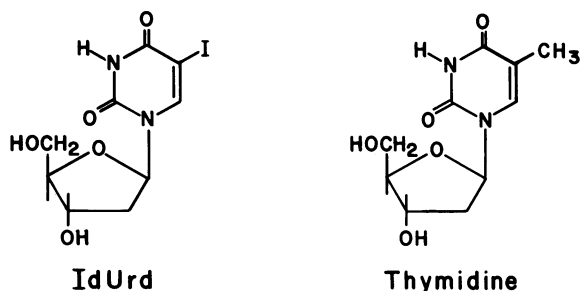


FIG. 2. Structures of IdUrd (idoxuridine) and thymidine.

DISCUSSION

A variety of approaches have been pursued for the development of antiviral drugs, as well as drugs in general, and these may be broadly classified as either the *rational* or the *empirical* strategy [12–16].

The *empirical* approach involves repeated structure modification of a lead compound until optimal antiviral activity is obtained, but with an acceptable therapeutic index. The probability of producing such a compound can be improved by combining structure-activity relationships with computer-graphic model building. This empirical approach perhaps could be termed also the “rational serendipity” approach, because structural modifications made are based on a rational extension of existing knowledge with other or similar compounds. A simple structural modification may not, however, produce the desired end product because any modification of structure will produce changes in size, shape, electronic distribution, partition coefficient, solubility, pKa, chemical reactivity, metabolism, and hydrogen-bonding capabilities [16]. The hope is that the change being made will result in improved potency, selectivity, duration of action, bioavailability, and reduced toxicity [16]. If the hope is realized, the achievement may be termed “rational serendipity.”

An example of a “rational serendipity” approach for development of drugs is that developed by Hitchings and his colleagues [17,18]. They were the first to study analogs of purines and pyrimidine bases as potential inhibitors of nucleic acid biosynthesis. The importance of nucleic acids for cellular replication had already been established. Their approach then was systematically to examine the effect of structural changes on the potency of a compound as an inhibitor of bacterial replication. Analogs of purines, pyrimidines, nucleosides, and nucleotides have since been of value in the elucidation of metabolic pathways as well as in the therapy of cancer, metabolic disorders, and therapy of various infectious agents.

Another example of the empirical or “rational serendipity” approach is the modification of the thymidine component of DNA, by replacement of the methyl group on carbon-5 of the pyrimidine moiety with an iodine atom (Fig. 2). Rationale dictated that since the van der Waals’ radii of the methyl group and the iodine atom were very similar, 2.00Å and 2.15Å, respectively, steric hindrance would not be a problem. The serendipity entered in when 5-iodo-2'-deoxyuridine (idoxuridine, IDU, IdUrd, IUdR), originally designed to be an anticancer drug, instead became the first antiviral compound to be approved by the FDA for therapy of a viral infection—herpetic keratitis.

There are many examples of structure modification which resulted in the formation of compounds with antiviral activity. Figure 3 depicts compounds that are substrates for the HSV-1 encoded thymidine kinase. The relationship to thymidine, the normal

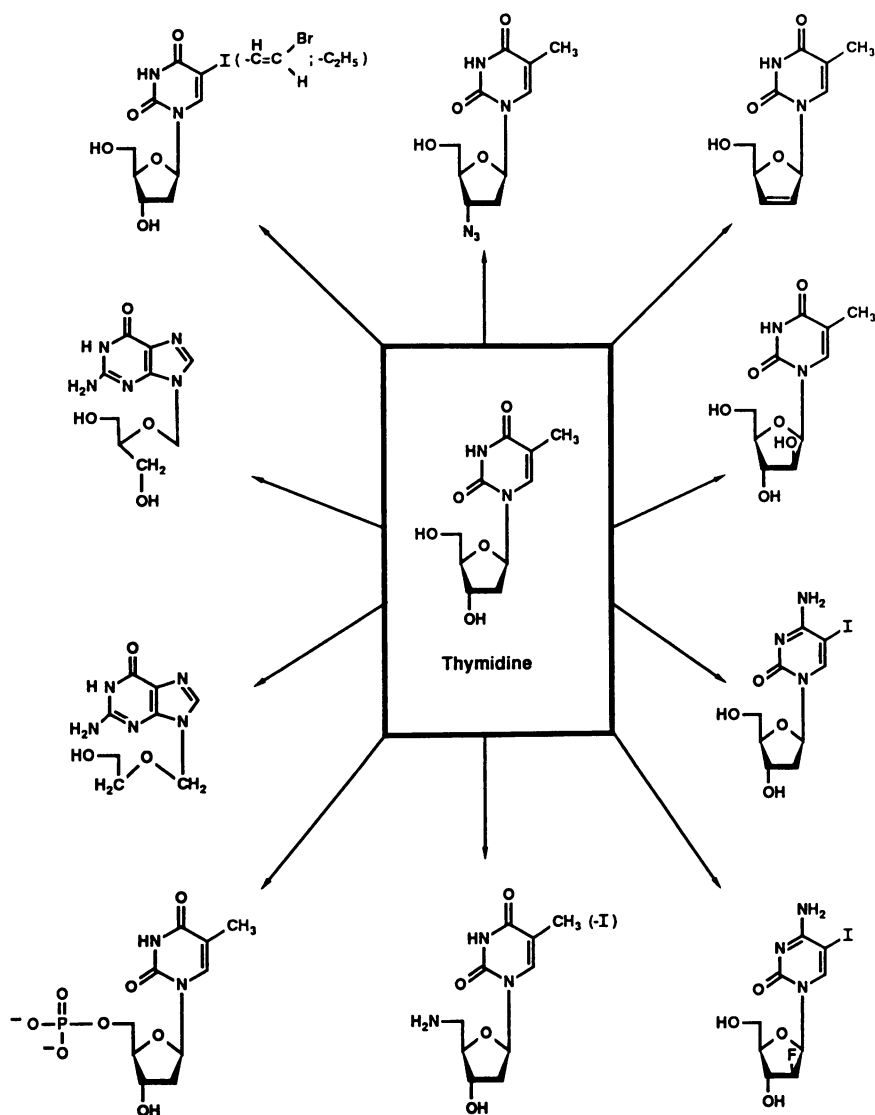


FIG. 3. Various compounds that are substrates of thymidine kinase encoded by herpes simplex virus type 1.

substrate, is reasonable; however, that of the guanine derivatives, acyclovir and ganciclovir, and that of thymidine monophosphate are rather surprising.

The second approach for drug development is the *rational* approach, and it requires a sophisticated knowledge of the target structure—be it the active site of an enzyme, or a receptor, or a macromolecule such as DNA, RNA, or a regulatory protein. Effective use of this approach requires a knowledge of the structural, spatial, and electronic requirements for a compound to interact uniquely with the target receptor. Today, not only do we have the potential to determine the three-dimensional atomic structure of target sites, but also how antiviral agents or drugs interact with these targets by use of X-ray crystallography and nuclear magnetic resonance. Knowledge of how the atoms

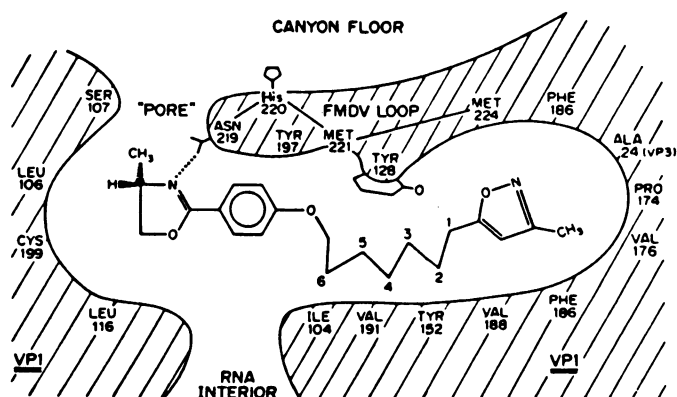


FIG. 4. Diagrammatic representation of the binding site of the antiviral agent WIN 52084 [19].

are arranged, when combined with molecular modeling and interactive computer graphics, affords the design of specific drugs [13–16].

This approach may also allow us to understand how a known antiviral drug exerts its effect. For example, the rhinovirus was crystallized and allowed to interact with a Sterling-Winthrop compound which is known to prevent viral uncoating, and then the complex was subjected to X-ray crystallography. Analysis afforded a computer-graphic picture of how the drug binds into a hydrophobic pocket beneath the canyon floor of the rhinovirus (Fig. 4). This interaction produced a large conformational change in 3-stretches of the VP-1 polypeptide chain, which increased the rigidity of the capsid proteins, with resultant inhibition of the disassembly of the virus [19–24]. Such knowledge allows the more intelligent modification of structure and also allows us to understand on an atomic level why these drugs are effective or ineffective.

An exciting approach for rational drug design is based on our understanding of gene structure and function [25–27]. Some success has already been achieved in the synthesis of “anti-sense” oligodeoxyribonucleotides, whose base pairs are complementary to critical regions of the viral genome or mRNA and, following specific hybridization, block viral expression [28–31] (Fig. 5). Thus, selective inhibition of gene expression is achieved. There are, however, several problems in the use of anti-sense oligonucleotides: (1) attainment of a high concentration in the cell; (2) rapid degradation by nucleases in plasma and cells; (3) transport into the cell; and (4)

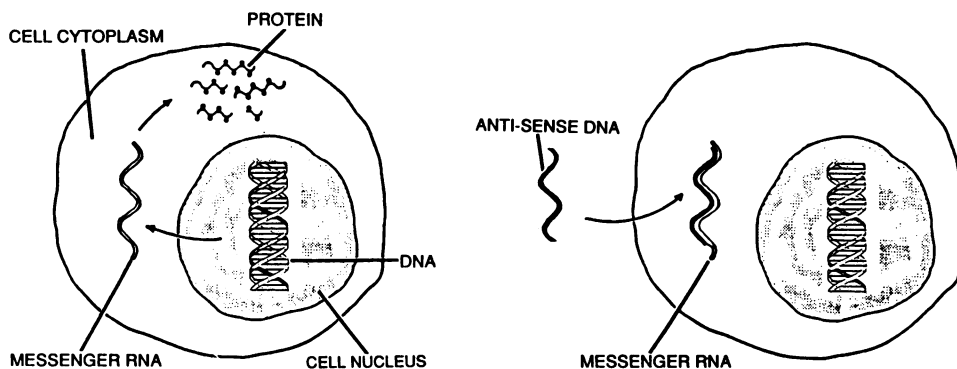


FIG. 5. Inhibition of messenger RNA translation by anti-sense oligodeoxyribonucleotide polymer [28].

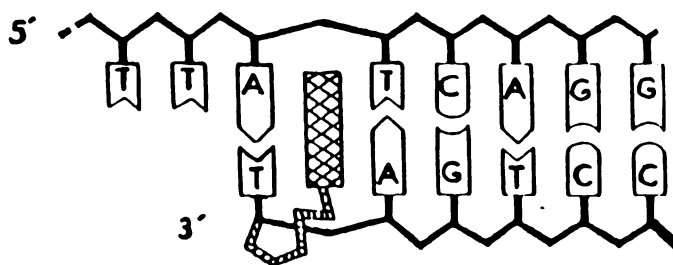


FIG. 6. Intercalation of an acridine derivative attached to anti-sense oligomer [35].

possible limited accessibility of the target nucleic acid sequence because of the tight binding proteins. Some of these problems have been approached in several laboratories and will be briefly described.

The phosphodiester linkage, for example, has been converted into non-ionic phosphotriesters, alkylphosphonates [32], and phosphorothioates [33], as well as non-phosphorous moieties such as amides, carbonates, carbamate, and siloxane [34]. The consequence of such conversion is a decreased sensitivity to enzymic degradation as well as improved transport into the cell because of increased lipophilicity (decreased

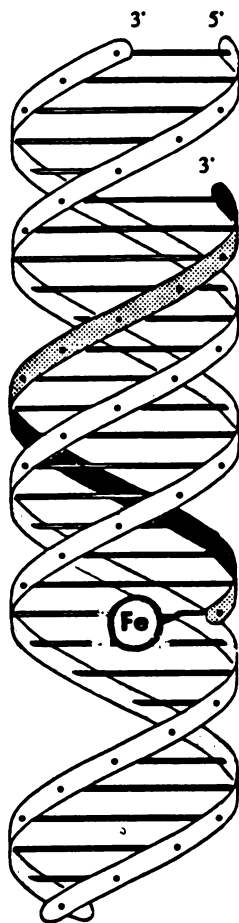


FIG. 7. DNA triple-stranded formation of an oligodeoxyribonucleotide with attached cleaving moiety (EDTA-Fe) to double-stranded DNA [37].

polarity). Unfortunately, the alkylphosphonates introduce an undesirable chiral phosphorus which complicates synthesis.

An increased stability of hybrid formation was achieved by the conjugation of an intercalating compound such as an acridine derivative to either the 3'- or the 5'-terminal of the oligomer via a pentamethylene tether. The intercalating acridine provides additional binding energy to stabilize the hybrid complex and also decreases susceptibility to endonuclease degradation [35] (Fig. 6).

Transport of the oligodeoxyribonucleotide was markedly increased by covalently linking cytidine to the 3'-terminus, oxidatively cleaving the 2', 3'-bond of cytidine, and by subsequent reaction with the epsilon amino moiety of poly-L-lysine forming an N-morpholino ring linking the oligomer to poly-L-lysine [36].

A potentially very important approach is termed "affinity cleaving." This technique involves the use of a complementary oligomer to carry a reactive moiety, which can, under appropriate conditions, destroy a specific portion of the nucleic acid genome. The oligomer-conjugate can react with double-stranded DNA at a specific DNA sequence, forming a triple helix, and a cleaving function, such as EDTA chelated to Fe^{++} , under appropriate redox conditions can generate highly reactive hydroxyl radicals from oxygen, which react and destroy the deoxyribose moieties. Where the deoxyribose moiety is cleaved on both strands of DNA, the gene is destroyed [37-39] (Fig. 7).

This method is truly a rational chemotherapeutic approach for inactivation of specific genes. One can visualize destruction of any viral gene which is integrated into cellular DNA. At present, the replicating virus but not the latent virus is susceptible to inhibition by our present armamentarium of antiviral drugs. Can this technique destroy the AIDS virus when it is integrated into cellular DNA as proviral DNA? Can this approach destroy the latent herpesvirus responsible for recurrences of genital herpes, or the varicella-zoster virus responsible for shingles, or, for that matter, an oncogene if involved in the progression of cancer?

In summary, it appears that some exciting new approaches for the design of antiviral agents are being pursued. We are, however, in the very early stages of development, and there are many obstacles which must be overcome before the "rational-serendipity" approach, or even what we optimistically term the "rational" approach, becomes a truly *rational* approach.

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