ADVANCES IN CHEMOTHERAPY OF AIDS

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Edited by

Robert B. Diasio, M.D.
Jean-Pierre Sommadossi, Ph.D.
Department of Pharmacology, University of Alabama, Birmingham, Alabama

PERGAMON PRESS
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Pergamon Press Offices:

U.S.A. Pergamon Press, Inc., Maxwell House, Fairview Park, Elmsford, New York 10523, U.S.A.

U.K. Pergamon Press plc, Headington Hill Hall, Oxford OX3 0BW, England

PEOPLE'S REPUBLIC OF CHINA Pergamon Press, 0909 China World Tower, No. 1 Jian Guo Men Wai Avenue, Beijing 100004, People's Republic of China

FEDERAL REPUBLIC OF GERMANY Pergamon Press GmbH, Hammerweg 6, D-6242 Kronberg, Federal Republic of Germany

BRAZIL Pergamon Editora Ltda, Rua Eça de Queiros, 346, CEP 04011, Paraiso, São Paulo, Brazil

AUSTRALIA Pergamon Press Australia Pty Ltd., P.O. Box 544, Potts Point, NSW 2011, Australia

JAPAN Pergamon Press, 8th Floor, Matsuoka Central Building, 1-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160, Japan

CANADA Pergamon Press Canada Ltd., Suite 271, 253 College Street, Toronto, Ontario M5T 1R5, Canada

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Library of Congress Cataloging in Publication Data

Advances in chemotherapy of AIDS / edited by Robert B. Diasio, Jean -Pierre Sommadossi.
p. cm.
Consists of revised and updated versions of lectures presented at the first symposium of Advances in Chemotherapy of AIDS held at the University of Alabama at Birmingham on Sept. 23, 1988.
Includes bibliographical references.
ISBN 0-08-040315-8 :
1. AIDS (Disease)--Chemotherapy--Congresses. I. Diasio, Robert B. II. Sommadossi, Jean-Pierre. III. Advances in Chemotherapy of AIDS Symposium (1st : 1988 : University of Alabama at Birmingham)
[DNLM: 1. Acquired Immunodeficiency Syndrome--drug therapy--congresses. WD 308 A244]
RC607.A26A343 1990
616.97'92061--dc20
DNLM/DLC
for Library of Congress 89-71127
CIP

Printing: 1 2 3 4 5 6 7 8 9 Year: 0 1 2 3 4 5 6 7 8 9

Printed in the United States of America

The paper used in this publication meets the minimum requirements of American National Standard for Information Sciences—Permanence of Paper for Printed Library Materials, ANSI Z39.48-1984

PREFACE

The chapters in this volume are revised and updated versions of lectures originally presented on September 23, 1988, at the first symposium of Advances in Chemotherapy of AIDS held at the University of Alabama at Birmingham. This symposium highlighted the most recent advances in basic research in the development of novel chemotherapeutic agents and pharmacologic approaches against acquired immunodeficiency syndrome (AIDS). These manuscripts describe major classes of drugs currently being examined in the treatment of AIDS.

We hope that this volume describing original research from several of the leading laboratories will prove useful to those involved in AIDS research including the molecular biologist, medical chemist, biochemist, pharmacologist, and clinician involved in the care of AIDS patients.

We would like to express our appreciation to the following companies for their generous support: Adria Laboratories; Beckman Instruments; Bristol-Myers Company; Lederle Laboratories; Mead Johnson Pharmaceutical Division; Sandoz Pharmaceuticals Corporation; Schering Corporation; Syntex Research; and The Upjohn Company.

ROBERT B. DIASIO
JEAN-PIERRE SOMMADOSSI

LIST OF CONTRIBUTORS

E. Michael August
Department of Pharmacology and
Comprehensive Cancer Center
Yale University School of Medicine
New Haven, CT

Yarlagadda S. Babu
Center for Macromolecular Crystallography
University of Alabama at Birmingham
Birmingham, AL

Isadore Brodsky
Department of Neoplastic Diseases
Hahnemann University
Philadelphia, PA

Robert B. Brown, Jr.
Department of Chemistry
Emory University
Atlanta, GA

Charles E. Bugg
Center for Macromolecular Crystallography
University of Alabama at Birmingham
Birmingham, AL

Ricarda Carlisle
Department of Pharmacology
Comprehensive Cancer Center
and Center for AIDS Research
Division of Clinical Pharmacology
University of Alabama at Birmingham
Birmingham, AL

William A. Carter
Department of Neoplastic Diseases
Hahnemann University
Philadelphia, PA

William J. Cook
Center for Macromolecular Crystallography
University of Alabama at Birmingham
Birmingham, AL

Pamela A. Crilley
Department of Neoplastic Diseases
Hahnemann University
Philadelphia, PA

Robert B. Diasio
Department of Pharmacology
Comprehensive Cancer Center
Division of Clinical Pharmacology
University of Alabama at Birmingham
Birmingham, AL

Steven E. Ealick
Center for Macromolecular Crystallography
University of Alabama at Birmingham
Birmingham, AL

Mahmoud H. el Kouni
Division of Biology and Medicine
Brown University
Providence, RI

Mahmoud Faraj
Department of Chemistry
Emory University
Atlanta, GA

Robert Fenning
Charlotte Memorial Hospital
Charlotte, NC

Arnold Fridland
Deparment of Biochemical and Clinical
Pharmacology
St. Jude Children's Research Hospital
Memphis, TN

Donald B. Harden
Department of Chemistry and Laboratory for
Microbial and Biochemical Sciences
Georgia State University
Atlanta, GA

Melody Hartnup
Department of Chemistry
Emory University
Atlanta, GA

Craig L. Hill
Department of Chemistry
Emory University
Atlanta, GA

Jean-Louis Imbach
Laboratoire de Chimie Bio-Organique
Université de Montpellier II,
Sciences et Techniques du Languedoc
Montpellier, FRANCE

Mark A. Johnson
Department of Biochemical and Clinical
Pharmacology
St. Jude Children's Research Hospital
Memphis, TN

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Miryam Kadkhodayan
Department of Chemistry
Emory University
Atlanta, GA

Katalin Kariko
Department of Biochemistry
Temple University School of Medicine
Philadelphia, PA

Shi Wu Li
Department of Biochemistry
Temple University School of Medicine
Philadelphia, PA

Tai-Shun Lin
Department of Pharmacology and
Comprehensive Cancer Center
Yale University School of Medicine
New Haven, CT

Claude Malvy
Institut Gustave Roussy
Villejuif, FRANCE

Maria Elena Marongiu
Instituto di Microbiologia
Universita Degli Studi di Cagliari
Cagliari, ITALY

Jerzy L. Mokrosz
Polish Academy of Sciences
Institute of Pharmacology
Cracow, POLAND

Maria J. Mokrosz
Department of Chemistry
Jagiellonian University
Cracow, POLAND

François Morvan
Laboratoire de Chimie Bio-Organique
Université de Montpellier II,
Sciences et Techniques du Languedoc
Montpellier, FRANCE

Sthanam V. L. Narayana
Center for Macromolecular Crystallography
University of Alabama at Birmingham
Birmingham, AL

Claude Paoletti
Institut Gustave Roussy
Villejuif, FRANCE

Jacques Paoletti
Institut Gustave Roussy
Villejuif, FRANCE

Edward C. Pequignot
Department of Neoplastic Diseases
Hahnemann University
Philadelphia, PA

Christina M. Prosser-McCartha
Department of Chemistry
Emory University
Atlanta, GA

William H. Prusoff
Department of Pharmacology and
Comprehensive Cancer Center
Yale University School of Medicine
New Haven, CT

Bernard Rayner
Laboratoire de Chimie Bio-Organique
Université de Montpellier II,
Sciences et Techniques du Languedoc
Montpellier, FRANCE

Nancy L. Reichenbach
Department of Biochemistry
Temple University School of Medicine
Philadelphia, PA

Raymond F. Schinazi
Veterans Affairs Medical Center and
Laboratory of Biochemical Pharmacology
Department of Pediatrics
Emory University School of Medicine
Atlanta, GA

William M. Shannon
Microbiology Research Department
Kettering-Meyer Laboratory
Southern Research Institute
Birmingham, AL

Robert W. Sobol
Department of Biochemistry
Temple University School of Medicine
Philadelphia, PA

Jean-Pierre Sommadossi
Department of Pharmacology
Comprehensive Cancer Center and
Center for AIDS Research
Division of Clinical Pharmacology
University of Alabama at Birmingham
Birmingham, AL

David R. Strayer
Department of Neoplastic Diseases
Hahnemann University
Philadelphia, PA

Lucjan Strekowski
Department of Chemistry and Laboratory for
Microbial and Biochemical Sciences
Georgia State University
Atlanta, GA

Robert J. Suhadolnik
Department of Biochemistry
Temple University School of Medicine
Philadelphia, PA

Mark Weeks
Department of Chemistry
Emory University
Atlanta, GA

Douglas A. Weidner
Department of Pharmacology
Comprehensive Cancer Center and
Center for AIDS Research
Division of Clinical Pharmacology
University of Alabama at Birmingham
Birmingham, AL

W. David Wilson
Department of Chemistry and Laboratory for
Microbial and Biochemical Sciences
Georgia State University
Atlanta, GA

Meng-Yu Xie
Department of Pharmacology
Comprehensive Cancer Center and
Center for AIDS Research
Division of Clinical Pharmacology
University of Alabama at Birmingham
Birmingham, AL

Zhou Zhu
Department of Pharmacology
Comprehensive Cancer Center and
Center for AIDS Research
Division of Clinical Pharmacology
University of Alabama at Birmingham
Birmingham, AL

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Diasio, R. B. and Sommadossi, J-P., editors.
(1990) *Advances in Chemotherapy of AIDS.*
Pergamon Press, Inc. (New York), pp. 1–9
Printed in the United States of America

CHAPTER 1

DEVELOPMENT OF ANTIVIRAL AGENTS FOR THERAPY OF AIDS

WILLIAM H. PRUSOFF,* TAI-SHUN LIN,* E. MICHAEL AUGUST,* AND MARIA ELENA MARONGIU†

**Department of Pharmacology and Comprehensive Cancer Center, Yale University School of Medicine, New Haven, CT; †Instituto di Microbiologia, Universita Degli Studi di Cagliari, Cagliari, Italy*

1. INTRODUCTION

The substances that have been approved by the Food and Drug Administration (FDA) for therapy of viral infections in humans include eight synthetic compounds plus interferon α. Idoxuridine, trifluridine, vidarabine, and acyclovir are approved for therapy of various herpesvirus infections; amantadine for therapy of respiratory infections caused by the influenza A virus, ribavirin for therapy of severe respiratory infections caused by the respiratory syncytial virus; zidovudine for therapy of autoimmune deficiency syndrome caused by human immunodeficiency virus 1 (HIV-1), and most recently interferon α for genital warts caused by the papilloma virus, and ganciclovir for therapy of cytomegalovirus retinitis.

There are a number of other very effective antiviral agents which could be clinically useful not only for therapy of acquired immunodeficiency syndrome (AIDS) but also for other viral infections if we could devise procedures for their preferential uptake into or activation in the infected cell, or restrict their action to only the infected cell.

The need for effective drugs is critical. The World Health Organization (WHO) estimates that over 5 million individuals may be infected with HIV-1 worldwide, and about 200,000 cases of AIDS have already occurred. In the United States alone, about 1 to 1.5 million are infected, and 88,000 cases of AIDS have been diagnosed and about 60% of these patients have died. Obviously, there is a strong need for the development of agents for therapy of AIDS, and this symposium was designed to explore some of the approaches for this end.

The drug azidothymidine (zidovudine, or AZT) is not the ideal drug for therapy of AIDS because of toxicities, primarily severe bone marrow depression. Although combination therapy is underway in an attempt to overcome this problem, there is a need for less toxic antiviral drugs. Hundreds of compounds have already been evaluated for potential activity against the HIV-1 virus. The National Institutes of Health (NIH) is setting up a program to screen about 10,000 natural and synthetic substances per year for anti-HIV-1 activity.

The development of antiviral agents for therapy of AIDS is being approached basically by two strategies: the empirical and the rational strategy, as well as modifications of both.

2. THE EMPIRICAL APPROACH

The empirical approach has provided most of the drugs in clinical use, and it would be prudent to continue this strategy. This approach entails the identification of a *lead* compound and subsequent repeated structure modification of the lead compound, and evaluation of activity, to produce a continual improvement until optimization is achieved. The lead compound may have been obtained by random screening (amantadine) or by structural modification of a critical metabolite, such as the replacement of the methyl moiety of thymidine with an iodine atom to produce idoxuridine (Prusoff,

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1959). The structural modifications of the lead compound that are made are dependent upon the chemists' experience and intuition, as well as serendipity. The probability of producing an active compound today can be greatly improved by combining structure-activity relationships with computer graphic model building.

Examples of a lead compound include idoxuridine (Prusoff, 1959) and AZT (Horwitz et al., 1964), both of which were first synthesized to be anticancer compounds but were found to have antiviral activity against herpesviruses (Herrmann, 1961; Kaufman, 1962) and retroviruses (Ostertag et al., 1974; Mitsuya et al., 1985), respectively. Numerous derivatives of idoxuridine have been synthesized: replacement of the iodine on carbon 5 of the pyrimidine moiety with a variety of substituents ($-CF_3$; $-C_2H_5$; $-CH=CHBr$); replacement of the uracil moiety with cytosine; replacement of the deoxyribose with other sugars (arabinose, xylose, etc.), cyclopentane, or acyclic moieties; or replacement of the 5′-hydroxyl with an amino function.

The finding that AZT is active against HIV-1, the virus responsible for AIDS (Mitsuya et al., 1985; Yarchoan et al., 1986), has also stimulated the synthesis of numerous nucleoside derivatives as well as evaluation of nucleosides previously synthesized.

The hope is that the modifications made in a compound will result in improved biological and physical properties such as potency, selectivity, duration of action, bioavailability, reduced toxicity, stability, and so forth. However, any modification of structure will produce a variety of physical and chemical changes, such as size, shape, electronic distribution, and metabolism (Baldwin, 1987). Any of these changes could markedly affect the biological properties desired, either positively or negatively. For example, Lin et al. (1987a,b; 1988) modified the structure of AZT by replacement of the methyl group with various substituents, or the addition of 3-oxopropenyl on nitrogen 3 of the pyrimidine, or replacement of carbon 6 with a nitrogen atom, but a more active compound was not produced; however, several compounds previously synthesized by Horwitz and colleagues (1964, 1967) such as 2′,3′-dideoxycytidin-2′-ene (d4C) and 3′-deoxythymidin-2′-ene (d4T) were found to be potent inhibitors of HIV-1 (Lin et al., 1987b,c) with very low levels of cytotoxicity (August et al., 1988).

Independently, several other laboratories have studied these compounds, and similar antiviral activities as reported by Lin et al. (1987b,c) were also found for d4T (Balzarini et al., 1987; Baba et al., 1987; Hamamoto et al., 1987) and for d4C (Hamamoto et al., 1987; Balzarini et al., 1986; 1987).

Our laboratory has worked closely with the scientists at Bristol-Myers Company for development of d4T, and d4T is at present in phase 1 clinical trial for the following reasons:

1. It has potent antiviral activity against HIV-1. The concentration required to inhibit viral replication by 50% is 0.0088 μM (Lin et al., 1987c).
2. It has very little cytotoxicity. The concentration required to inhibit uninfected H9 cells is 250 μM (August et al., 1988).
3. It is 100-fold less toxic than AZT to normal human granulocyte-macrophage progenitor cells, but has similar effects on erythroid progenitor cells (Mansuri et al., 1989).
4. d4T is almost completely absorbed on oral administration (Russell et al., 1989).
5. d4T crosses the blood-brain barrier at an antiviral concentration (Russell et al., 1989).
6. d4T, unlike AZT, does not form the glucuronide, but is excreted in the urine unchanged (Russell et al., 1989).
7. Bristol-Myers scientists (personal communication) have found that:
   a. d4T, in a comparative toxicity study with AZT, has a more favorable toxicity profile;
   b. d4T has less tendency to cause anemia.
   c. Toxicity studies in rats and monkeys are in progress.

8. Phase 1 trials are in progress and preliminary results are very encouraging relative to efficacy and low toxicity.

3. THE RATIONAL APPROACH

The second approach for drug development is the *rational approach,* which is a greater challenge, but is intellectually more satisfying. Rapid progress is being made in this direction, and hopefully the rational approach will soon be the productive way to go. This approach requires a sophisticated knowledge of the target structure—be it an active site of an enzyme, a receptor, or a macromolecule such as DNA, RNA, or a regulatory protein—as well as a knowledge of the biochemical pathways involved. Once the target to be attacked is identified, the structural, spatial, and electronic requirements for a compound to uniquely interact with the target receptor must be determined.

3.1. HIV-1 Targets

What are some of the targets that are unique to the AIDS virus, and hence appropriate for development of a drug? Examination of the structure of HIV-1 as well as our present understanding of the biochemistry of how it replicates will identify potential targets for drug design.

Two molecules of viral RNA genome plus reverse transcriptase are surrounded by a shell composed of p24 proteins, and then by an outer shell of p18 proteins which lies just beneath the lipid bilayer. Glycoprotein, gp120, lies on the outside of the bilipid membrane, and it is hooked into the interior by the glycoprotein gp41.

The replication of HIV-1 is initiated by its adsorption to a susceptible cell by interaction with a specific cell-surface receptor termed CD4. After adsorption to the cell surface, the virus penetrates either by fusion with the cell surface or by endocytosis. Either way the viral lipid bilayer and core proteins are removed with release of the two molecules of RNA, which are bound to several molecules of the reverse transcriptase. The reverse transcriptase transcribes the viral RNA to form a complementary DNA, which hybridizes with the RNA. The RNA of the RNA-DNA hybrid is enzymically degraded and the DNA is replicated, forming a DNA duplex which circularizes and is integrated into the cellular DNA. The proviral DNA may remain in a latent state for an unknown period of time, and when the proviral DNA is activated, RNA is transcribed from the DNA, forming genomic RNA and mRNA, and the mRNA is translated into enzymatic and structural proteins. A complicated series of events are involved that include intragene regulation as well as polyprotein processing which involves proteolytic cleavage, glycosylation, and myristylation. The stage is now set for assembly in the host cell cytoplasm and release of the virion by budding through the host cell membrane, where a bilipid envelope is acquired.

Thus the possible targets include:

1. Adsorption
2. Penetration—fusion or endocytosis
3. Uncoating
4. Reverse transcription:
   a. RNA → RNA ~ DNA
   b. Degradation of RNA in the RNA-DNA hybrid (RNase H)
   c. DNA → linear double stranded DNA (dsDNA) → circular dsDNA
5. Integration of viral dsDNA into host cell genome
6. Transcription of proviral DNA into mRNA and RNA genome
7. Replication of proviral DNA

8. Translation of viral mRNA into protein and its regulation
9. Cleavage of polyprotein by protease
10. Processing of protein
    a. Glycosylation
    b. Myristylation
11. Assembly of the formed macromolecules into a virion
12. Release of virion by budding

There are at least eight genes in the HIV-1 genome, and each makes products that are potential targets. These products include regulatory proteins; enzymes such as reverse transcriptase, endonuclease, RNase H, and protease; structural proteins; and proteins of unknown function.

Hundreds of compounds have already been examined for anti-HIV activity. Most compounds have little or no activity or are too toxic, and some look promising and are in various stages of development. It would not be feasible to list all the various compounds that have been evaluated, and hence I will mention only a few and their targets.

Adsorption of the virus to a susceptible cell has been interfered with by use of (1) a monoclonal antibody to bind with the cellular CD4 receptor, (2) soluble CD4 antigen to bind the viral gp120, (3) peptide T, an octapeptide, to block the CD4 receptor, (4) dextran sulfate to block binding of the virus to the cell surface or to prevent fusion-dependent events, and (5) AL-721 to extract sterols from viral or cellular membranes, thereby increasing fluidity which may prevent adsorption or penetration.

Reverse transcriptase is a popular target, either for inhibition by compounds such as suramin, HPA-23, phosphonoformate, or ansamycin; or for preferential terminal incorporation of nucleoside analogs into HIV-1 DNA as exemplified by 2′,3′-dideoxynucleoside analogs (AZT, d2C, d4C and d4T).

Viral proteins are another target for inhibition. These targets include the viral encoded enzymes responsible for polyprotein cleavage (protease inhibitors) and processing of glycoproteins (castanospermine), function of regulatory proteins, myristylation of protein, translation of HIV-1 mRNA (antisense oligodeoxyribonucleotides), and so forth. Antisense oligomers can inhibit not only viral translation but also viral transcription.

3.2. Applications of Rational Drug Design

Today we have the potential to determine not only the three-dimensional atomic structure of target sites such as receptors, or viruses, or macromolecules but also how antiviral agents or drugs interact with these targets by use of x-ray crystallography and nuclear magnetic resonance (NMR). Knowledge of how the atoms are arranged, when combined with molecular modeling and interactive computer graphics, has the potential to afford the rational design of specific drugs. X-ray crystallography and computer modeling have been used very effectively to understand the mechanism of action of several compounds which prevent uncoating of the rhinovirus (T. J. Smith et al., 1986; Rossmann et al., 1987). For example, the rhinovirus was crystallized and allowed to interact with the drug and then subjected to x-ray crystallography, and a computer-graphic picture was made of how the Sterling-Winthrop compound binds into a hydrophobic pocket beneath the canyon floor of the virus (Colonno et al., 1988). The insertion of the compound into the hydrophobic pocket produces a large conformational change in three stretches of the viral VP-1 polypeptide chain, and the consequence is that the protein shell is less flexible and thereby inhibits disassembly of the virus (Badger et al., 1988).

This finding may be considered to be a "lead," since now the potential is available to modify structure in order to enhance the interaction between virus and drug. The reverse situation is more difficult, that is, to design a drug based on knowledge of the structure of the macromolecule whose function you wish to inhibit—whether it be an enzyme or a regulatory protein or something else.

There are two major problems. One is to obtain large amounts of protein, and the second is to prepare single crystals of the protein that are large enough so that the three-dimensional structure can be elucidated by x-ray diffraction. This is not a trivial task; however, large amounts of protein can be obtained by genetic engineering utilizing, for example, bacteria in which the appropriate gene has been inserted. A novel approach to obtain large crystals is to take advantage of space research. Under conditions of microgravity in space, single crystals of β-galactosidase and lysozyme were formed which were 27 and 1000 times larger, respectively, than those obtained under conditions of normal gravity (Littke and John, 1984).

However, even though a drug is designed based on the x-ray structure of the macromolecule, this does not ensure its clinical utility. There are problems of absorption, distribution, metabolism, excretion, and unpredictable toxicities. But this is true of any new compound, whether obtained by the empirical or the rational approach.

Rational design of antiviral drugs may be based on our understanding of gene structure and function. Some success has already been achieved in the synthesis of antisense oligodeoxyribonucleotides, whose base pairs are complementary to critical regions of the viral genome or mRNA and following specific hybridization block their expression (Green et al., 1986; Loose-Mitchell, 1988; Stein and Cohen, 1988).

Zamecnik and Stephenson (1978) found a single-stranded 13-base oligodeoxyribonucleotide, complementary to a region of the terminally redundant sequences of Rous sarcoma virus, that inhibits viral production. More recently Zamecnik and colleagues (1986) found that the replication and expression of HIV-1 is also inhibited by an antisense oligonucleotide, presumably by hybridization competition.

However, there are several problems in the use of antisense oligonucleotides; (1) rapid degradation by nucleases in plasma and cells; (2) a need for transport into cells; (3) a need for high concentrations for the inhibition to occur; (4) the presence of tight binding proteins at the target site; and (5) a need for oral delivery. These challenges have been approached by several groups. Enzymatic degradation of oligodeoxyribonucleotides has been decreased by converting the phosphodiester linkage into nonionic links such as phosphotriester or alkylphosphonates (Miller et al., 1979, 1981; Miller and Ts'o, 1987), phosphorothioates (Matsukura et al., 1987), or nonphosphorous moities such as amides, carbonates, carbamate, or siloxane (Cormier and Ogilvie, 1988). Thus an oligodeoxyriboside methyl phosphonate that is complementary to a section of HSV-1 mRNA, and has a chain length of 8 nucleotidyl units, produced a two-log reduction in virus yield (C. C. Smith et al., 1986). Furthermore, the methyl phosphonates, being less polar, are transported more readily into cells.

Another approach to circumvent the problems of transport of the oligomer into the cell as well as the stability of the formed hybrid involves the conjugation of acridine derivatives, via a pentamethylene tether, to either the 3′ or the 5′ end of the oligonucleotide (Helene et al., 1985). Thus, binding specificity toward the complementary sequence is retained, while the intercalating acridine provides additional binding energy to stabilize the hybrid complex (Thuong et al., 1987). In addition, the acridine decreases susceptibility of the oligomer to exonuclease degradation, and also facilitates cellular uptake. Furthermore, because of the increased binding stability, the size of the oligonucleotide can be shortened (Asseline et al., 1984a, Helene et al., 1985). Thus inhibition of *exo*nuclease degradation was achieved by the addition of the terminal acridine, and protection from *endo*nuclease was achieved by modification of the phosphodiester linkage (Asseline et al., 1984a,b; Miller et al., 1979).

Transport of oligonucleotides into the cell has been increased by attachment of the oligomer to a positively charged polymer, such as poly-L-lysine [poly(LLys)]. Lemaitre and colleagues (1987) conjugated a 13- to 15-base oligonucleotide which was complementary to a region of the vesicular stomatitis virus genome to poly(LLys), and they found antiviral activity at a concentration of 0.1 μM.

The precise mechanism for inhibition of viral replication by oligonucleotides, how-

ever, may be more complex than inhibition of transcription or translation. Matsukura and colleagues (1987) found that phosphorothioate analogs of oligodeoxyribonucleotides are potent inhibitors of the AIDS virus at a concentration of 0.5 μM, comparable to that of dideoxycytidine. They found significant inhibition of purified HIV reverse transcriptase. Therefore, these compunds may have multiple sites of inhibition, which may vary with the specific composition and length.

3.3. Affinity Cleaving

An interesting "rational approach" with great potential is termed *affinity cleaving* and involves the use of a complementary oligomer to carry a moiety which can destroy a specific region of the DNA genome. Information about gene sequences which would be required for this approach is rapidly being accumulated and is being stored in computerized data banks. We now have the instrumentation to synthesize specific complementary oligodeoxyribonucleotides based on the knowledge of gene sequences. There are many advantages in attacking the DNA genome rather than the mRNA. Specifically, it is desirable to attack the AIDS virus when it exists as the provirus incorporated into cellular DNA. If you destroy the proviral DNA, you prevent formation of a critical mRNA, but if you inactivate the mRNA by hybridization, the proviral DNA can make more mRNA, which will have to be continually inactivated.

The concept of using a complementary oligodeoxyribonucleotide which carries a special reagent for modification of a desired region of the nucleic acid polymer was first discussed as by Belikova et al. (1967) and by Grineva and Karpova (1973) and is now being pursued by Moser and Dervan (1987) and Miller and Ts'o (1987). The latter two groups also make use of an oligodeoxyribonucleotide complementary to a specific sequence of dsDNA, which is conjugated with a DNA-cleaving moiety in order to destroy a specific gene. The oligodeoxyribonucleotide conjugate can react with dsDNA, forming a triple helix at a specific DNA sequence. The cleaving function is ethylenediaminetetracetic acid (EDTA) with chelated ferrous ion (Fe II). Under appropriate redox conditions, Fe^{2+} generates highly reactive hydroxyl radicals from oxygen, and the hydroxyl radical (HO·), being short-lived, oxidizes only those deoxyribose groups that are close to where it is bound in the DNA. The consequence is cleavage on both strands of DNA.

The objective of this approach is a rational chemotherapeutic approach for inactivation of specific genes. Can this approach be used to attack the AIDS virus when it is integrated into the cellular DNA? Of perhaps equal importance is whether this technique of DNA cleavage is applicable to the destruction of the latent herpesvirus, thereby preventing recurrences of genital herpes, or destruction of the latent varicella virus, thereby preventing the recurrence or even occurrence of shingles, or destruction of a specific oncogene were it to be involved in the progression of cancer. Can other cleaving moieties be identified which are not dependent on redox potential?

A very important pharmacologic problem is delivery of the oligomer in vivo. Conjugation of oligonucleotides to poly(LLys) appears to increase cellular transport (Lemaitre et al., 1987), but can such a conjugate be administered orally? An interesting study which may be applicable to the delivery of oligonucleotides concerns the *oral* delivery of drugs which are normally degraded before they can be absorbed from the gastrointestinal tract. Peptide agents are potentially very important antiviral drugs, not only for interaction with receptors to prevent attachment of the virus to the cell, or viral transport into the cell, or viral uncoating, but also to inhibit viral proteases as well as to prevent the function of viral regulatory proteins. When given orally, peptide drugs are inactivated by enzymes in the stomach and small intestine, but recent studies have involved giving the peptide coated with a polymer cross-linked with azoaromatic groups. Although protected from digestion in the stomach and small intestine, the diazo bonds are reduced by the microflora normally present in the large intestine. The cross-links are broken and the

drug released and absorbed: ($R-C_6H_4-N=N-C_6H_4-R \rightarrow 2R-C_6H_4-NH_2$). It was reported that when vasopressin or insulin was so administered to rats orally, the expected biological responses of antidiuresis and hypoglycemia, respectively, were indeed produced (Saffron et al., 1986).

4. DISCUSSION

In summary, several strategies for antiviral drug design and delivery have been discussed. No doubt there are other modifications of the empirical and rational approaches to drug design. The antiviral compounds under development appear at best to interfere with the progression of AIDS as well as its transmission. Since such a drug would not produce a cure and would require continuous intake, it would of necessity have to be nontoxic. However, the latency problem is still a concern. DNA cleaving as a solution is attractive, but as of today it is highly theoretical and problematic. However, it does offer hope of a cure.

Acknowledgments—Supported by USPHS grants CA-05260 and CA-44094 from the National Cancer Institute and a grant from Bristol-Myers Company.

REFERENCES

ASSELINE, U., DELARUE, M., LANCELOT, G., TOULME, F., THUONG, N. T., MONTENAY-GARESTIER, T., and HELENE, C. (1984*a*) Nucleic acid-binding molecules with high affinity and base sequence specificity: Intercalating agents covalently linked to oligodeoxynucleotides, *Proc. Natl. Acad. Sci. USA*, **81**: 3297–3301.

ASSELINE, U., TOULME, F., THUONG, M. J., DELARUE, M., MONTENAY-GARESTIER, J., and HELENE, C. (1984b) Oligodeoxynucleotides covalently linked to intercalating dyes as base sequence–specific ligands. Influence of dye attachment site. *Embo. J.* **3**: 795–800.

AUGUST, E. M., MARONGIU, M. E., LIN, T.-S., and PRUSOFF, W. H. (1988) Initial studies on the cellular pharmacology of 2′,3′-dideoxy-2′,3′-didehydrothymidine: A potent and selective inhibitor of human immunodeficiency virus. *Biochem. Pharmacol.* **23**: 4419–4422.

BABA, M., PAUWELS, R., HERDEWIJN, P., DE CLERCQ, E., DESMYTER, J., and VANDEPUTTE, M. (1987) Both 2′,3′-dideoxythymidine and its 2′,3′-unsaturated derivative (2′,3′-dideoxythymidinene) are potent and selective inhibitors of human immunodeficiency virus replication *in vitro*. *Biochem. Biophys. Res. Commun.*, **142**: 128–134.

BADGER, J., MINOR, I., KREMER, M. J., OLIVEIRA, M. A., SMITH, T. J., GRIFFITH, M. P., GUEEIN, D. M. A., KRISHNASWAMY, S., LUO, M., ROSSMANN, M. G., MCKINLAY, M. A., DIANE, G. D., KUTKO, F. J., FANCHER, M., RUECKERTT, R. R., and HEINZ, B. A. (1988) Structural analysis of a series of antiviral agents complexed with human rhinovirus 14. *Proc. Natl. Acad. Sci. USA*, **85**: 3304–3308.

BALDWIN, J. J. (1987) Drug design. In: *Drug Discovery and Development*, pp. 33–71, Williams, M. and Malick, J. B. (eds), Humana, Clifton, NJ.

BALZARINI, J., PAUWELS, R., HERDEWIJN, P., DE CLERCQ, E., COONEY, D. A., KANG, G.-J., DALAL, M., JOHNS, D. G., and BRODER, S. (1986) Potent and selective anti-HTLV-III/LAV activity of the 2′,3′-unsaturated derivative of 2′,3′-dedeoxycytidine. *Biochem. Biophys. Res. Commun.*, **140**: 735–742.

BALZARINI, J., KANG, G. J., DALAL, M., HERDEWIJN, P., DE CLERCQ, E., BRODER, S., and JOHNS, D. G. (1987) The anti-HTLV-III (anti-HIV) and cytotoxic activity of 2′,3′-dideoxyribonucleosides. *Mol. Pharmacol.*, **32**: 162–167.

BELIKOVA, A. M., ZARYTOVA, V. F., and GRINEVA, N. I. (1967) Synthesis of ribonucleosides and diribonucleoside phosphates containing 2-chloroethylamine and nitrogen mustard residues. *Tetrahedron Lett.*, **37**: 3557–3562.

COLONNO, R. J., CONDRA, J. H., MIZUTANI, S., CALLAHAN, P. L., DAVIES, M., and MURCKO, M. A. (1988) Evidence for the direct involvement of the rhinovirus canyon in receptor binding. *Proc. Natl. Acad. Sci. USA*, **85**: 5449–5453.

CORMIER, J. F., and OGILVIE, K. K. (1988) Synthesis of hexanucleotide analogues containing diisopropylsilyl internucleotide linkages. *Nucleic Acid Res.*, **16**: 4583–4594.

GREEN, P. J., PINES, O., and INOUYE, M. (1986) The role of antisense RNA in gene regulation. *Annu. Rev. Biochem.*, **55**: 569–597.

GRINEVA, N. I., and KARPOVA, G. G. (1973) Complementarily addressed modification of mRNA with *p*-(chloroethylmethylamino) benzylidene hexanucleotides. *FEBS Lett.*, **32**: 351–355.

HAMAMOTO, T., NAKASHIMA, H., MATSUI, T., MATSUDA, A., UEDA, T., and TAMAMOTO, N. (1987) Inhibitory effect of 2′,3′-didehydro-2′,3′-dideoxynucleosides on infectivity, cytopathic effects, and replication of human immunodeficiency virus. *Antimicrob. Agents Chemother.*, **31**: 907–910.

HELENE, C., MONTENAY-GARESTIER, T., SAISON, T., TAKASUGI, M., TOULME, J. J., ASSELINE, U., LANCELOT, G., MAURIZOT, J. C., TOULME, F., and THUONG, N. T. (1985) Oligodeoxynucleotides covalently linked to intercalating agents: A new class of gene regulatory substances. *Biochimie*, **67**: 777–783.

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