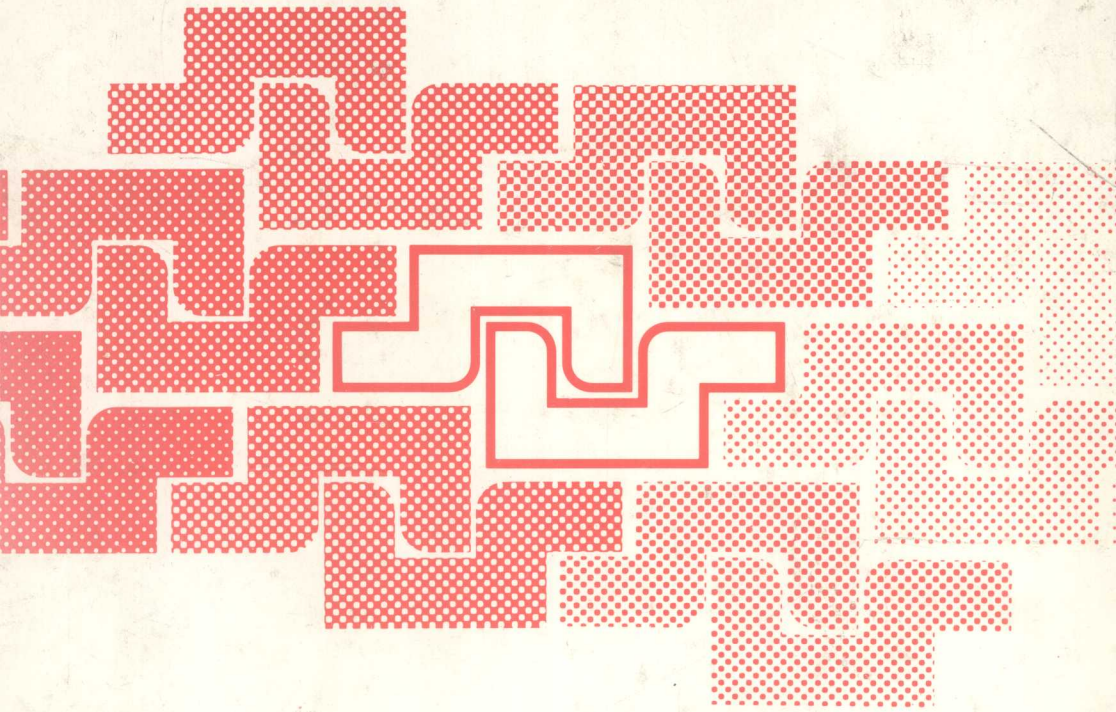

Targets for the Design of Antiviral Agents

Edited by

E. De Clercq and

R. T. Walker

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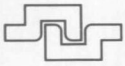

Series A: Life Sciences

PREFACE

This publication contains the Review Lectures presented at a joint NATO Advanced Study Institute and FEBS Advanced Study Course held at Les Arcs, Bourg-Saint-Maurice, France, from the 19th June - 2nd July 1983. The Course, entitled "Targets for the Design of Antiviral Agents" was in some ways a sequel to the NATO-FEBS Course held at SOGESTA (near Urbino), Italy from the 7th - 18th May 1979 and published as volume A26 in this series. During the subsequent four years, we have witnessed the first of the "new generation" of antiviral compounds, which are more efficacious and less toxic than the "classical" antiviral drugs, reach the clinic and we felt that it was the right time to assess the future prospects of this very important and exciting field.

The vast majority of the drugs developed recently have proved active against various members of the herpesvirus family and elsewhere in this publication we learn that the cure for only rather few viral diseases, such as the common cold, influenza and herpes, promises the return on investment required by the pharmaceutical industry. However, the aim of this Course was for eminent virologists to identify possible targets among the various virus classes against which the chemists could then design suitable therapeutic agents.

Recent advances with antiherpesvirus drugs have shown that a far greater selectivity and therapeutic index can be obtained than was previously thought to be possible. Now that the mode of action of many of these antiherpes drugs is being resolved, it is hoped that the information gained from these serendipitously discovered drugs could be applied to other virus classes so that a more rational approach might lead to the development of the next generation of antiviral drugs. Many chemists have only a very hazy idea of the mode of virus replication and thus one of the intentions of the meeting was to acquaint the chemist engaged in drug synthesis, with the potential targets in the different virus groups upon which antiviral drugs could act. Such an approach should hasten a more rationalized drug design.

As before, the aim of the meeting was to gather together many of the experts in different scientific disciplines, to enable senior scientists in one discipline to learn from their colleagues working in other related and relevant disciplines, and to present the opportunity to younger scientists who have just entered the field to assimilate current knowledge and make personal contacts with the more senior people. In the design of new antiviral agents, a coordinated effort of virologists, chemists, pharmacologists, toxicologists and clinicians is required and such an effort can be afforded only by a strong cooperation between academic institutes, pharmaceutical industry and governmental organizations. This is precisely the kind of scientists which assembled for the meeting. An important feature was the interest shown by many pharmaceutical companies, as can be seen from the Acknowledgments.

This publication only contains the review lectures which cover in depth the possible potential targets for attack within the various virus classes. Other review articles are focussed on the antiviral compounds which are presently in use or being assessed. As the molecular biologists and virologists continue to unravel the mechanism of viral replication, the structure of the viral genomes and the enzymes for which the viral genes code, this will increase considerably our insight into the targets available; and for some viruses we already have a reasonable basis on which to plan an attack. One exception is the class of slow viruses, where it is still not known whether the infective agent contains nucleic acid, and although this class of viruses hardly constitutes a commercially viable target, the mechanism by which these infections are transmitted may be of vital concern to us all.

The initial euphoria which was apparent four years ago has already started to evaporate. Serendipity still rules, nothing of fundamental significance has yet been designed and our knowledge of the detailed mechanism of viral replication is woefully inadequate. On a molecular level, our understanding of the mode of action of the virus-specified enzymes is even less well known but at least a start has been made. Several relatively non-toxic and specific antiviral agents have been developed and some of these agents have reached the market and what holds for one class of viruses (particularly herpes) may well hold for others. It is unlikely that the antiviral "penicillin" will ever exist but the coming years will undoubtedly reveal many new antiviral agents with a useful spectrum of activity combined with low toxicity.

In conclusion, the Directors of the Course would like to acknowledge the help given by Bernard Croise and his staff of Seminarc and by Charles Arnould and his staff of the Hotel du Golf.

We were able to enjoy the superb scenery and remove the effect of the excess of calories by trying to follow the national ski speed champion Pascal Budin up and (very occasionally) down dale or by using some of the many other sports facilities available without being worried by organizational difficulties. More importantly, we were able to concentrate on the science, renew contacts with old friends and set up new collaborative projects, the results of which should become apparent in the years to come.

We finish with a quotation used by one of our review lecturers (J.S. Oxford) and taken out of the context in which it was originally used 40 years ago but which perhaps encapsulates the current position in the field of the rational design of antiviral drugs:

"This is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning"

W.S. Churchill, 1943

July 1983

E. De Clercq

R.T. Walker

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CONTENTS

OVERVIEW OF THE POSSIBLE TARGETS FOR VIRAL CHEMOTHERAPY

William H. Prusoff, Tai-Shun Lin, William R. Mancini, Michael J. Otto, Scott A. Siegel and J.J. Lee

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The ideal target for prevention of viral infections is eradication of either the virus or the host, since both are required to produce an infection. For a variety of reasons, mostly personal, we would all agree to preferably eliminate the virus. Such an approach appears to have been accomplished with the world-wide elimination of the virus responsible for smallpox. This was achieved by a highly successful campaign of surveillance and containment by vaccination in order to prevent its spread, rather than by universal routine immunization.

A number of live attenuated vaccines are available not only for prevention of small pox, which has been eradicated, but more importantly for rubeola (measles), rubella (German measles), mumps, and poliomyelitis. Inactivated vaccines are also available for potential prevention of influenza, poliomyelitis, and rabies (1). In 1981 the U.S. Food and Drug Administration approved a new vaccine for hepatitis B which is produced from hepatitis B surface antigens (HB_5Ag) found in human blood (2).

Of interest is the development of a vaccine against foot and mouth disease in cattle, swine, sheep and goats caused by a picornavirus. By use of recombinant DNA techniques, the viral RNA coding for VP_3, a coat protein, was inserted into an *E. coli* plasmid, and the desired antigenic protein was produced and purified (3). Two injections of 0.25 mg of this protein produced protection in animals from a challenge dose of virus. However since there are many strains of this virus, about 15 different proteins are expected to be eventually included in order to prepare a comprehensive foot and mouth disease vaccine (3).

An exciting development is the recent chemical synthesis of the first synthetic vaccine to be produced. Peptides were synthesized which corresponded to several regions of the foot-and-mouth disease virus, and one of the peptides (20 amino acids) produced neutralizing antibodies which protected guinea pigs against a challenge with this virus (4). The potential to develop synthetic vaccines for other virus infections is obvious and studies are in progress for development of synthetic vaccines (5-7).

Unfortunately vaccines are not available for all viruses that infect humans and there are a variety of reasons for this. The rhinoviruses consist of over 100 different serotypes and therefore it is most unlikely that vaccination would be a successful procedure for prevention because of the specificity of the immune reaction. A number of compounds however are under consideration for potential therapy of the rhinoviruses and these include arildone, enviroxime, dichloroflavin and interferon.

The influenza A virus presents another problem, in that it continuously changes its antigenic composition, so that this year's vaccine may be less effective against next year's emerging virus. Although amantadine is available for prophylactic or early use against respiratory infections caused by the influenza A viruses, the primary approach is use of the vaccine.

The family of herpesvirus are responsible for a number of clinically important infections:

HSV-1: herpes keratitis, herpes encephalitis, mucocutaneous herpes and more recently genital herpes.

HSV-2: genital herpes, and possibly cervical cancer.

Varicella-Zoster: Varicella (chicken pox) in children, herpes zoster (shingles) in adults and in immunocompromised cancer and organ transplant patients.

Cytomegalovirus: Congenital CNS infections. In adolescents and adults an infectious-mononucleosis-like syndrome. In immunocompromised cancer and organ transplant patients it is a major cause of death.

Epstein Barr Virus: Infectious mononucleosis, nasopharyngeal cancer, Burkitt's Lymphoma.

The development of a herpes simplex virus vaccine is under investigation. Inactivated virus cannot be used because of potential oncogenicity. The use of protein-subunits, free of DNA

has been evaluated, but they lack potency. An interesting approach, discussed in a workshop at the NIH concerned with genital herpes vaccines (8), was the use of recombinant herpesvirus, free of those genes believed to be responsible for oncogenicity or the induction and maintenance of latency.

In the absence of an effective vaccine for interaction with the target virus, we are dependent upon the development of antiviral drugs. Ideally the drug should exert its antiviral activity as a consequence of interaction with a target that is unique to the virus, and hence of little or no concern to the uninfected host tissues. The interaction could consist of an inhibitory event unique for the virus, or of a preferential activation of the drug in the virus infected cell.

What are some of the targets that one can consider as potential sites for antiviral drug development? What are some of the strategies that one might employ to take advantage of the availability of these targets? What are some of the approaches that one might take to develop antiviral agents for specific interaction with known target sites, as well as those target sites yet to be uncovered as a consequence of future acquired knowledge of the biochemistry of viral replication, the physiology of host cell-virus interrelationships, and the physical chemistry of drug-target interactions.

At this time we know very few targets that we have been able to take advantage of, and so it is not surprising that we have as of today very few clinically useful drugs. Only 5 drugs have been approved for use in humans by the Food and Drug Administration in the United States, and these include: (1) amantadine (Adamantanamine) for prevention or very early treatment of infections caused by the influenza A viruses, (2) 1-(2-deoxy-β-D-ribofuranosyl)-5-iodouracil (5-iodo-2'-deoxyuridine, Idoxuridine, IdUrd), (3) 1-(2-deoxy-β-D-ribofuranosyl)-5-trifluoromethyluracil (5-trifluoromethyl-2'-deoxyuridine, Trifluridine, F_3dThd), (4) 9-β-D-arabinofuranosyl-9H-purine-6-amine (Vidarabine, adenine arabinoside, ara-A) for topical therapy of herpetic keratitis, for systemic therapy of herpes encephalitis and pending is FDA approval for neonatal herpes, and (5) 9-(2-hydroxyethoxymethyl)guanine (Acyclovir, ACV) for topical therapy of primary genital herpes and non-life threatening mucocutaneous herpes, as well as for intravenous administration for therapy of severe cases of initial genital herpes and cutaneous and mucosal herpes-simplex (HSV-1 and HSV-2) infections in immunocompromised patients.

In Europe 5-ethyl-2'-deoxyuridine (Aedurid) and 5-iodo-2'-deoxycytidine (Cebe-Viran) are available for therapy of herpes keratitis.

A number of compounds which are in clinical trial, or look promising, or are of interest include E-5-(2-bromovinyl)-2'-deoxyuridine (BVdU), 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC), phosphonoformate (foscarnet, PFA), 5-methoxymethyl-2'-deoxyuridine, 5-n-propyl-2'-deoxyuridine, (S)-9-(2,3-dihydroxypropyl)adenine ((S)-DHPA), 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG, BIOLF-62, 2'-NDG), 2-deoxyglucose, L-lysine, arildone, ultrasound + "Hepergon", enviroxime, zinviroxime, dichloroflavin, isoprinosine, interferon, etc.

Table 1. Viral Targets

1.	Extracellular
	A. Antibody
	B. Enzymes
	C. Chemicals
	1. Organic Solvents
	2. Detergents
	3. Formaldehyde
2.	Adsorbtion of virus to cell surface
3.	Transport across cell wall
4.	Uncoating of virus
5.	Transport of genome into cytoplasm or nucleus
6.	Transcription (RNA formation)
7.	Methylation of RNA
8.	Protein synthesis or processing
9.	Enzymes
10.	Maturation (assembly of macromolecules into a virion)
11.	Release of virion from cell

Table 1 indicates various targets for which antiviral drugs have been demonstrated to exert an effect, or are potential targets for future drug development. We have already discussed the use of vaccines which interact with the target virus, when it is present in the blood stream, or during the phase of reversible adsorption to the cell prior to penetration.

Extracellular Target

The virus prior to penetration into the host animal is a target for inactivation by a variety of biological, chemical and physical agents and some of these have been of value in preparation of inactivated vaccines (9).

Proteolytic enzymes can decrease the infectivity of enveloped viruses by removal of their glycoprotein spikes, and phospholipases can inactivate by hydrolysis of phospholipids present in such viruses. Steele and Black (10) found 2-thiouracil decreased the infectivity of polio virus by a direct reaction of the oxidized form of this compound with the capsid sulfhydryl groups.

Chemical agents which inactivate the virus directly include kethoxal (3-ethoxy-2-oxobutyraldehyde), calcium elenolate, and certain dihydroisoquinolines references are cited by Smith et al. (11). The first two exert an antiviral effect on both RNA and DNA viruses and the latter against the influenza and parainfluenza viruses, but none have proven to be clinically useful. Retinoids (12-14), long-chain unsaturated monogylcerides and alcohols (15) also inhibit viral replication by direct interaction.

Anionic detergents (sodium dodecyl sulfate) and nonionic detergents (Triton X-100, etc.) inactivate viruses by solubilizing the viral envelope. Compounds such as guanidine, urea or phenol dissociate the polypeptides of the viral capsids into their individual components. Formaldehyde interacts with amino groups of nucleic acid bases as well as free amino moieties of proteins.

Some viruses such as myxovirus and oncogenic RNA virus, are sensitive to heat inactivation, whereas many others are not. X-ray, gamma-ray and ultraviolet radiations also may produce a loss of infectivity. Whereas X-ray and gamma-ray radiations produce single stranded breaks in the DNA, ultraviolet radiations produce butane dimers between DNA pyrimidines.

Viral Attachment and/or Penetration of Virus as Targets

Reproduction of a virus requires that the virus adsorb to the cell membrane and penetrate into the cell. This involves an initial reversible attachment of the virus to a receptor on the host cell membrane, which quickly undergoes a change such that the virus can no longer be dissociated from the cell by mild procedures. The nature of the conversion from reversible to irreversible binding is not clear, but it may involve an increase