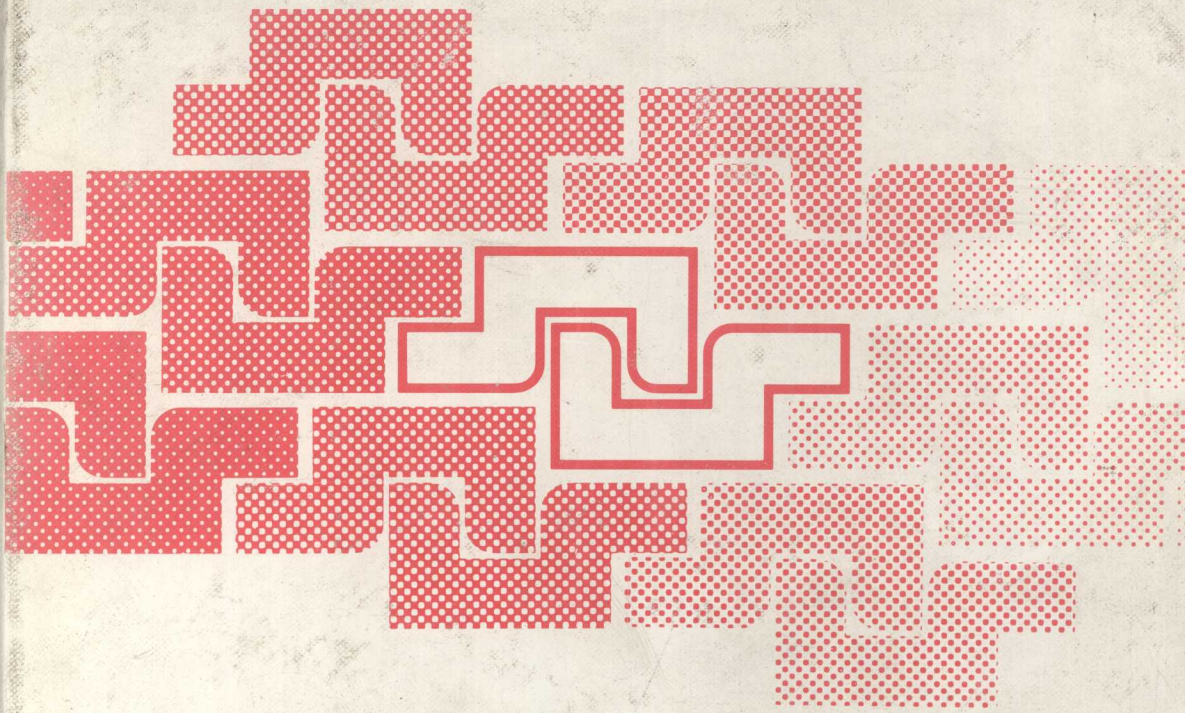

Antiviral Drug Development

A Multidisciplinary Approach

Edited by

Erik De Clercq and

Richard T. Walker

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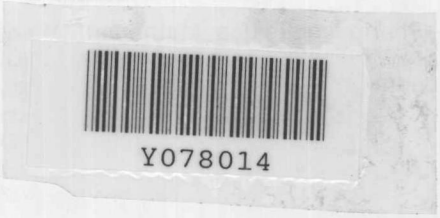

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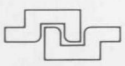

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CONTENTS

ANTIVIRAL CHEMOTHERAPY: AN INTRODUCTION AND REASONS FOR THE SLOW PROGRESS, PARTICULARLY TOWARDS RATIONAL DESIGN

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INTRODUCTION

Six years ago, Sir Charles Stuart-Harris opened a Beecham Colloquium on Problems of Antiviral Therapy[1] with a lecture entitled "Antiviral Chemotherapy: an Introduction and Apology for the Slow Progress". Today, I think we no longer have to apologise; progress is still very slow but as I hope to show, there are many reasons for this, some of which are outside the control of scientists and given the constraints, no one can reasonably expect progress to be any faster.

In this introduction, I wish to examine the current status of antiviral chemotherapy and set the scene for the review lectures which are to follow and in which specific areas will be discussed in more detail.

This is the third Course in this series, the first being held eight years ago.[2] Already at that time, even in this pre-AIDS period, it was becoming clear that the old-fashioned view concerning antiviral chemotherapy, which was a safe and specific antiviral agent would be unlikely to exist, was not correct. Acyclovir[3] had been synthesised and shown to be effective and there were rumours of other compounds being discovered in many laboratories.

Four years later,[4] the initial enthusiasm had waned somewhat. The progress of acyclovir continued unabated but most of the other compounds were running into difficulties and few if any new leads had been discovered. However, we were learning more about how the so-called second generation of antivirals might work and in an elegant introduction to our meeting four years ago, Bill Prusoff[5] showed us some of the specific viral targets which could be attacked so that efficacious and safe antiviral drugs could be de-

veloped. Now, four years later, acyclovir is on the market but apart from the area of anti-AIDS compounds, progress has been extremely disappointing. Why is this ?

WHY HAS PROGRESS BEEN SLOW ?

In order to generate discussion, I will be deliberately provocative and I would suggest that one of the main reasons for lack of progress is that in general, industry and government are not interested in preventing or alleviating the effects of viral infection by means of chemotherapy. The lawyers have got such a domination of this area, that what was already a high-risk area in terms of money invested when balanced against the possible return, is now so unattractive that it is unlikely that there are many areas where the risk is worth taking.

That governments and industry are not interested has been highlighted in the last few years by their attitude to the spread of HIV. Suddenly they have realised that viral diseases could not be cured with penicillin and now that a lethal viral disease is at large among the populations of the industrialised nations, it has had the effect of focussing the minds of politicians on the necessity to find a cure. Thus, in a remarkably short space of time,[6] a drug (AZT) which has been around for years, has been tested, synthesised in very large quantities and licensed for use. This alternative of no treatment is not acceptable. However, outside the industrialised countries there are many other viral infections which cause death and misery and in which little interest has been shown.

Until recently, it was not possible to develop a vaccine against hepatitis B virus. This problem has now been solved[7] but no successful antiviral compound had been found to prevent the disease, despite the fact that currently there are 200 million carriers of hepatitis B virus in the world of whom 40 million will die of cirrhosis and 10 million of hepatocarcinoma. It will be several years, if ever, that deaths from AIDS will reach this level.

Thus I have to conclude that with such a high-risk future, it is unlikely that many antiviral compounds will ever reach the market. It has been shown that when public demand insists, compounds can be found very quickly, most of the safety and testing standards can be bent and a compound with a very low therapeutic index can be licensed, but most viral diseases can be allowed to run their natural course.

With that as introduction, I would now like to detail some of the ma-

jor reasons why advance in antiviral chemotherapy has been so slow. These reasons follow in no particular order, but taken together they all contribute to a general atmosphere which is not encouraging.

Firstly, there is a general impression that the most reasonable way to cure viral diseases is by their prevention by using vaccines. Vaccines in many areas have had outstanding success and apart from total victory over smallpox, many other of the common childhood viral diseases are almost unknown in countries like the USA where vaccination is taken seriously by the health authorities. Until recently there have been some problem areas but we have already referred to one, hepatitis B virus, which has recently succumbed to the molecular virologist. However, there are still some outstanding examples of economically important viral diseases where no ideal vaccine yet exists such as influenza virus, rhinovirus, rotavirus, herpesvirus and HIV. Herpesvirus may have its own problems because of latency, the others cause problems because of the diverse spectrum of epitopes present and because mutants continuously evolve. Thus, there are few virus diseases which present a commercially viable target once vaccines have been discussed.

However, consideration of vaccines raises two other problems which are equally applicable to antiviral chemotherapy and these are costs of development and use, and safety. Although presumably someone undergoing chemotherapy already has the disease, whereas by definition someone being vaccinated does not have it, it is rare in a developed country (with the exception of HIV) for people to die or suffer permanent damage from viral infections (at least on a scale which governments notice). This means that the safety regulations for licensing are correctly very stringent, but then the product is going to be very expensive, can only therefore be used in developed countries where it has to be supremely safe because, left to itself, the virus will probably not do too much damage and the lawyers are ever-ready to take advantage of any possible side-effects which might be the result of treatment.

Another problem which retarded research in the early days into antiviral chemotherapy was the competing research on interferon. Many large pharmaceutical companies invested large amounts of money into research in this area in the hope of finding an "antiviral penicillin". This resulted in lack of funds for antiviral chemotherapy and yet despite all this investment in interferon, very little in terms of clinically useful products has resulted, certainly as far as the antiviral field is concerned; and the future does not look bright.

As I have previously mentioned, the existence of specific viral tar-

gets for attack has been recognised for some time and yet with amantadine as an exception, all the licensed antivirals are nucleosides and are targeted against virally coded enzymes. None of the other potential targets seem to have been tackled and no non-nucleosides have been found to be specific enzyme inhibitors. I suggest that the main reason for this is that because of the limited resources that are available, we do not have sufficient information for any designed attack to be made on targets other than the virally coded enzymes. Even in the HIV field, all the initial success has been with nucleoside analogues with attempts to produce reverse transcriptase inhibitors or chain terminators. Yet, almost inevitably, nucleosides are likely to have some side effects as the compounds or their metabolites may be recognised by other enzymes in the cell. Even when the HIV target is not the reverse transcriptase,[8] compounds designed to prevent translation of the tat-III gene are still nucleoside derivatives in the form of antisense DNA. However, with our current lack of knowledge, when it is still difficult, if not impossible, to design rationally even a nucleoside analogue, it is clearly too much to expect a rational design of non-nucleoside molecules.

Before I discuss further the prospects for the design of antivirals, there are more problems which contribute to the lack of success in antiviral chemotherapy, which will no doubt be covered in some detail during this Course. Among these are the relevance of test systems and toxicity assays and the problems of early viral disease diagnosis. The latter, with the help of molecular biology is reasonably easily overcome nowadays if the money is available. However, it requires a change in attitude of many clinicians, which is often that "if it is a viral disease, it really doesn't matter too much which one it is because little treatment is available for any of them".

The relevance and use of testing and screening assays is altogether another problem. Antiviral chemotherapy is (usually) aimed at preventing or curing a viral disease in a human patient; yet, with the exception of anti-HIV compounds, that is the last thing which one is allowed to do. The close association of a virus with its host, not only brings problems in the design of safe antivirals but it also makes the design of relevant test systems very difficult. Because of cost and the quantity of material available, one is usually initially restricted to in vitro assays in cell culture. Some viruses cannot so be cultured, other systems are so artificial

that their relevance to the clinical disease is tenuous at best and yet this is the sort of system we often have to use to find leads. Sometimes it is relatively easy to stop viral growth in infected cells but it proves to be much more difficult when the compound is used in vivo. This can be because either transport or cell targeting or uptake is very different or that the drug is metabolised before it reaches the target site. In vitro cell lines usually do not contain a full complement of functional enzymes and thus a compound like E-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) works even better in vitro than it does in vivo where it is susceptible to nucleoside phosphorylase which is absent or present at only low levels in many cell lines. However, this doesn't have to be an all or none effect. Should a compound require activation by one or more enzymes before the active metabolite is produced, then small changes in concentrations of intermediates could mean that the K_m for subsequent enzyme-mediated transformations may not be achieved and so the active metabolite is not produced or some intermediate is side-tracked down another pathway.

Animal models often have precisely the same problems. Usually one is trying to cure or prevent a human disease and the animal model, apart from being very costly, may or may not be relevant. Uptake, distribution and metabolism of the drug may be very different, as is the nature of the viral infection.

Toxicity assays also may not be relevant. Recently it was reported that 1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC)[9] is poorly deaminated by dogs used for toxicity studies, whereas in humans it is rapidly deaminated to the corresponding uracil derivative, FIAU. What then is the purpose of doing toxicity studies of this compound in dogs when one is concerned with a different compound in the human situation ? It is a very naive assumption to make, that all metabolism of compounds in test animals and humans is likely to be the same or even to be similar, particularly when huge doses of compounds are used.

Thus all these factors add up to give the present situation where antiviral chemotherapy is still regarded as a high risk business. Although targets are clearly presented, we do not have sufficient information for rational design. The chance of getting anything clinically-useful from an in vitro screen which will then show promise in animal models, pass the toxicity tests and yet still work against the clinical disease is very small and the number of viral diseases worth commercial consideration is also small.

RATIONAL DESIGN

Let me finally turn to the problem of design and I will restrict myself to an area where I have had some experience and which is probably one of the areas in which the most information is available; the design of an antiherpesvirus nucleoside.

We have several lead compounds: 5-iodo-2'-deoxyuridine (IDU), acyclovir, FIAC and BVDU. How do they work, do they have anything in common and ought we to be able to design something better ? All these compounds require phosphorylation by a kinase.[10] The latter three compounds are only phosphorylated by the virally coded thymidine kinase, whereas IDU shows little specificity, is also phosphorylated by the normal cellular kinase and is therefore much more toxic.

It is known that acyclovir monophosphate is further taken to the diphosphate by a cellular guanylate kinase and presumably the triphosphate is a substrate for the viral polymerase and acts as a chain terminator.

This at least is a plausible mechanism because it is not clear to me how the other nucleosides exert their antiviral effect. They too apparently require further phosphorylation (BVDU is relatively ineffective against HSV-2 and here the second phosphorylation step is less efficient although this may not be cause and effect).

For many years it was a tenet of faith that incorporation of these analogues into viral DNA was responsible for the cessation of viral replication. For instance, DNA containing BVDU has been claimed to be more susceptible to single-stranded breaks than normal DNA[11] although *in vitro* it is known that DNA containing quite large quantities of 5-substituted pyrimidine nucleosides can be replicated and transcribed. Such incorporation may be mutagenic (although BVDU is not mutagenic in the Ames test and other test systems[12]) but should not necessarily be responsible for the very efficient way in which viral DNA synthesis is stopped. It is also possible that the nucleoside analogue triphosphates are viral DNA polymerase inhibitors rather than alternative substrates but again one has to question whether the effects seen *in vivo* are likely to be caused by such inhibition at the concentrations of analogue likely to be achieved.

Recently[13,14] it has become possible to study the effect of the activation of BVDU to the monophosphate in a mammalian carcinoma cell line. Murine mammary carcinoma cells have been transformed with the thymidine kinase gene from HSV-1[13] or HSV-2[14] and the effect of BVDU investigated. If one assumes that what one now sees is due exclusively to the production of BVDUMP in the cells, the result is indeed dramatic as BVDU is now toxic

with an ID_{50} of 500 pg/ml for the HSV-1 transformed line and 50 pg/ml for the HSV-2 transformed line. Thus, BVDU is 5 orders of magnitude more toxic than it is for the wild-type cell line and yet acyclovir is only 100-fold more toxic for these transformed cells. Why is BVDU now so toxic and does this have any relevance to its antiviral activity ? At the moment, we do not have the answers to these questions but at least it should begin to raise doubts in our mind as to whether a compound which is toxic at 50 pg/ml can conceivably exert this effect, whether against a virus or a transformed cell line, by either being incorporated into DNA or by inhibiting a viral polymerase.

This discussion is relevant to the problem of design because we now have to bear in mind that we do not definitely know how some of the antiviral agents available now, actually work. However, for the purpose of further discussion let us make the most simple and naive assumption that we require a pyrimidine analogue 5'-triphosphate which will inhibit a viral polymerase and let us examine the metabolic pathway open to the nucleosides as it enters a cell.

Here, even in our present ignorant state, we can see that at least eight enzymes are involved; four of which have to be avoided and for four of which the analogue or its metabolites need to be substrates. The enzymes in the first category are : 1) nucleoside phosphorylase; 2) cellular thymidine kinase; 3) thymidylate synthetase (for the analogue 5'-monophosphate); and 4) cellular DNA polymerase. The first enzyme inactivates the analogue; reaction with the others would almost certainly cause toxicity. The enzymes in the second category are : 1) viral thymidine kinase; 2) viral (or cellular ?) thymidylate kinase; 3) nucleoside diphosphate kinase; 4) viral DNA polymerase. We know almost nothing about the substrate specificity of any of these enzymes and some of the information we do have seems to lack any chemical logic (why does the viral thymidine kinase phosphorylate specifically both acyclovir and BVDU ?). Thus I would submit that, at present, it is hopelessly optimistic to expect to achieve a rational pathway as those compounds which already show activity. This, however, would be a minor task when considering what needs to be done if the design of a non-nucleoside is required or that of a product which is to be aimed at one of the other possible targets.

To highlight how little interest has been taken in the design of antiviral drugs, let me suggest an example where design could have been started years ago but presumably it has been thought to be not worthwhile, even though the product would be aimed at herpesvirus infections which are one of the few commercially viable targets for antiviral chemotherapy.

It is known (and there are many examples available), that the specificity of the thymidine kinases of normal human cells, HSV-1 and HSV-2 are different. It is likely that a thymidine kinase inhibitor might show antiviral properties and it should be possible to use the difference in specificity to design HSV-1 thymidine kinase - or HSV-2 thymidine kinase - inhibitors (not nucleosides) which are not inhibitors of the normal cellular enzymes. As a start to this project, if any rationality is to be brought into the design, we need to know how these three key enzymes work. The genes for these enzymes can all be cloned, expressed, probably crystallised and their 3-dimensional structures determined and active site located. Then perhaps an inhibitor could be designed but as far as I am aware, little has been done in this direction even though the chance of success would seem to be reasonably high. So far, with very few exceptions, most nucleosides investigated are either not recognised by the kinase or are substrates for it, but it should surely be possible to design a non-nucleoside inhibitor if the information discussed above were available ?

However, I suspect that no one takes the problem seriously enough to take up the challenge. Hopefully during this meeting it will be shown that this pessimistic view I have taken of the current status of antiviral chemotherapy is wrong and that in the post-AIDS era there will be a resurgent interest in the rational design of antiviral compounds.

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