EDITORIAL

Quo vadis antiviral agents for herpes, influenza and HIV?

The discovery of the nucleoside analogue acyclovir and the establishment of its biochemical mode of action as an anti-herpes agent were the scientific foundation stones for medical investigations which have proved its wide clinical usefulness over the last two decades. I remember clearly the initial launch of the drug in London and it was with considerable interest that I attended recent meetings to discuss the antiviral activity of “sisters” of acyclovir, namely, famciclovir, penciclovir and, later vancyclovir. It did not appear likely in the 1970s that a nucleoside analogue, or at least the triphosphate derivative, could have such a narrow specificity against a viral enzyme—namely herpes DNA polymerase—without inhibiting cellular DNA polymerases. Countless scientific investigations have since demonstrated the relative safety of the compound, its antiviral spectrum, its ability to prevent viral spread and recurrent infection, and to alleviate the symptoms following tissue destruction by the virus. Drug-resistant herpes viruses have been slow to appear.

Given the success of acyclovir do we need alternative anti-herpes compounds or could the efficacy or even the antiviral range of acyclovir be improved? The answer to the first question must be “yes”. New antiviral agents are always required, if for no other reason that to treat persons infected with drug-resistant viruses, who include immunosuppressed patients such as those receiving transplants or those infected with HIV. The antiviral agent foscarinet has a mode of action completely different from that of acyclovir and, as expected, cross-resistance is not a major problem. The drug is used to treat immunosuppressed patients suffering from serious infections caused by acyclovir-resistant herpes viruses and CMV.

A narrow antiviral spectrum has been a problem with every antiviral agent. Amantadine inhibits only influenza A virus replication and not influenza B virus; zidovudine inhibits only HIV-1; acyclovir may be very potent against HSV-1 but is much less so against VZV. Could this problem be circumvented by increased dosages of acyclovir or, alternatively, by discovering closely related analogues which may produce high plasma levels in the body because of increased half-life or different dosage scheduling? The task is to discover compounds which are similar enough to acyclovir in molecular structure to give confidence that they will have the same safety profile, but different enough to have new antiviral properties and to be patentable.

Ganciclovir has a different molecular structure, a different antiviral spectrum to include CMV but also, unfortunately, a much less favourable toxicological profile. SmithKline Beecham chemists synthesised famciclovir and penciclovir whilst Burroughs Wellcome have produced valaciclovir. Famciclovir is a pro-drug which is de-acetylated and oxidised to penciclovir giving high bioavailability of the latter molecule. The half-life is somewhat prolonged and so dosages may be wider apart. Similarly valaciclovir, the L-valyl ester of acyclovir, is hydrolysed by acyclovir giving, after oral administration, plasma concentrations equivalent to those obtained after intravenous acyclovir. At present all three appear to be hopeful antiviral agents with a useful future.

Can we draw from the herpes success some meaningful conclusions to help us with two more intractable viral candidates for extinction or, at least, containment: influenza A and HIV-1? The chemists' favourite molecule, the nucleoside analogue, still has much potential, but the incredible mutation rates of RNA viruses is an inherent problem. Ho et al. have described the emergence of drug-resistant mutants within days of onset of antiviral chemotherapy of AIDS patients with a protease inhibitor, but the same study also described the milieu in which some antiviral compounds exist; this has been described as a “mean street” with billions of virions being eradicated daily by millions of activated T cells. At first sight, the situation may appear completely hopeless for any but the most powerful inhibitor. Although herpes viruses also undergo massive replication in the body and can be stopped by antiviral agents, as demonstrated clearly with acyclovir, DNA viruses do not have the propensity for the high mutation rates of RNA viruses and so constitute easier targets. A trip back in time to the ice age would be required to discover as many herpes mutants as one would discover with influenza or HIV-1 in a journey lasting a single year. Nevertheless there is no overwhelming evidence that a patient would succumb to an infection with drug-resistant HIV or, indeed, a drug-resistant influenza A virus. Rather, at least with HIV, mixed populations of pro-viral genomes exist in a patient treated with zidovudine and even after long periods of drug treatment, zidovudine-sensitive genome sequences can still be detected.

Amantadine has been shown to exert a strong and reproducible antiviral effect when used as a prophylactic agent against human sub/types of influenza A virus including H2N2, H1N1 and H3N2. Drug-resistant viruses can be selected in the laboratory and in the patient and yet treated patients continue to benefit from therapy. Amantadine- or rimantadine-resistant strains of influenza A were isolated even
before the drug was used\textsuperscript{17} whilst viruses resistant to
zidovudine or non-nucleoside inhibitors of HIV can be isolated from a minority of patients before drug
therapy is commenced.\textsuperscript{18} Pre-existing polymorphisms exist in the particular nucleotides of HIV reverse
transcriptase or protease coding for amino-acid substi-
tution which, in the corresponding viral protein,
would result in a configuration change and drug
resistance.

Given these achievements, where will antiviral
agents proceed in the next few years? Inevitably, the
solution of further X-ray structures of viral proteins
and computerised imaging will be keys to new develop-
ments. Eleven years ago a group of scientists in
Melbourne published the first data on the atomic X-
ray structure of influenza A virus neuraminidase. This
enzyme is not unique to viruses; similar ones are
widespread in the animal kingdom. These enzymes are
glycohydrolases which cleave terminal sialic acid from
glycoproteins, glycolipids or oligosaccharides. In viro-
logical terms the enzyme functions at the stage of
influenza virus release from an infected cell. The main
study of anti-neuraminidase drugs commenced a
quarter of a century ago with the synthesis of the
Neu5Ac2en, a derivative of sialic acid which inhibited
viral, bacterial and mammalian sialidases. A series of
these compounds was tested as anti-influenza virus
drugs\textsuperscript{18} and although a number of analogues of
Neu5Ac2en inhibited influenza virus replication in cell
culture, no antiviral activity was detected in animal
models. In retrospect, this negative finding may be
attributed to the rapid metabolism of the early
compounds tested; newly synthesised analogues pro-
duce a specific anti-influenza effect in vitro\textsuperscript{19} and
clinically,\textsuperscript{20} after topical application.

Influenza sialidase is a tetramer of identical sub-
units. There are six four-stranded anti-parallel $\beta$-
sheets arranged as if in the blades of a propeller. The active
enzyme site is a deep cavity on the neuraminidase
protein surface and is lined entirely by amino acids
that are invariant in sialidases of all strains of influenza
A and B which have been characterised. In contrast,
variable amino acids are found next to and encircling
the active site and these are antigenic determinants
reacting with post-infection anti-neuraminidase anti-
odies. The scientific trick is to avoid inhibitors binding
to these variable amino acids but rather to select molecules to the invariant amino acids.

Sialic acid, the product of the enzyme-catalysed
reaction, binds to the active site of the enzyme in a
"boat" configuration. The carboxylate interacts with
three invariant arginine residues on the neuraminidase
enzyme. In this conformation it resembles the ge-
ometry of the potent sialidase inhibitor (Neu5Ac2en),
as noted above. Importantly, strain invariance extends
to a number of other amino acids which do not
themselves make contact with sialic acid, but which
provide a scaffold on which amino acids contacting the
bound sugar are supported.

The Melbourne group made predictions from X-
ray-derived structures of energetically favourable
substitutions to the unsaturated sialic acid analogue
Neu5Ac2en. The most interesting of these chemical
modifications was the replacement of the hydroxyl
group at the four position on Neu5Ac2en by an amino
group. Substitutions of the 4-hydroxyl group by an
amino group should produce a significant increase in
the overall binding interaction due to a salt bridge
formation with the side chain carboxylic acid group of
Glu 119 in the enzyme. Similarly, the replacement of
the same hydroxyl groups by the significantly more
basic guanidine group was predicted to produce an
even tighter affinity of the substituted Neu5Ac2en for
the active site as a result of lateral binding through the
terminal nitrogens of the guanidine group with both
Glu 119 and Glu 227. Direct measurements of viral
neuraminidase enzyme inhibition showed that the 4-
amino and 4-guanidino-substituted Neu5Ac2en were,
indeed, high-affinity inhibitors for the influenza virus
enzyme. Moreover, the compounds inhibited influenza
virus plaque formation in cell culture. Most excitingly,
the compounds had antiviral effects in vivo: Hayden et
al.\textsuperscript{20} using intranasal prophylaxis, were unable to
recover virus from 16 drug-treated volunteers, whereas
virus was recovered from 65\% of 17 untreated
controls. The compound looks most interesting.

But I have an important reservation about current
searches for inhibitors of HIV. With influenza
infections of the respiratory tract there is clear evidence of
viral replication in the trachea, bronchi and alveoli.
The disease is clearly related to known pathological
changes and to viral replication, and inhibitors of viral
replication have a logical scientific basis for clinical
action. This has been shown with amantadine and with
the anti-neuraminidase compound. Similarly, herpes
viruses cause cellular destruction in the skin, eye or
brain and these pathological changes are stopped by
antiviral compounds. But is the pathology of HIV so
clear cut? Ho et al.\textsuperscript{10} clearly believe it is. In their
important study the antiviral effects of ABT 538, an
inhibitor of HIV protease, were quantified by the
reduction in viral genomes and, in half the patients, by
an increase in CD4 cell count. In approximately one-
third of the treated patients, no increase in CD4 cell
count was noted, whilst the remaining patients formed
an intermediate group. Thus uncertainty remains as to
whether viral replication per se is destroying CD4 cells.
What if the exquisitely hostile environment created by
the immune system in AIDS patients\textsuperscript{41} is also directed
to self? Habeshaw et al.\textsuperscript{21} have steadfastly maintained
that a strong auto-immune component can be
recognised in AIDS patients by virtue of stimulation of
subsets of autoreactive T cells. Supporters of the
hypothesis point to activation of CD4 lymphocytes as
a key feature of the pathogenesis. There is evidence
that these T cell subsets kill uninfected CD4 cells as
well as virus-infected cells. These autoreactive T cells,
which are normally suppressed, recognise in an activ-
vated T cell a portion of the C terminus of gp120,
which when presented on a class I molecule mimics the
normal presentation of an allo-epitope HLA class II β chain.\textsuperscript{52} If auto-immunity contributes significantly to the pathogenesis of AIDS then new vistas appear for chemotherapists. Certainly new inhibitors of viral replication will still be the cornerstone of treatment but would need to be used as soon as the patient is infected to reduce the opportunity of recognition of class II β chain presented on HLA class 1 by these pre-existing autoreactive T cells. Later possibilities for chemotherapy would be drugs to mask the cross-reactive allo-epitope on the C terminus of the gp160 viral spike or, more controversially, the use of selective immunosuppressive drugs to counteract the auto-immune reaction. But perhaps most exciting would be the novel possibility of tolerising an AIDS patient with high concentrations of the mimic peptide or by transfusing with antibodies to the mimic peptide.

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References