Chemotherapy of Influenza and Herpes Virus Infections

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There is general agreement that active immunisation is the most effective measure for prevention of many virus diseases such as measles, rubella and poliomyelitis, and that chemoprophylaxis is unlikely to be used on a widespread scale where vaccine is already available[1]. However, certain virus infections of man are, for diverse reasons, unlikely to be controlled by vaccines in the near future and these include herpes virus (HSV), influenza and common cold viruses. The herpes virion is complex, with at least 24 structural proteins[2,3], is difficult to purify for production of a sub-unit or inactivated virus vaccine, and the exact proteins inducing protective immunity are not clearly established[1,3]. In contrast, common cold viruses are less complex immunologically, but exist as many serotypes, which would make the formulation of vaccines very difficult. In addition, influenza vaccines have a less than optimum efficacy, which is largely attributed to antigenic variability of the most important protective antigen, the haemagglutinin. Antiviral chemotherapy may have a role to play in the prevention of this important pandemic disease.

For treatment rather than prevention of virus diseases, specific virus inhibitors including interferon, and also, in certain circumstances, immune globulin, are more important than vaccines. Much of the available interferon in the near future is likely to be used in trials with cancer patients, although the recent demonstration of in vivo efficacy of interferon produced by cloning the interferon gene and subsequent insertion of the genetic information into an Esch. coli plasmid may lead to more rapid production of large quantities of the molecule[4]. Compared to studies with antiviral molecules, only a few well controlled trials of interferon with respiratory viruses, herpes virus and hepatitis B virus have been carried out[5] and so the precise degree of antiviral effect will have to be established in future trials. The application of monoclonal antibody techniques has expanded rapidly and the first human monoclones have now been described[6]. This may open new approaches to the treatment of certain rare virus infections, such as Lassa fever, by passive antibody.

This article reviews briefly the current prospects of treatment and prevention of certain virus infections with inhibitors (Fig. 1). To illustrate recent developments in antiviral prophylaxis, inhibition of influenza and herpes virus will be discussed. Other important virus infections awaiting some form of prevention or treatment are hepatitis A and B, and arena viruses.

Influenza virus

The longest laboratory and the most comprehensive clinical experience with antiviral agents has been obtained with the amantadine series of molecules, which have an inhibitory activity against influenza A viruses[7], although arenaviruses are inhibited in vitro to a low degree. The prophylactic and therapeutic effects of aman-
amantadine have recently been confirmed by trials in Europe, the USSR[8] and the USA, and extended with the related compound α-methyl-l-adamantane methyleneamine or rimantadine. To date, clinical trials with amantadine or rimantadine have been carried out in at least eight countries, involving many thousands of volunteers, and the overall protection rate in prophylactic trials against illness caused by H2N2, H3N2 or H1N1 viruses has varied between 50-100 per cent. In the majority of controlled therapeutic studies with amantadine, a demonstrable reduction in the severity and duration of influenza-caused fever was reported in those groups receiving amantadine, compared to the control groups, provided treatment was begun within two days of the onset of clinical symptoms[7].

Present and Future Clinical Use of Amantadine

At present amantadine or rimantadine have not found widespread use as antiviral compounds except in the USSR. This situation may have resulted from the absence of a clear directive for the use of a prophylactic agent against influenza A virus or confusion about the usefulness of amantadine in the general population or, alternatively, concern about possible subtle toxic effects versus a relatively mild antiviral efficacy. A recent consensus report in the USA has encouraged a more widespread clinical application of the compound[9]. Jackson[10] has suggested several clinical applications for the use of amantadine:

1. Household contacts of an index case of influenza to prevent virus spread to the rest of the family. The trials in the UK organised by Galbraith et al.[11-13] have established the usefulness of this approach both prophylactically and therapeutically.

2. Hospital patients and personnel, to prevent hospital spread when patients with influenza A virus infections are admitted.

3. Persons in institutions, such as old persons’ homes.

4. Unvaccinated adults with underlying disease which places them in a potentially high mortality group following an attack of influenza, e.g. persons with pulmonary, cardiac, metabolic or immunological deficiencies.

5. Vaccinated persons. Amantadine-supplemented protection would be expected to increase the protective effect of vaccine alone[14,15]. Single doses of sub-unit or split virus vaccine against a new pandemic virus subtype would not be expected to give significant protection[1]. Amantadine might be administered prophylactically during the three-week period of development of vaccine-induced immunity.

6. Adults, such as hospital workers, public transport personnel, etc., in the face of an epidemic caused by a new influenza A subtype when insufficient vaccine is available.

7. Persons presenting with influenza within 48 hours of the onset of clinical signs.

Clinical Studies with Amantadine Aerosols

Although amantadine has been shown to have a significant degree of prophylactic activity in man against influenza A viruses of three subtypes (H2N2, H3N2, H1N1), its possible role in the management of more severe illness such as primary influenza virus pneumonia remains undefined. Animal studies have shown greater therapeutic activity when the compound is administered as an aerosol than when given orally, and such topical application attempts to provide local high concentrations of drug and to minimise drug toxicity. Recent studies using volunteers have been undertaken to establish the safety and acceptability of delivery of amantadine by small particle aerosol[16,17]. In one study, thirty-minute treatments were given twice daily for 12 days[16]. One hour after aerosol treatments with a 1.0g/100ml solution, amantadine levels in nasal wash samples (mean 30.3 μg/ml) greatly exceeded blood and nasal wash levels following oral administration of the compound. Thus, following administration of 200 mg/day amantadine orally, blood levels of less than 1 μg/ml were detected. (With a plaque inhibition test, 0.3 μg/ml amantadine is required for inhibition of virus.) Pulmonary function studies showed no evidence of abnormalities after amantadine inhalation with five normal volunteers, although two persons, one asthmatic, had mild episodes of bronchospasm after prolonged inhalation[17]. The authors concluded that although normal subjects tolerated amantadine inhalation extremely well, the compound may occasionally produce mild adverse effects in susceptible patients with reactive airways. In the three young adults with influenza, amantadine aerosol was started at 6, 24 and 36 hours in 2-4 hour courses for 10-11 hours daily for three days. Recovery was rapid in all cases. Despite acute influenza, there was no evidence of irritation of the respiratory tract by the inhalations.

Mode of Action of Amantadine

Studies have indicated a point of action of amantadine at the stage of virus penetration of cells or early uncoating[7,8,18]. Analysis of influenza recombinants with mixed genes from amantadine-resistant and amantadine-susceptible parental viruses indicated that amantadine resistance was predominantly controlled by gene 7 coding for virus matrix protein, thus indirectly implicating M protein as a site of action of the drug. However, recent studies indicate a more complex derivation of resistance involving several genes. This would be consistent with current ideas that multiple rather than single genes are responsible for the virulence of influenza virus[19].

Amantadine and other amines may be lysosomotropic and can increase the intralysosomal pH[20]. Ammonium chloride increases the intracellular pH of mouse peritoneal macrophages from pH 4.5 to pH 6.2, whereas amantadine raises the pH to 5.5. Fusion of Semliki Forest Virus (SFV) membrane with lysosome membranes occurs in vitro and is strictly pH-dependent, fusion only occurring at pH 6.0 or lower. Thus, amantadine could prevent this fusion in the lysosome by increasing the pH. Experimental evidence points to the lysosomes as the point of entry of the SFV genome into the cytoplasm, and the inhibitory effect of amantadine on the replication of this
virus may result from interference with an essential fusion event. Experiments are now required to investigate the possible relevance of these observations to the uncoating of influenza virus in the presence or absence of amines.

**Inhibition of recent Influenza A virus Isolates by Amantadine**

The viruses of the H1N1 antigenic subtype now circulating throughout the world appear to form a genetically heterogeneous group and this may have important biological implications[21-23]. It was of interest, therefore, to examine the sensitivity of the viruses to amantadine, particularly as previous studies indicated some differences between influenza A viruses of different subtypes in regard to their inhibition by this compound[7].

In these quantitative experiments, cells were treated with varying twofold concentrations of amantadine and infected with different influenza A viruses. After 6 hours' incubation, infected cells were pulsed for 20 minutes with 35S methionine and virus-induced polypeptides were analysed by SDS-polyacrylamide gel electrophoresis (Fig. 2). The bands on the autoradiographs were quantified by densitometry and the degree of inhibition of virus-induced polypeptides by amantadine was established by analysis of peak areas of the densitometer tracing (Table 1). A dose response effect was noted with each influenza A virus when tested with varying concentrations of amantadine. Analysis of the degree of inhibition of synthesis of NP, NS1 or M polypeptides by 25 μg/ml amantadine showed that many recent influenza A virus isolates tested were inhibited to a similar degree. As anticipated, the influenza B virus was not inhibited by amantadine. Future studies are required to monitor the degree of inhibition of a wider range of influenza A viruses from geographically different epidemics to check for any emergence of amantadine-resistant viruses.

**Herpes Virus**

It is currently estimated that 7 per cent of the population in the USA have more than one episode of herpes labialis per year[24]. In addition, the incidence of venereal infections caused by HSV types 1 and 2 is increasing, as are more generalised varicella zoster and herpes infections in persons treated with immunosuppressive drugs. Considerable effort has been directed to the search for specific inhibitors of this virus and some compounds with demonstrated efficacy in initial experiments in volunteers are now under more detailed investigation in the clinic, including trifluorothymidine, ara-A[25], acyclovir[26], phosphonoformate (PFA)[27] and bromovinyl deoxyuridine (BVDU)[28].

Herpes virus must exert a precise control at the levels of virus DNA transcription and also translation of virus mRNA in the infected cell in order to direct the synthesis at the correct time of over 24 structural and 23 non-structural proteins and to co-ordinate the assembly of a complex infectious virion[3]. The virus also directs the synthesis of certain enzymes, including a thymidine kinase (TK) and a DNA polymerase, for the replication of the virus DNA. These virus enzymes, particularly the latter, are being used currently as targets in the search for new HSV inhibitors. Another attraction for the chemotherapist of such HSV infections as herpes labialis and keratitis is their superficial location and hence amenability to topical treatment[29].

**Acyclovir (9-(2-hydroxyethoxy methyl) guanine or acyclic guanosine)**

This antiviral compound is undergoing extensive clinical
Table 1. Inhibition of polypeptide synthesis of representative influenza variants by amantadine.

| Virus            | µg amantadine | Inhibition of virus polypeptide synthesis (%) |
|------------------|---------------|-----------------------------------------------|
| A/Brazil/11/78   | 25            | Not done                                      |
| (H1N1) A/Alaska/78 | 25            | 96.5                                      |
| (H3N2) A/Leningrad/549/80 | 25 | 73.9                                      |
| (H2N2) B/HK/72   | 25            | 59.2                                          |

Note that A/Brazil/78 and A/Alaska/78 are typical representatives of the two antigenic subtypes. A/Leningrad/549/80 is a recent virus isolate closely resembling viruses of the H2N2 subtype such as A/Singapore/57 by RNA and oligonucleotide mapping. The virus may have no epidemiological significance but has been used here as an example of a recent human isolate of this antigenic subtype.

trials in the UK, Europe and USA at present[26]. At the cellular level acyclovir is converted to the monophosphate by the viral thymidine kinase enzyme and the monophosphate is subsequently converted to the di- and triphosphate derivatives. The triphosphate is a specific inhibitor of the herpes virus DNA polymerase[30]. Thus, at a concentration of 100 µM of acyclovir, Vero cell DNA synthesis was reduced by 50 per cent at 48 hrs[30] whereas concentrations of 1 µM or less of the compound were required to reduce viral DNA synthesis to the same extent. Under certain conditions, acycloguanosine triphosphate can serve as a substrate for the α-DNA polymerase of the cell and is incorporated into a DNA template[30]. The incorporation of acycloguanosine monophosphate, which lacks the 3'-OH group required for chain elongation, may prevent further DNA chain growth by blocking the attachment of additional nucleoside residues. Therefore, the compound, in common with others to be described below, such as adenine arabinoside (ara-A), BVDU and PFA may have longer-term toxicity problems. Virological and biological experiments have established that the replication of HSV types 1 and 2 is inhibited by 0.1 µmol of the compound, whereas the growth of Vero cells is inhibited by 300 µmol, an effective therapeutic index of 3,000[31,32].

Higher concentrations of 2.0 µmol are required to inhibit varicella zoster virus and strains may vary in their degree of inhibition by acyclovir. The multiplication of Epstein-Barr viruses is inhibited, but this may result from a highly sensitive DNA polymerase rather than any thymidine kinase induced by this virus. The compound has only marginal inhibitory effects against cytomegalovirus and vaccinia virus, whilst adenovirus type 5 and a variety of RNA viruses are not inhibited. Mutants of HSV type 1 lacking thymidine kinase are resistant to acyclovir.

Animal experiments have also given encouraging results; herpes virus B is inhibited in vitro and in vivo[32]. The death of herpes B infected rabbits was prevented by administration of acyclovir at 200 mg/day for 14 days and treatment was effective even when delayed for 72 hours after infection. Herpetic keratitis induced by HSV-1 in rabbits was cured by two-hourly application in ophthalmic ointment. Local application cured model HSV-1 infections of guinea-pig skin, while subcutaneous administration of 40-100 mg/kg/day to mice infected intracerebrally with herpes virus reduced mortality to 33-73 per cent. Perhaps not unexpectedly, prolonged treatment failed to eradicate latent infections in mice.

A double blind placebo controlled trial was carried out at Moorfields Eye Hospital, with encouraging results[33]. Topical therapy with either 3 per cent acycloguanosine eye ointment or placebo five times a day was given to patients whose dendritic ulcers had been treated by minimal wiping debridement. There were seven recurrences of typical corneal herpetic lesions within one week in the 12 placebo patients. In contrast, no recurrences were detected in 12 patients who received acyclovir. These studies have been extended by others[34]. The first systemic studies of acyclovir have now been carried out in man[26]. Intravenous administration showed a satisfactory pharmacokinetic profile, with a mean plasma halflife of 2.9 hours, and no adverse toxic effects were noted. In man, oral doses up to 600 mg result in a peak plasma level of 2.6 µmol—some 26 times the concentration required to inhibit HSV-1 and -2 replication. A number of patients with severe herpetic infections, including systemic herpetic zoster or herpes simplex, were treated with 5 mg/kg acyclovir eight-hourly for five days and the progress of the infection was arrested. In a recently reported double blind placebo controlled study of acyclovir against HSV in recipients of bone marrow transplants, no lesions developed in 10 patients who received acyclovir intravenously, whereas lesions developed in 7 out of 10 patients who received placebo[34a].

Phosphonoformate (PFA or Foscarnet)

This compound is related to the parent phosphonoacetic acid (PAA), but has the advantage of inducing no local reactions following application to the skin. In addition, PFA has a rather different antiviral spectrum to that of PAA[35]. As well as anti-herpes type 1 and 2 activity, PFA inhibits the DNA polymerase of hepatitis B virus. Laboratory studies have established that PFA selectively inhibits the DNA polymerase of HSV-1 and HSV-2 viruses, possibly by acting as a non-competitive inhibitor at the pyrophosphate binding site on the enzyme itself[35]. Different strains of HSV show a varying degree of sensitivity to PFA in plaque assays. Human cytomegalovirus was less well inhibited, but marked inhibitory effects were found with Marek's disease herpes virus, pseudorabies and infectious rhinotracheitis virus. RNA viruses, including influenza A, polio type I, measles, rubies and visna, were not inhibited significantly. Work in animal model infections, particularly infection of guinea-pig skin, has established the therapeutic activity of PFA[36]. In this model infection a depilated area of the skin is punctured lightly with a gun and infected with HSV-1, and 24 hours later an erythema is detected. Addition of 2 per cent PFA as a topical cream six times daily thereafter results in a therapeutic activity, measured...
both as a cumulative score and on a time to healing parameter.

Preliminary clinical trials have described a therapeutic effect of PFA in man[35]. The therapeutic efficacy of 3 per cent ointment (corresponding to 18 µg PFA) used six times daily for 4 days was studied in a multicentre double blind placebo controlled trial in patients with a history of recurrent herpes labialis; 200 episodes of current disease have been studied. At the time of the first episode, treatment was begun within 24 hours, whereas after cross-over and with the next episode the patients used self-medication immediately immediate clinical signs were obvious. Lesion size and stage were measured at the clinic at the first episode and, in addition, each patient kept a record of pain score and lesion development. PFA shortened the popular and vesicular stages of the disease and more patients in the control group developed new vesicles. Of particular interest for future clinical trials was the observation that many patients were able to discriminate between active and placebo treatment in spite of a high comparability of objective measurements. These initial trials, which show a relatively mild antiviral effect, will have to be confirmed using larger numbers of patients. A potential toxicity problem with PFA concerns the ability of PFA to bind tightly, but not irreversibly, to the inorganic matrix of bone in animal studies[35]. Approximately 30 per cent of the systemically available drug is deposited in bone and cartilage. Further studies are now required to determine the precise degree of adsorption of PFA from the skin in man, and hence to quantitate the tissue distribution and binding.

Adenine Arabinoside (ara-A or Vidarabine)

In common with the other anti-herpes compounds described, the active metabolite araA-triphosphate inhibits herpes virus DNA polymerase at significantly lower concentrations than cell DNA polymerases. Ara-A is also incorporated into viral and cellular DNA and may act as a chain terminator for newly synthesised virus DNA strands[37,38]. Most herpes simplex and varicella zoster virus strains are inhibited by less than 3 µg/ml of ara-A, whilst vaccinia virus is inhibited by 0.5 µg/ml.

A practical clinical problem with the compound is its poor solubility, hence ara-A has to be administered in extensive intravenous infusions. More soluble derivatives such as ara-AMP are currently under investigation. Ara-A is rapidly deaminated to arabinosyl hypoxanthine, which is less antiviral than the parent compound. Ara-A is used at present mainly for life-threatening infections such as HSV encephalitis, severe varicella zoster or generalised neonatal HSV infection. Short-term clinical studies have indicated few toxic effects at 10 mg/kg for 5 days (a total of 3.5 g per 70 kg person) when used for the treatment of varicella zoster skin infections[38]. In contrast, in studies with HSV encephalitis, higher doses of 15 mg/kg for 10 days (total of 10.5 g) were given, which approached the toxic dose of 20 mg/kg where tremors, weight loss, and bone marrow megaloblastosis have been described[38]. The large volumes of intravenous fluid required because of the low solubility of the compound may worsen existing cerebral oedema[39]. An extensive and well documented double blind study of biopsy-proven cases of HSV encephalitis indicated that ara-A treatment reduced mortality from 70 to 28 per cent[39]. Ocular penetration of the compound is poor and so ara-A is not used for the treatment of deep stromal disease or herpetic uveitis, but the compound is effective as a 3 per cent ointment for HSV-induced acute keratoconjunctivitis and recurrent epithelial keratitis[40]. Topical preparations of ara-A or the more soluble ara-AMP have not proved beneficial in genital or labial herpes virus infections.

Bromovinyldeoxyuridine (BVDU)

Preliminary experiments in vitro have indicated that this thymidine analogue is one of the most active inhibitory molecules against HSV type 1 (ID_{50} approximately 0.01 µg/ml) with a therapeutic index of 10,000 although 100-fold less activity is noted with HSV type 2[41]. The HSV-1 coded thymidine kinase enzyme phosphorylates BVDU, and the 5'-triphosphate has a selective action on the virus DNA polymerase. As anticipated, TK+ mutants of HSV are not inhibited by BVDU. Thus, the mode of action appears to resemble that of acyclovir and ara-A except that evidence of incorporation of BVDU into viral or cell DNA is lacking at present. The compound is also active against varicella zoster virus in vitro and inhibits cytopathic effects at concentrations as low as 0.04 µg/ml.

It is essentially inactive against vaccinia virus and a representative RNA virus (SVS) and is unlikely to inhibit cytomegalovirus since this virus is not known to induce a TK enzyme[42]. In vivo experiments established that BVDU as a 0.5 per cent ointment reduced the severity of HSV keratitis in rabbit model infection[42].

The compound appeared to be active in herpetic keratitis that was unresponsive to idoxuridine (IDU) or ara-A[43]. Further placebo controlled trials will be necessary to quantify this effect. Briefly, 37 patients were treated with 0.1 per cent BVDU eye-drops at hourly intervals during the day. Twenty-eight of these patients had been treated unsuccessfully before with IDU or ara-A. All patients responded clinically to BVDU therapy, with an average healing time of 7.8 days for dendritic ulcer and 10.8 days for geographic ulcers. BVDU caused a brisk healing of deep stromal keratitis. In additional clinical studies, oral BVDU treatment (7.5 mg/kg/day for 5 days) caused prompt recovery in four patients with severe herpes zoster[44]. Circulating plasma levels of the drug were 1-1.5 µg/ml, which are well above the concentration required to inhibit virus replication in vitro. Existing lesions regressed within the first few days after the start of the treatment and no new lesions developed while patients were under therapy.

Conclusions

We have recently summarised the current attempts at antiviral therapy as ‘the end of the beginning’[45]. This emphasises the large amount of detailed work carried out in the last thirty years which has now established the
scientific basis for the investigation of antiviral compounds in the laboratory and the clinic. Defined model systems have been established that give data relevant to subsequent antiviral activity in man. Examples are the infection of guinea-pig skin with HSV-1 virus, the demonstrable curative effects of PFA, and the mouse models of influenza-induced pneumonia which first demonstrated the therapeutic effectiveness of amantadine. In addition, good correlation has been noted between in vitro sensitivity of influenza A strains to amantadine and antiviral activity in clinical trials.

The earlier antivirals, methisazone, idoxuridine, ara-C and amantadine, were discovered by random selection[46-48]. More recently, with increased knowledge of the details of virus replication at the molecular level, it has become apparent that viruses, like all living organisms, have Achilles’ heels—in this case virus specific enzymes. These enzymes are now being used with some initial success in routine laboratory screening for new antivirals. This is semi-rational chemotherapy, but the selection of specific tailor-made antivirals may now be feasible. The rapid techniques of DNA sequencing are currently being applied to a wide variety of virus genomes. With certain viruses with predominantly mononucleic messenger RNAs, such as influenza, nucleotide sequence data from DNA transcripts can be reconstructed as amino acid sequences for important proteins such as the haemagglutinin. The short peptides Z-Gly-L-Leu-L-Phe-Gly and Z-Gly-L-Phe-L-Phe-Gly mimic the sequences at the N-terminus of the HA2 polypeptide of influenza A and B viruses and inhibit virus plaque formation in vitro[49]. The N-terminus of the HA2 polypeptide may be involved in viral penetration and hence the synthetic peptides would be expected to act as competitors at this stage. Equally, the establishment of RNA or DNA polymerase enzyme binding sites should lead to the selection of specific inhibitors for a number of viruses.

From the clinical point of view, the last few years have shown the necessity for well-controlled (both clinically and virologically) trials. Many of the poorly controlled earlier trials with herpes infections and idoxuridine, ara-C, ether, ultra-violet light and 2-deoxyglucose have been repeated under more stringent conditions and the compounds have been shown to have marginal or no activity. But a number of antiviral compounds with proven efficacy remain, including amantadine for the prophylaxis and treatment of influenza A virus infections, and intravenous ara-A for the treatment of severe life-threatening herpes infections. Several controlled trials have demonstrated the effectiveness of acyclovir against eye infections caused by herpes viruses, while, in a single controlled trial, PFA has shown effectiveness as a topical ointment against herpes labialis. Combination therapy with more than one antiviral compound, or with antivirals and interferon, may be more widely used. Supplementation of trifluorothymidine treatment with daily local instillation of two drops of leukocyte interferon containing 3 x 10⁷ units/cm² has resulted in more rapid healing of dendritic keratitis and faster cessation of virus shedding[50].

The laboratory systems can therefore predict antiviral activity with a reasonable degree of confidence. Clinical trials, in spite of the greater difficulties in organisation and subsequent scientific analysis, can be conducted to a correspondingly high standard to give unequivocal data of efficacy or otherwise.

Acknowledgements

I would like to thank Doctors B. Oberon, E. Helgstrand, E. De Clercq and M. Falcon for details of unpublished data for inclusion in this article. Mrs T. Corcoran and D. I. Meredith provided excellent technical and photographic assistance respectively.

This article is based on a paper read at the Conference on Infection in Britain Today held at the Royal College of Physicians in November 1980.

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