SPECIFIC ANTIVIRAL THERAPY OF RESPIRATORY VIRUSES

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21.1 INTRODUCTION

In this last decade of the twentieth century we inhabit a world of molecules which are being investigated for the first time at the atomic level, while biologists have begun to manipulate them to our advantage. The current scientific literature abounds with descriptions of transgenic animals, of chimaeric viruses, of viral proteins and enzymes analysed by X-ray crystallography, synthetic genes and, most recently, the ability of biologists to manipulate genes even to a point of fusion of the sperm and ovum.

The respiratory viruses co-inhabit this world of host DNA and RNA and indirectly manipulate host genetic machinery to produce new viral offspring. The entanglement of replication of viral nucleic acids, either RNA or DNA, with host cell nucleic acid and transcription and replication mechanisms has made the discovery of viral inhibitory molecules exceedingly difficult. In terms of specific inhibitors of influenza virus, only two compounds (namely amantadine and ribavirin) have been discovered to date. They have gained unequivocal acceptance as drugs for clinical use (reviewed in [69]) and no other drug against any other respiratory virus is used in the clinic. There remains, therefore, a unique opportunity for the development of a novel programme to discover new drugs. Indeed, the search for a small synthetic or a naturally occurring molecule, the virological counterpart of the antibacterial penicillin molecule, and which could target and inhibit the replication of a wide

range of viral pathogens is perhaps more intense now than ever before.

21.2 THE MEDICAL RESEARCH COUNCIL COMMON COLD UNIT

The MRC Common Cold Unit in Salisbury, UK played a central role in early studies of antiviral chemotherapy, including the first controlled demonstration of the clinical activity of interferon against influenza and the clinical effectiveness of a virion-binding drug against the rhinovirus.

The research unit in Salisbury was established before the discovery of the first antiviral compounds in the mid-1950s. At this time two antivirals were being studied, namely methisazone against smallpox and idoxuridine against herpes infections of the eye (reviewed in [48]). However, the scientific attractions of specific molecules binding to virion proteins or inhibiting virus-specific enzymes were recognized as immense. It was also well recognized that the discovery of specific virus inhibitors could lead to fundamental discoveries about viruses themselves and this is particularly so when a drug-resistant virus can be selected.

Therefore, as the search for respiratory virus inhibitors quickened during the next decade, the Common Cold Unit (CCU) began to occupy a central stage position in this growing area of virology. By 1963 the first anti-influenza virus compound had been reported [16], while during the next two decades new anti-rhinovirus inhibitors came under scrutiny and investigations commenced of inhibitors to coronavirus [35,36] and respiratory syncytial (RS) virus. But during this period the most important contribution of the CCU was undoubtedly the clear demonstration that interferon could inhibit a virus in a clinical situation [42]. In later studies, the group investigated the effectiveness of purified interferon [60] and interferon alpha$_{-2}$ produced by recombinant DNA [62] technology as well as other interferons produced in cell culture and administered by the intranasal route [54]. Not all interferons were found to be clinically effective [61]. Undoubtedly it was a source of satisfaction to the staff of the CCU and to the scientific community worldwide when a compound which showed clear effects against the common cold virus was reported [1,4].

A wider and perhaps the major contribution to the developing science of antiviral chemotherapy to emerge from the Salisbury Unit was the establishment of a base for

controlled clinical studies in a defined scientific environment [7]. The combination of clinical and laboratory science was a key element at the CCU. To my knowledge only two such clinical volunteer units now exist worldwide, one in the CIS (St Petersburg) and the other in the USA. Respiratory viruses are rather unique in the sense that true volunteers come forward for clinical experiments, in marked contrast to the other target viruses of antiviral chemotherapists: HIV, papillomavirus, hepatitis B virus and herpes simplex virus. Beare and Reed [7] described in a detailed chapter the day to day clinical and scientific work of the CCU.

In the last 18 months a new anti-influenza molecule has been discovered (Figure 21.2), or at least refined using the most modern methods of X-ray crystallography and computer graphics [72]. Normally the CCU was the first centre chosen for clinical experiments and it may be presumed that work would have been initiated there in volunteers with the new drug. The absence of the Unit means that there is now a serious gap for EC countries in the provision of clinical facilities to bring a new antiviral molecule to the registration stage. As the following discussion will illustrate, respiratory viruses are still very important pathogens and the need for a co-ordinated scientific and clinical approach for new inhibitors has not diminished since the inauguration of the CCU, rather it has increased. The chapter will highlight major discoveries in respiratory antiviral chemotherapy over the last three decades, with particular emphasis on influenza virus. The volunteer work carried out with this virus may be considered to be the most challenging for

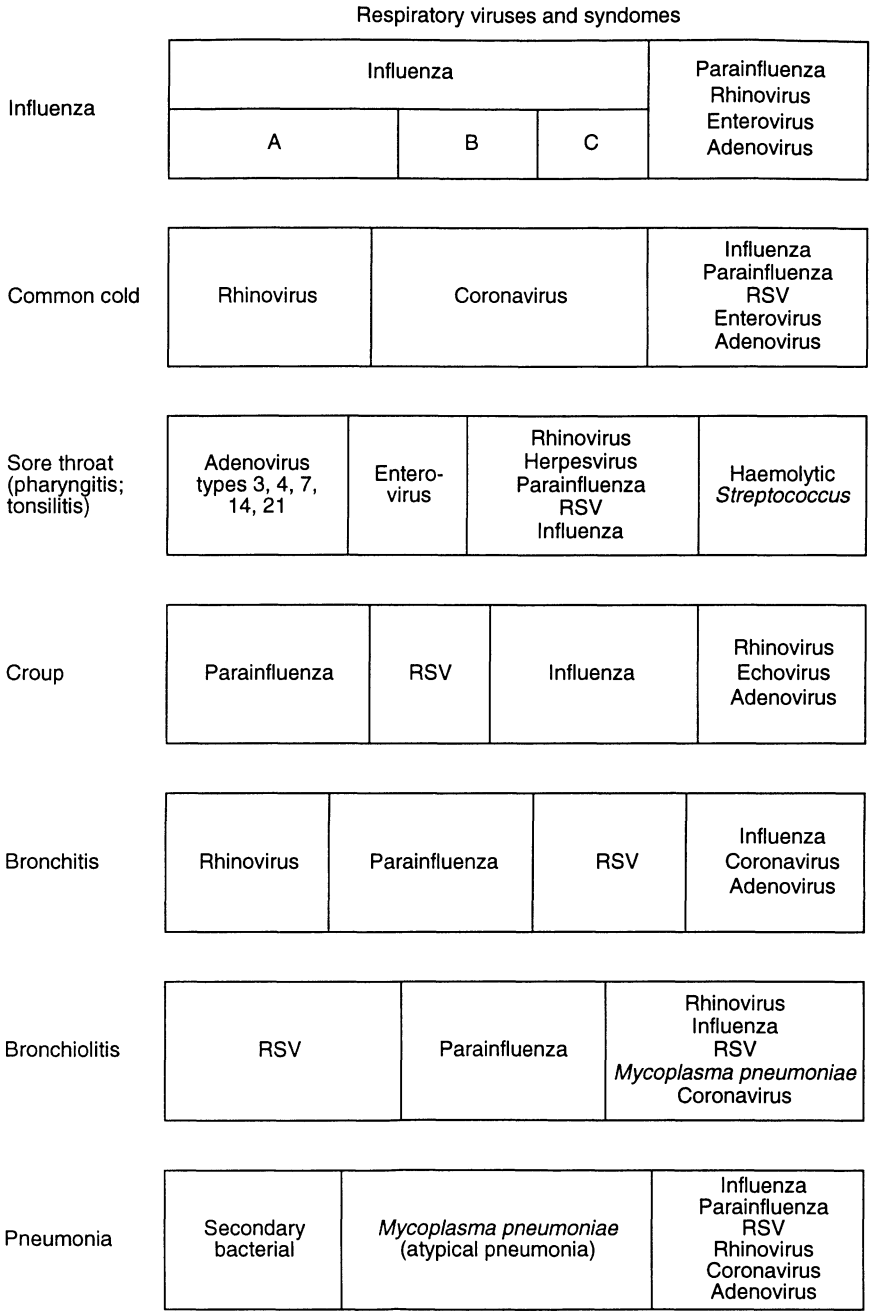

Figure 21.1 The range of respiratory viruses correlated with clinical disease. The relative size of the blocks is related to the proportion of viruses causing the disease. (From [66].)

the scientists and yet worrying for the clinicians. Unlike the other viruses utilized at the CCU, influenza virus has the ability to cause very serious infection in any person, volunteers included. It is a credit to the Salisbury Unit that volunteers came forward confidently for these experiments and the expertise at the Unit ensured that there was no clinical detriment to the volunteers. It should be clearly appreciated that basic studies into the genetic determinants of virulence of influenza viruses were also proceeding in parallel to studies of inhibitors (see Chapter 14) which may themselves lead in the near future to the discovery of new compounds.

21.3 CLINICAL NEED FOR ANTIVIRALS

A continued clinical need for the discovery and refinement of new antivirals against respiratory viruses is clearly apparent in the 1990s.

21.3.1 EPIDEMIC INFLUENZA

Influenza A and B viruses are notably the single major cause of several lower respiratory tract infections and death throughout the world and are seasonal in their impact (Table 21.1). For example, in the UK alone 26 000 excess deaths were caused by influenza A virus in the winter of 1975–1976 while in the winter of 1989–1990 there were in excess of 19 000 deaths. The viruses also cause severe economic disruption and in 1975 there were 900 000 excess new claims for sickness benefit in the UK alone. Not all sections of the community are at equal risk of mortality. Certain 'special risk' groups including older persons, diabetics of any age and persons of all ages with chronic heart, kidney or respiratory disease have been defined and are at a ten-fold greater risk of mortality following an infection with either influenza A or B virus. Not surprisingly, these groups of vulnerable persons in our community are the major target for vaccines and new chemotherapeutic agents.

21.3.2 SERIOUS LOWER RESPIRATORY INFECTIONS CAUSED BY RESPIRATORY SYNCYTIAL VIRUS

Another important virus causing severe lower respiratory tract infection and even death in

Table 21.1 The myriad of viruses causing pathology of the respiratory tract

Viruses causing respiratory tract infection	*Target group*	*Antiviral agents which are clinically active*
Upper respiratory tract		
Rhinoviruses	Young and old	Natural interferon, recombinant α-2a interferon*; virion binding molecules
Coronaviruses	Young and old	None (but see [35, 36])
Lower respiratory tract		
Influenza A, B and C	Young and old but mortality in 'at risk' group	Amantadine*, rimantadine* ribavirin*, neuraminidase inhibitor
Adenoviruses	Young and old – mortality rare	None
Parainfluenza types I, II, III and IV	Young and old – mortality rare	None
Respiratory syncytial virus	Young and old with mortality in both	Ribavirin*

* Licensed antiviral: clinically active.

the elderly and particularly in infants and young children, is respiratory syncytial (RS) virus. For example, in the USA alone RS virus causes 90 000 hospitalizations and 4500 deaths per year. RS virus is the most frequent cause of bronchiolitis and pneumonia in infants (Table 21.1). More recently [22], deaths have been reported in the elderly which may have been inadvertently attributed to influenza A virus. Like influenza virus, RS virus is seasonal and causes outbreaks worldwide but probably with greater regularity than influenza virus with, for example, yearly outbreaks in temperate climates for 3 months each winter. A unique feature of the epidemiology of RS virus is the ability of the virus to re-infect a patient. Since natural immunity seems short-lived it could be surmised that vaccination would be unsuccessful in controlling this disease. Therefore antiviral chemotherapy is expected to play an important role in prevention and cure of RS virus infections.

21.3.3 VIRUSES CAUSING UPPER RESPIRATORY TRACT INFECTION

A galaxy of viruses causes upper respiratory tract infection (Figure 21.1), including the 150 or so small RNA viruses, the rhinoviruses, while additional important viral pathogens of the upper respiratory tract are the coronaviruses and four types of parainfluenza virus (Figure 21.1). In the USA alone in 1985, colds accounted for 17% of all episodes of acute illness requiring medical attention, while 23 million days of lost work were recorded. The rhinoviruses circulate all the year round and are not seasonal. The antigenic variability among the rhinoviruses would appear to argue against the usefulness of vaccines were it not for the presence of certain common amino acid sequences in the virus attachment site. Although the latter respiratory viruses are more commonly inhabitants of the upper respiratory tract, they can predispose a patient to serious secondary bacterial infections of the paranasal sinuses and middle ear and possibly contribute to increased mortality of children in third-world countries.

21.3.4 THE CHEMISTRY OF INHIBITORS OF RESPIRATORY VIRUSES

It would be difficult to give an accurate estimate of the number of antiviral molecules already screened *in vitro* against respiratory viruses. Over 100 000 molecules have been tested and the handful of molecules shown in Figure 21.2 are those with some clinical activity. It has been a gigantic task, and many first-rate chemists have devoted their life's work to this area of science. The objectives of this difficult but rewarding work are summarized in Table 21.2.

The molecules which have been discovered to date include natural products of plants, such as alkaloids, and chalcones and short peptides, benzimidazoles, nucleoside ana-

Table 21.2 The ideal requirements of an antiviral agent against respiratory viruses

Absence of toxicity and side effects
Broad antiviral spectrum to inhibit influenza A and B viruses, respiratory syncytial virus, rhinoviruses and coronaviruses*
Administered orally
Cheap to synthesize and heat stable for storage
Chemical variation possible to cope with subsequent emergence of drug-resistant viruses

* Ribavirin and interferon partially meet this requirement

logues, simple amines and thiosemicarbazones. It should be clearly recognized that none of these compounds has been 'designed' as an antiviral: all have been found as a result of random screening. Most large pharmaceutical companies will possess a 'compound library' of molecules synthesized over the years for many areas of medical science. Such libraries may contain 250 000 molecules, but for a screen only a portion of these would be tested in cell cultures against a target virus. Following the identification of a 'lead' compound, considerable chemical ingenuity has been and is still used to synthesize more effective derivatives. A particularly fruitful source of antiviral compounds, respiratory viruses not excluded, are the nucleoside analogues of purines and pyrimidines.

Thousands of analogues of the naturally occurring nucleosides have now been synthesized and tested in the laboratory and most pharmaceutical companies have large libraries of such compounds alone. These nucleoside analogues are particularly useful to chemists since they can be modified extensively. It will be appreciated that even a single atomic substitution may change an active to an inactive molecule or vice versa. Frustratingly, however, it has been a common experience that, *after* the initial discovery of an active molecule, subsequent chemical modification more often leads to compounds with less antiviral activity than the reverse. It is not often appreciated that many of these compounds are difficult to synthesize, even by experienced chemists, and may require many months of work. Often, therefore, only small quantities of drug are available for *in vitro* tests and in only a few cases is enough synthesized for animal experiments. In the particular case of drugs active against respiratory viruses which replicate at mucosal surfaces, the delivery vehicle can assume very great importance. In fact the use of nasal sprays and aerosols may avoid the ingestion orally of large quantities of the drug which would be required to achieve inhibiting drug levels in the respiratory tract.

21.4 INFLUENZA

21.4.1 AMANTADINE – THE FIRST ANTI-INFLUENZA A VIRUS DRUG

One of the first antivirals [16] against a respiratory virus to be tested extensively in humans was the primary acyclic amine, amantadine or Symmetrel (Figure 21.2), which inhibits influenza A virus but not influenza B virus replication. Addition of a methyl grouping to give the analogue rimantadine alters the pharmacological distribution of the drug and prevents its entry into the brain, thus reducing the particular side effect of amantadine described as 'jitteriness' but without affecting the antiviral activity or antiviral spectrum. Many hundreds of similar amantadine molecules have been synthesized (reviewed in [47]), but none apparently greatly exceeds the original compounds in its inhibitory effect against influenza virus.

Clinical studies

Worldwide clinical studies have demonstrated the prophylactic effectiveness of amantadine against influenza A viruses. From the time of the great influenza pandemic in 1918 chemotherapists have attempted to find and use selective inhibitors of the virus, but not until the discovery of the primary amine amantadine [16] did effective chemoprophylaxis and even chemotherapy of influenza A virus infections become a reality. It had been known for some time that simple amines and ammonium compounds inhibited the replication of influenza virus in cell cultures and in eggs but successful clinical application was only achieved with amantadine, presumably because its unusual 'birdcage' structure (Figure 21.2) confers pharmacological stability. Initially, Jackson and colleagues [65] showed that the drug could prevent the development of respiratory symptoms in volunteers. It

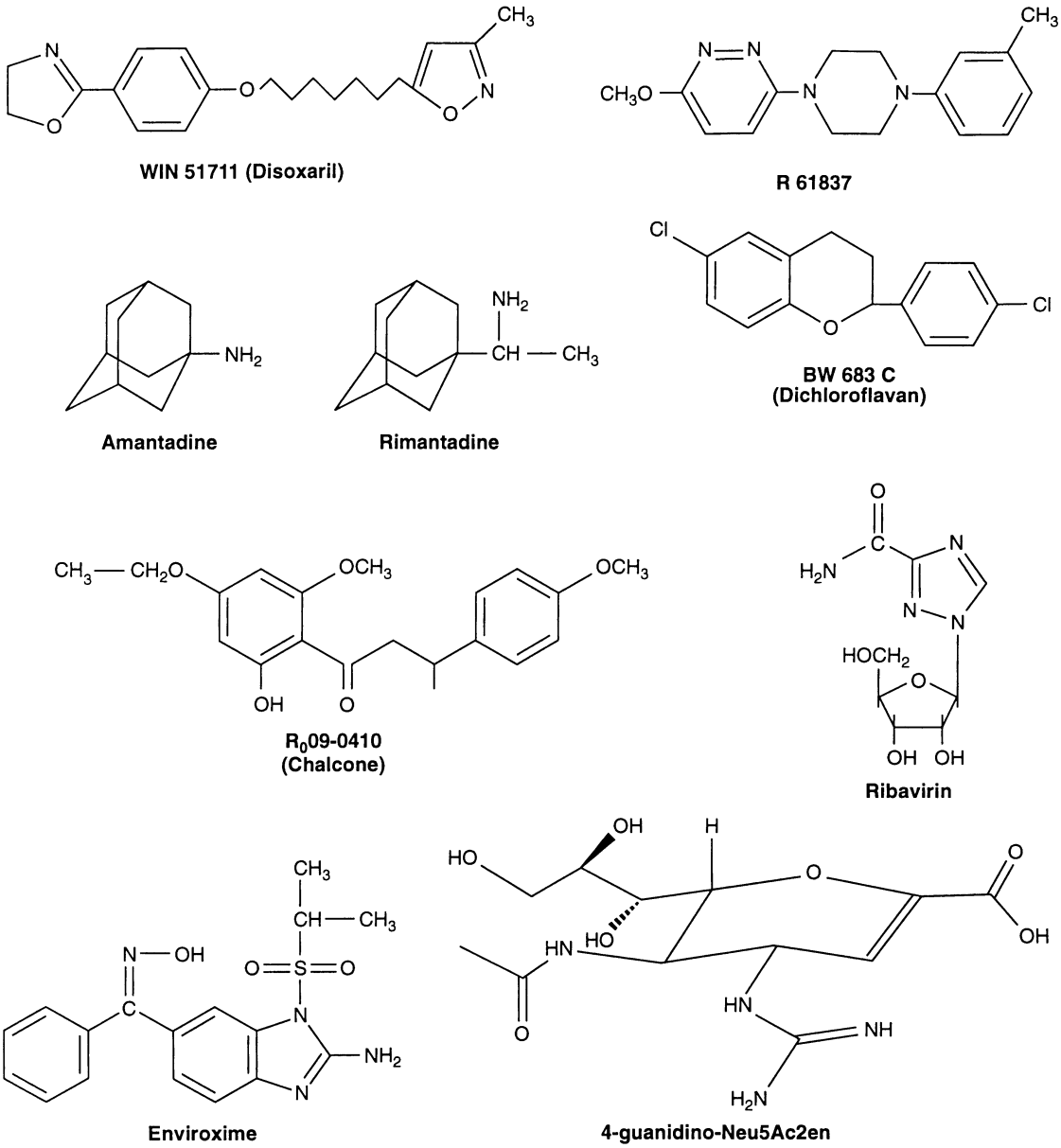

Figure 21.2 The range of molecules which inhibit the replication of respiratory viruses.

should be remembered that all these and subsequent clinical studies were pioneering in the sense that no drug had previously been shown to be active clinically against a respiratory virus, nor was it clear how such a compound could be used in the community to prevent disease. The properties of an ideal antiviral compound are summarized in Table 21.2 and it must be acknowledged that to date such a perfect molecule has not been found.

A study of amantadine in conjunction with the Royal College of General Practitioners

[24,25] is worthy of more detailed description because it was probably the first trial planned as a practical use of an antiviral, as well as a scientifically controlled study. Medical practitioners throughout the UK were asked to identify clinical cases of influenza and take blood (for serology) and nasal wash samples (for virus isolation) from this 'index case'. The index case was left untreated but immediate family contacts of the index case were given amantadine or placebo daily for 10 days in a double-blind trial. Then, the spread of virus from the untreated index case to the rest of the family was monitored. It was found that 14% of members of the families in the placebo group were subsequently infected with influenza A virus compared with 3.6% in the amantadine group. However, none of the latter persons had serologically confirmed influenza, whereas 10 (14.5%) of 69 persons in the placebo group had serologically confirmed influenza. Thus, amantadine used in a practical way successfully pre-

Table 21.3 Clinical trials with amantadine and rimantadine involving H3N2 and H1N1 viruses

Virus	*Amantadine or rimantadine*	*Trial situation*	*Result*	*References*
A/Shanghai/11/87/(H3N2)	Amantadine	Persons in nursing homes who had been vaccinated Prophylaxis	Viruses inhibited *in vitro* before prophylaxis. Drug-resistant viruses also isolated. Amantadine apparently reduced mortality	[41]
A/Bangkok/1/79 (H3N2)	Rimantadine	Double-blind, placebo-controlled therapeutic trial	Prompt reduction in virus titre in nasal secretion	[30]
A/USSR/77 (H1N1)	Rimantadine	Placebo-controlled trial in students		[70]
A/Leningrad/87 (H3N2)	Rimantadine	Placebo-controlled community prophylaxis study (low dosage 100 mg)	Low-dose rimantadine; 86% efficacy in illness reduction	[10]
A/Texas/1/85 (H1N1)	Amantadine	Prophylactic experimental challenge with wild type virus Adult volunteers (low dosage 100 mg)	78% protection against illness; 100-fold reduction in virus shedding	[63]
A/Bethesda/1/85 (H3N2)	Amantadine	Prophylactic experimental challenge with reduced dosage (100 mg)	Significant reduction in virus shedding Significant reduction in illness	[58]
Influenza A/H3N2 presumed 1985	Rimantadine	Therapeutic trial in children.	Viruses inhibited *in vitro* Significant reduction in fever and improvement in symptom scores. Resistant viruses also isolated	[12]
A/Philippines/2/82 (H3N2)	Amantadine	Prophylaxis in a partially vaccinated nursing home in reduced dosage (100 mg)	Beneficial effect on the decline of influenza	[5]
Typical influenza/A/H3N2 1986 virus	Amantadine	Uncontrolled trial in a boarding school (100 mg/day)	Significant reduction in illness compared with previous year's outbreak at the school	[15]
A/Philippines/82/(H3N2) A/Panama (H3N2) Influenza A H1N1	Rimantadine	Double-blind, placebo-controlled prophylactic study in children in families	73% reduction in rate of infection and 78% reduction in influenza illness	[13]
Typical influenza A/H3N2 1988 viruses	Rimantadine	Double-blind, placebo-controlled therapeutic study in families	Significant reduction in days of fever and symptoms Drug-resistant viruses isolated	[32]

vented spread of influenza A virus in persons in a family setting. With the advent of a new subtype of influenza virus the following year, namely A/Hong Kong/1/68 (H3N2) virus, a new trial was conducted which failed to show clinical efficacy [23].

However, numerous clinical trials, both in volunteers given attenuated influenza A viruses [67] and in the community where persons were infected with naturally virulent influenza A viruses, including influenza A (H3N2) viruses, confirmed and extended the initial studies of the prophylactic effect of amantadine (Table 21.3). Of particular significance has been the observation over the succeeding decades that all such subtypes of influenza A virus so far encountered in humans, namely H2N2, H1N1 and H3N2, have been inhibited both in the laboratory and also in clinical trials (see below).

The results of some studies recently have raised the question of whether amantadine has a mild suppressive effect on the development of immunity following infection. A lower dose of amantadine (50 mg) had no effect on local IgA production in the respiratory tract whereas a suppressive effect was noted in the systemic IgG response. For clinical use a recommended prophylactic and therapeutic dose of amantadine is 200 mg daily, reduced to 100 mg in those aged 10–15 years or over 65 years. The suggested dose in children aged 1–9 years is 2–4 mg/kg [26,45]. It is a truism that much of the pharmacology data for amantadine was collected using healthy volunteers whereas different problems occur in the use of any drug in the frail and elderly. In these, a 50 mg dosage of amantadine may lead to inadequate steady-state trough serum concentrations whereas 100 mg may lead to excessive levels and adverse effects, particularly in the presence of renal impairment. Some clinicians therefore recommend individualization of dosages in the elderly.

Therapeutic effect

Arguably the most significant discovery, at least for the future development of antivirals against respiratory viruses, was that amantadine had a *therapeutic* effect. In the initial studies, volunteers in prison in the USA who were infected with influenza A virus and were showing the first signs of clinical influenza were given amantadine or placebo (reviewed in [47]). The influenza illness, including objective parameters such as temperature subsided more rapidly in persons to whom amantadine was administered. Subsequently, Galbraith and his colleagues [47] planned a therapeutic trial in the UK on naturally infected patients in families and confirmed the therapeutic effect of oral amantadine administered not more than 24 hours after the first influenza symptoms appeared [47]. Perhaps, not surprisingly, the use of the antiviral later than this time failed to abrogate symptoms of the disease. It should be mentioned that before these trials most virologists considered that, concomitant with the peak of respiratory symptomatology, influenza virus replication would also reach a peak and therefore the therapeutic use of an inhibitor at this stage of the disease would be fruitless. Therefore, the demonstration of the therapeutic effect of amantadine, as well as being of considerable scientific interest, also emphasizes the need to perform experiments rather than placing too much emphasis on hypothesis. Few studies have actually been performed on the quantity of influenza virus excreted in the lower respiratory tract of infected humans. Postmortem examination of trachea specimens by immunofluorescence and cytology during the influenza A (H2N2) virus pandemic showed that virus replication was patchy and that many cells remained uninfected. This observation gives credence to the concept of the theoretical usefulness of therapeutic intervention since antiviral drugs would be expected to prevent spread of virus further down into the respiratory tract.

It must be acknowledged that the precise degree of clinical impact of amantadine or, indeed, any other antiviral drug against respiratory virus infection in the community has yet to be calculated. For example, it is quite possi-

ble that early use of the compound could prevent spread of the influenza A virus into the lower respiratory tract and lungs and so abrogate mortality [52]. Pharmacological experiments both in animals and humans have shown a somewhat preferential accumulation of amantadine in respiratory tissue including the lung (reviewed in [47]). To date, such clinical investigations of possible effects of amantadine on influenza virus-induced mortality in the elderly [14] have not been undertaken widely and in our opinion are urgently needed.

Amantadine is under-used as an antiviral

At present amantadine is not widely used in clinical medicine. For the future development of new antivirals it is important to understand how this situation has arisen. No pharmaceutical company would wish to invest scientific endeavour to discover a drug which perhaps for logistical reasons could not be used to its full potential. Three contributory reasons for the under-use of the drug are: concern about the mild side reactions of jitteriness noticed by 7% of patients taking the drug; the problem of self-administration (the ideal situation for rapid use of the drug upon contact with an infected person) and the presumed absence of very dramatic clinical effects, such as prevention of mortality. In an event, which must be unique in the history of antimicrobials, two scientific meetings were convened to make suggestions about the clinical use of amantadine (Table 21.4). Both scientific panels recommended more extensive clinical use of amantadine and rimantadine. Amantadine, therefore, is a pioneering drug and the anticipation is that it and subsequent inhibitors might be more effectively used in near future.

The discovery and progression to clinical use of rimantadine, a drug closely related to amantadine, is not without relevance and interest to the future selection of new drugs. Firstly, rimantadine differs chemically from the parent molecule by the addition of a methyl group, but this change is sufficient to alter the pharmacological distribution of the drug without compromising effective antiviral action. Thus, rimantadine cannot penetrate the blood–brain barrier and so does not induce any neurological side effects sometimes noted with amantadine. Tissue and plasma levels are normally two-fold higher following oral

Table 21.4 Recommended usage of amantadine and rimantadine in prophylaxis and therapy*

1. There should be clear epidemiological and virological evidence of influenza A virus infection in the community
2. Amantadine or rimantadine should be used prophylactically each day for 6–8 weeks
3. Groups recommended for special consideration:†
   (a) High-risk persons, such as diabetics, the elderly and those with chronic heart, respiratory or circulatory disease, whether vaccinated or not
   (b) Special community groups such as public transport employees, doctors, nurses
   (c) Persons who may wish to be given prophylaxis or therapy
4. Drug prophylaxis should not replace vaccination because amantadine does not inhibit influenza B virus
5. Therapy should be initiated within 24 hours of first symptoms and given for 10 subsequent days

* These recommendations are an amalgam of those first published following the NIAID meeting (1980) and the Vienna WHO meeting [8]

† Amantadine or rimantadine cannot be used as a replacement for influenza vaccine in the elderly or those at special risk [14] because the drugs do not inhibit influenza B virus

administration of rimantadine (compared with amantadine) and thus dosage is reduced two-fold to 100 mg daily. The rimantadine compound was first discovered in the USA at the du Pont laboratories, but its apparent advantages in the clinic were first noted in the former USSR, where the drug has since been used extensively [38]. In the last few years scientific interest in rimantadine has been revived in the USA and the compound is now licensed there for clinical use. In spite of very large searches by different groups throughout the world no amantadine-like molecule with significantly improved antiviral efficacy has been discovered and, moreover, consensus opinion and also side-by-side comparison [20] would be that amantadine and rimantadine have a comparable clinical effect.

A potential advantage of rimantadine compared with amantadine is a lower risk of central nervous system effects such as light-headedness, difficulty in concentrating, nervousness and insomnia, which can be a problem in a minority of patients treated with amantadine. Dose-related, reversible gastrointestinal complaints such as nausea and vomiting are similar to those with amantadine. Since 200 mg/day doses can lead to high plasma levels of amantadine and an increased incidence of adverse effects in elderly nursing home residents, lowering the dosage to 100 mg/day may be advisable in such patients. Safety in pregnancy has not been established for either drug. There is increasing evidence that amantadine may be used as an adjunct to influenza vaccine in those persons at special risk.

Antiviral activity *in vitro*

In vitro sensitivity testing of influenza A virus subtypes demonstrates that most influenza A viruses are inhibited by amantadine or rimantadine. Earlier reported studies of the inhibitory activity of amantadine for influenza A viruses showed near universal inhibitory effects against all subtypes tested [59]. However, sensitivity testing has continued up to the present time and modern ELISA techniques have facilitated the testing of larger numbers of' isolates. In a very extensive study, Belshe *et al.* [9] analysed the susceptibility of current influenza A viruses to amantadine and rimantadine. Some 65 influenza A (H1N1) viruses and 181 influenza A (H3N2) viruses isolated in rhesus monkey kidney cell culture from patients between 1978–1988 and 25 strains between 1984–1985 were tested. All were susceptible to inhibition (see Table 21.5). All of the 65 influenza A (H1N1) viruses isolated between 1980–1987 were susceptible to inhibition by amantadine and rimantadine, including A/England/80, A/Victoria 83 and A/Taiwan/85 like viruses. Similarly, all 96 influenza A (H3N2) viruses, namely A/Taiwan/79, A/Oregon/80, A/Philippines/82 and A/Mississippi/85-like viruses, tested between 1980–1986 were inhibited by the drugs. In addition, others have tested field isolates directly for resistance, including the following strains: A/Shanghai/l 1/87 (H3N2) [41] and influenza A (H3N2) viruses circulating in the USA in 1985 (Table 21.5).

Five drug-resistant viruses (A/Sichuan/87) were detected among 85 tested in 1987 and 1988 and these were from patients participating in a rimantadine trial. Therefore, sporadic and very occasional influenza A viruses when tested *in vitro* may show a lesser degree of inhibition to amantadine. The great majority of influenza A (H1N1, H2N2 and H3N2) viruses tested to date have been well inhibited by amantadine or rimantadine.

Emergence of drug-resistant viruses

Soon after the discovery of the anti-influenza activity of amantadine, emergence *in vivo* of drug-resistant influenza A (H2N2) viruses which were also resistant to other amantadine-like molecules was noted [49,51]. Early work indicated that gene 7 (matrix) was responsible for conferring sensitivity or resis-

Table 21.5 Susceptibility to amantadine and rimantadine of influenza A viruses from 1978–1988

Virus, epidemic year	*No. susceptible to amantadine and rimantadine/No. tested*	*Antigenic characteristics of virus*
H1N1		
1978–79	2/2	Not tested
1980–81	7/7	England/80-like
1982–83	11/11	England/80-like
1983–84	5/5	Victoria/83-like
1986–87	40/40	Taiwan/85-like
Total	65/65	
H3N2		
1980–81	32/32	Taiwan/79-like
1982–83	37/37	Oregon/80-like*
1984–85	25/25	Philippines/82-like
1985–86	2/2	Mississippi/85/-like
1987–88	80/85†	Sichuan/87-like
Total	176/181†	

* Some isolates were Texas/77-like

† Five resistant viruses were from patients participating in rimantadine versus placebo study (data from [9])

tance to amantadine [27,40]. In a wider virological context the selection of drug-resistant viruses is expected and, indeed, acknowledged to confirm the specific antiviral activity of a drug, thus indicating that the compound is not inhibiting viral replication indirectly via an effect on the host cell. In all other virus–drug combinations including HIV and zidovudine [39, 44], herpes simplex viruses and Zovirax [50] and rhinoviruses [73], drug-resistant viruses can be detected but, to date, they usually have had little clinical impact.

Influenza viruses resistant to amantadine or rimantadine have been detected at low frequency in the field, even in countries such as Germany, which have not utilized amantadine-like drugs [33] and also have been specifically isolated from ill patients undergoing prophylactic or therapeutic treatment with rimantadine [29, 31, 32] or amantadine [17]. Drug-resistant viruses are detected more frequently during treatment than prophylaxis [29]. However, even in such treated patients the drug appears to continue to exert beneficial clinical effects [32]. Drug-resistant variants occur with a frequency of one particle among 10 000 virions in cell cultures but do not appear to have any selective advantages in nature because their recovery is so rare [29]. It is quite possible that natural selection of antigenic variants and the disappearance of previous variants might prevent the emergence of amantadine-resistant viruses with changes in the M2 and HA genes.

Mechanism of action

The block to virus replication mediated by amantadine is thought to occur after the virus has bound to the cell but before uncoating occurs [11,19,21]. In the presence of the drug, intact complexes of the ribonucleoproteins (RNPs) and the viral membrane protein (M_1) can be isolated from cells, but these cannot be found in the absence of the drug [11]. It has been assumed that M_1 protein is selectively removed from the RNP structure at acidic pH

(pH 5.5) in the endosomal compartment. However, as the M_1 protein resides inside the viral lipid bilayer, a mechanism, sensitive to amantadine, of making the interior of the virion accessible to a pH change is required. The M_1 protein is a peripheral membrane protein that associates with both the RNPs and the cytoplasmic face of the lipid bilayer. In contrast, the M_2 integral membrane protein (97 amino acids in length) is abundantly expressed at the plasma membrane of virus-infected cells but only a few (on average 23–60) molecules are incorporated into virus particles. The M_2 protein spans the lipid membrane once and is orientated such that it has a 23 amino acid N-terminal extracellular residue and a 54 residue C-terminal cytoplasmic domain; thus, M_2 is a model type III integral membrane protein. The native form of the M_2 protein is minimally a homotetramer, consisting of either a pair of disulphide-linked dimers or disulphide-linked tetrameters, the disulphide bonds acting to stabilize the oligomer.

A most interesting study by Pinto, Holsinger and Lamb [57] provided direct evidence that the influenza virus M_2 protein forms an ion channel connecting the two sides of the lipid membrane. Experimental injection of wild-type M_2 mRNA into oocytes produced an inward current that could be blocked by addition of the anti-viral drug amantadine. However, amantadine did not block the conductance of oocytes expressing M_2 proteins that contained mutations that confer viral resistance to amantadine. In the virus particle, M_2 may be an ion channel permitting the flow of protons from endosomes into the virion interior to facilitate removal of M_1 protein from RNPs during virion uncoating in endosomes.

Rimantadine benefits against drug-resistant viruses

The recovery of amantadine or rimantadine-resistant influenza A virus from drug-treated children has been documented, and this finding has been confirmed in adults (reviewed in [29]). The transmission of drug-resistant influenza A virus from treated patients to contacts receiving drug prophylaxis has been described in the household setting during rimantadine use [31] and in nursing home outbreaks managed with amantadine.

The findings of a study by Hayden *et al.* [32] show that drug-resistant influenza A virus can be recovered from children or adults treated with rimantadine. Resistant virus was recovered from samples taken from one-third of rimantadine-treated patients collected on the fifth day of treatment. In the adult treatment study, which incorporated frequent samplings for virus isolation, resistant virus was detected in three of six rimantadine recipients and shedding began as early as the second day of treatment. Rimantadine recipients from whom virus was recovered on day four or five of treatment were likely to shed resistant virus in both the family-based (80%) and adult treatment (100%) studies. The results of the adult treatment study [32] confirmed earlier observations in children that once shedding of resistant virus develops, subsequent viral isolates from the same patient are also drug resistant. However, the clinical importance of recovering drug-resistant virus from treated patients is still unresolved. In the adult treatment study, rimantadine administration was associated with significant reductions in virus titres in the upper respiratory tract, despite the recovery of resistant virus from some patients. Thus, the drug still appeared to be exerting an antiviral effect. Patients who remain virus-positive after several days of drug treatment appeared to be extremely likely to shed drug-resistant virus, and one of the patients in the Hayden trial continued to shed high titres of drug-resistant virus during therapy (2.5 $\log_{10}$ $TCID_{50}$/0.2 ml) on treatment day five. Of course the clinical concern is that rimantadine-treated individuals may transmit resistant virus to close contacts, and this problem has been considered in households during rimantadine use and in a nursing home during

amantadine use. In the household setting, avoiding treatment of ill index cases, specifically young children, would appear to reduce the likelihood of failure of drug prophylaxis as a result of the apparent transmission of resistant virus.

A sensible approach is to recommend that the presence or absence of drug-resistant viruses should continue to be monitored in clinical trials but to acknowledge that at present the problem at the clinical level is minimal and does not require changes in already published recommendations from the WHO consensus meeting [8] on the clinical use of amantadine or rimantadine. Essentially similar conclusions have been presented already in the UK [45], the USA [9, 43] and in the former USSR [38].

21.4.2 GENERAL RECOMMENDATIONS FOR THE CLINICAL USE OF AMANTADINE

A number of recommendations have been made from International and National Committees and Health Authorities regarding the clinical use of amantadine.

WHO consensus meeting (Vienna)

The committee recognized no significant difference in the degree of inhibition of human influenza A virus subtypes by amantadine [8]. It was concluded that the major deciding factor for the clinical use of amantadine was the prior isolation of influenza A virus (regardless of antigenic subtype) in the community. Under these circumstances prophylaxis of 6 weeks' duration or, alternatively, therapy of 10 days' duration, with amantadine was recommended.

Similarly, the NIH consensus meeting on the clinical use of amantadine recommended the drug's increased use (and now presumably also rimantadine) to combat influenza A infection in the community, not only particularly in the 'special risk' groups, but also in other groups in the community. No scientific discrimination was made between antigenic subtypes of influenza A virus as regards sensitivity to inhibition by amantadine or rimantadine and all influenza A strains of different subtypes were considered sensitive. The consensus opinion is:

1. Amantadine and rimantadine are effective inhibitors of naturally occurring influenza A virus of all subtypes.
2. The efficacy of amantadine or rimantadine is equivalent or exceeds that of the influenza A virus component of currently licensed inactivated influenza virus vaccines. However, the antivirals do not inhibit influenza B virus which can cause mortality in the 'special risk' groups.
3. The compounds could with benefit be used more widely to provide extra protection in already immunized 'at risk' persons or in non-immunized 'at risk' persons or in special community groups.
4. Drug-resistant variants have emerged but are not at present a clinical problem and since the beneficial clinical effects of amantadine and rimantadine are apparent the compounds should continue to be used as recommended.
5. Monitoring should continue for drug-resistant variants.

Amantadine or rimantadine use in an influenza pandemic

In the event of a pandemic of influenza it is recognized that the Public Health Laboratory Service (PHLS) and other major laboratories will play a major role in isolating and identifying the new virus in the UK, monitoring its spread and initiating studies on vaccines and antivirals. The PHLS 'pandemic report' recognizes six phases in the emergence of a pandemic:

- phase 0 interpandemic period
- phase 1 emergence of a pandemic virus, presumably outside of the UK

- phase 2 epidemic or pandemic caused by the virus outside the UK
- phase 3 new virus isolated in UK – pandemic imminent
- phase 4 pandemic influenza in UK
- phase 5 return to 'background' influenza activity

The report specifically identifies amantadine, or rimantadine, as the compound to test in high-risk populations in phase 3. At present, amantadine is the only antiviral compound licensed for use against influenza in the UK. The report also recognizes the usefulness of assessing the degree of inhibition that amantadine exerts on a new influenza A virus during phase 2 before the new virus has arrived in the UK and caused clinical outbreaks. This is consistent with present policies of continued and voluntary monitoring of influenza A virus strains for sensitivity to inhibition by amantadine.

A minimal period of 36 weeks elapses from the isolation of a new influenza virus to the time of production of the first batches of inactivated vaccine. Therefore, it is apparent that a vaccine would not be available for high-risk individuals during the important first wave of the pandemic. Chemoprophylaxis with amantadine or rimantadine would have an important role to play both during the initial outbreak and in the succeeding waves.

Possible amantadine use for the first wave of an influenza pandemic

The EC licensing of amantadine and rimantadine for use against 'all influenza A viruses' as in the USA would permit rapid deployment of the drug in 'special risk' groups during the potentially dangerous first wave of the pandemic when no vaccine would be available. It is generally acknowledged that amantadine and rimantadine would have an important role to play during the evolution of a virus pandemic. It is also recognized [43] that the potential clinical benefits of the drug were compromised by previous restrictive licensing in the USA. Although *in vivo* studies had shown that all influenza A viruses of different subtypes were inhibited by amantadine the drug had, nevertheless, been licensed in 1966 in the USA for use against type A (H2N2) strains only. Re-licensing for use against the newly emerged pandemic influenza A subtype H3N2 in 1968 was not accomplished until 1976 when amantadine was approved in the USA for prophylactic and therapeutic use against *all* type A influenza viruses. These factors restricted the use of the drug during the important influenza A (H3N2) pandemic in 1968, but the change in licensing, was accomplished in time for the drug to be used effectively against the newly emerged influenza A (H1N1) virus in 1977.

Will amantadine and rimantadine inhibit new pandemic strains of influenza A virus?

There are two conflicting, and as yet unresolved, hypotheses of the origin of new pandemic influenza A strains. Firstly, a recycling theory whereby a virus re-emerges from elderly persons who many years previously have been infected with other subtypes [37] or, secondly, a theory of the emergence from 'mixing bowl' situations in pigs of new reassortant viruses with virulence for humans (reviewed in [66]). The latter hypothesis has most current scientific support, although the recycling theory is not discounted. In either case, however, it is apparent that a pandemic strain may not be 'new' as such but will have an HA or NA, either from an old human strain (e.g. H2) or from an existing avian or animal virus (H4–H13). In either case, evidence at present would suggest potential susceptibility of all subtypes of influenza A virus to inhibition by amantadine [59]. Recent studies in our laboratory have confirmed the *in vitro* inhibition of influenza A viruses of the majority of the avian subtypes (H1–H13) (J.S. Oxford and M. Wigg, unpublished data).

As noted above, the mode of action of amantadine would appear to be closely related to the M_2 protein of influenza A virus. Of the 120 isolates of amantadine or rimantadine-resistant influenza A viruses that have been characterized genetically so far, all have changes restricted to amino acids 27, 30, 31 or 34 in the M_2 protein [9,29]. With a change in antigenic subtype and the advent of a pandemic virus, the HA and NA proteins are completely different whereas other gene products including M_2 (coded by gene 7) may or may not remain unchanged.

The M_2 protein appears to be highly conserved among influenza A virus strains of all known subtypes (reviewed in [66]) and this would seem to provide the scientific basis of their universal susceptibility to amantadine. Therefore, it could be hypothesized that the emergence of a 'new' pandemic strain with completely novel HA and NA proteins for the human population would be expected to have few or no implications as regards susceptibility to amantadine, which at the low dosages used in human administration would be dependent on the amino acid sequence of the much less variable M_2 protein.

21.4.3 'DESIGNED' ANTI-NEURAMINIDASE DRUGS

Eleven years ago a group of scientists in Melbourne published the first data on the atomic X-ray structure of influenza A virus neuraminidase (reviewed in [66]). The latter enzyme is not virus-specific *per se* and similar enzymes are widespread in the animal kingdom. These enzymes are glycohydrolases which cleave terminal sialic acid from glycoproteins or glycolipids or oligosaccharides. In virological 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. Palese and Schulman (reviewed in [48]) tested a series of these compounds as anti-influenza virus drugs 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 compounds tested in this early work, and now with newly synthesized, more effective analogues which can be applied topically, a specific anti-influenza virus effect can be measured *in vivo*. The new team of chemists, X-ray crystallographers and virologists have, in their own words, 'rationally designed a series of sialidase-based inhibitors of influenza virus' [72]. Firstly though it is advisable to review the basic structure of the neuraminidase molecule itself.

Influenza sialidase is a tetramer of identical subunits. There are six four-stranded, anti-parallel B-sheets arranged as if on the blades of a propeller (reviewed in [66]). 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 characterized. 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 antibodies.

Sialic acid, the product of the enzyme-catalysed reaction, binds to the active site of the enzymes in a 'boat' configuration. The carboxylate interacts with three invariant arginine residues on the neuraminidase enzyme. In this conformation it resembles the geometry of the unsaturated sialic acid analogue (Neu5Ac2en), a potent sialidase inhibitor, as noted above. An important aspect of the active site is that strain invariance extends to a number of other amino acids which do not themselves make contact with sialic acid but which provide substructure or a scaffold on which

amino acids contacting the bound sugar are supported.

The Melbourne group made predictions of energetically favourable substitutions to the unsaturated sialic acid analogue Neu5Ac2en [72]. Among the most interesting of these chemical modifications was the replacement of the hydroxyl group at the four position on Neu5Ac2en by an amino group. In theory, 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 Glu119 in the enzyme. Similarly, the replacement of the same hydroxyl group 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 guanidino group with both Glu119 and Glu227. As predicted, direct measurements of viral neuraminidase enzyme inhibition showed that the 4-amino and 4-guanidino-substituted Neu5Ac2en (Figure 21.2) were 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* (Figure 21.3). Thus, inhibition of influenza virus replication was noted in both mice and ferrets, with both 4-amino and 4-guanidino-Neu5Ac2en inhibitor and, to a lesser extent, with Neu5Ac2en itself. The most comprehensive data have been obtained with 4-guanidino-Neu5Ac2en in mice infected with influenza A/Singapore/1/57 virus, comparison being made with amantadine and ribavirin used as positive control compounds.

Results obtained with 4-guanidino-Neu5Ac2en administered both before and during infection of ferrets at a dose of 50 μg/kg twice daily (b.d.) are shown in Figure 21.3. In this experimental system, virus shedding is observed typically in nasal washes from 3 to 5 days after inoculation, and an elevated body temperature recorded on days 2–3 after inoculation. At 50 μg/kg (b.d.) 4-guanidino-Neu5Ac2en effectively abolished virus shedding and reduced pyrexia fully in all of five animals. 4-Guanidino-Neu5Ac2en was approximately 1000 times more effective than amantadine when given intranasally to either species. The compound has now reached phase II and III clinical trials in various countries and has been shown to have prophylactic activity in students infected with an influenza A (H1N1) virus.

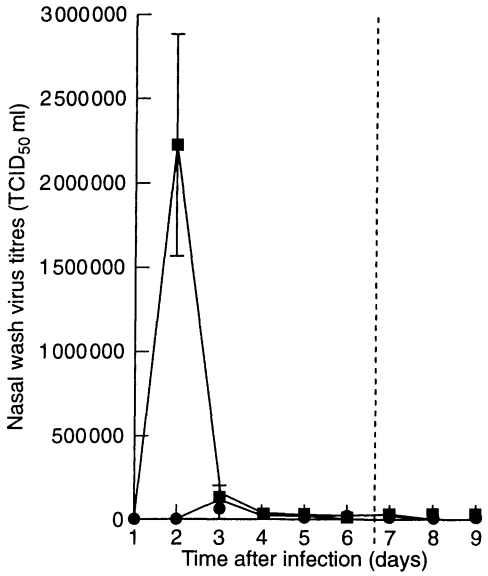

Figure 21.3 Treatment of influenza-infected ferrets with 4-guanidino-Neu5Ac2en. Influenza virus titres were measured in nasal washings from ferrets treated with 4-guanidino-Neu5Ac2en (treated, •) or distilled water (controls, ■). Ferrets were infected intranasally with 10^{50} $TCID^{50}$ influenzaA/Mississippi/1/85. Treated and control groups (n=5) was treated twice daily (8 hours apart) from the day before infection to day 5 after infection. On the day of infection the first treatment dose was given 2 hours before infection (from [72]).

21.5 RESPIRATORY SYNCYTIAL (RS) VIRUS

21.5.1 INHIBITION BY RIBAVIRIN

Just as amantadine acted as a novel pioneer drug for a respiratory virus, namely influenza

A virus, ribavirin was also pioneered as a nucleoside analogue for possible long-term prophylaxis and therapy against RS virus and influenza virus (reviewed in [71]). When the compound was first discovered, only relatively toxic nucleoside analogues, such as iododeoxyuridine and adenine arabinoside, had been used for antiviral chemotherapy of serious herpesvirus infections. Now, 15 years later and with the additional extensive clinical experience of using the antiviral nucleoside analogue, acyclovir, it is generally acknowledged that not all analogues of naturally occurring purines or pyrimidines are inherently toxic for replicating mammalian cells.

Nevertheless, because of the relatively low therapeutic margin, ribavirin has been more extensively tested in aerosol form than other antivirals. As seen with the novel influenza anti-neuraminidase inhibitor above, aerosol administration has the theoretical advantage of direct deposition of the drug onto the tissues of the upper and lower respiratory tract (reviewed in [7]) without compromising the function of other organs where the presence of the drug is, in any case, not required for antiviral action. A potential disadvantage of aerosol use is the complicated equipment required, particularly for use of ribavirin in infants suffering from RS virus infection. Nevertheless, ribavirin is the only drug to have shown effectiveness in the clinic against potentially serious RS virus infections, although other compounds may be effective *in vitro* or in animal models. Pioneering studies were carried out by Hall *et al.* (reviewed in [48]) in controlled clinical trials, firstly in adults and then in children. The studies involved normal infants who were hospitalized with pneumonia or bronchiolitis but were otherwise normal, or infants with more serious underlying conditions, placing them at high risk from serious RS virus infection. Infants receiving ribavirin showed a significantly greater amelioration of their illness than those in the placebo group and improvement on the first day of therapy as well as on subsequent dates. The results of these important studies show that antiviral therapy of RS virus is possible and gave encouragement and impetus in the search for other inhibitors of this otherwise uncontrolled virus infection, including interferons [34]. More recently, Fleming and Cross [22] have provided evidence of RS virus causing life-threatening infections in the elderly and ribavirin may have a clinical role to play in this group.

21.6 RHINOVIRUSES

21.6.1 ANTIVIRAL DRUGS INTERACTING WITH VIRUS STRUCTURAL PROTEINS

It might be expected that viruses are most vulnerable to interception when in the extracellular or transmission phase of their life cycle. Indeed, it is precisely at this point that many vaccines exert their effects via the induction of neutralizing antibody. However surprisingly, few antiviral compounds are known to inhibit or inactivate extracellular virus. Bile salts have been investigated recently [46] as a means of inactivating lipid-enveloped viruses. Some activity against influenza A virus infection was detected in an animal model system, but the main impetus of the research was towards direct inactivation of HIV. However, virologists interested in chemotherapy of rhinoviruses have discovered some remarkable molecules which bind to these viruses. A good example of molecules acting at this stage is the series of drugs which bind to the external VP1 coat protein of picornaviruses; typical examples are the arildone series of antiviral inhibitors dichloroflavan and chalcone (reviewed in [18,68]). These compounds are considered to *stabilize* the virus and so prevent the uncoating stage whereby the virus releases its nucleic acid intracellularly. In an extremely innovative study of the interaction of a virus with an antiviral (the first to be carried out at the atomic level using X-ray diffraction studies) Smith *et al.* [64] described the binding of WIN 51711 (Disoxaril) within a small canyon

at the base of the receptor-binding pocket of rhinovirus type 14. It is hypothesized that the antiviral molecule blocks the entry, into the inner part of the virion, of ions which would normally participate in the process of uncoating of the rhinovirus RNA soon after the virion has entered the cell. Similarly, the cell receptor-binding site on influenza HA has been identified following X-ray crystallography studies of the protein; it is situated as a shallow saucer-like indentation at the tip of the molecule and in close proximity to the important antigenic site B. The precise configuration and spatial relationships of amino acids constituting the sides and floor of the site are known but to date this knowledge has not been exploited to develop antiviral inhibitors of influenza virus.

It is calculated that 72 molecules of WIN 51711 bind per rhinovirus virion. Fortunately, the electron density of WIN 51711 was unequivocal with the oxazoline and phenoxy groups appearing as two flattened bulges separated from the bulge of the iso-oxazole group. The oxazoline phenoxy (OP) groups were located in the hydrophilic region at an opening on the floor of the canyon of the receptor-binding site on the rhinovirus particle. The OP end may orientate by hydrogen bonding between the nitrogen in the oxazoline ring and a hydrogen of Asn219 amide group in VP1. The methyl iso-oxazole group inserts itself into the hydrophobic interior of the VP1 barrel. In this case, as mentioned above, the compound could act by blocking the entrance of ions into the channel which normally acts as a conduit to the inside of the virion. An interesting analysis was performed to determine the minimum inhibitory concentration (MIC) of WIN 51711 for two human rhinoviruses, namely HRV-2 and HRV-14. Also, the amino acid composition of their receptor-binding pockets was compared with those of other picornaviruses including meningovirus, and foot and mouth disease virus of cattle (FMDV) which are not inhibited by WIN 51711. A possible explanation for this latter negative observation is the replacement of Asn219 in the WIN 51711-insensitive viruses by serine or glycine which does not allow correct orientation of the oxazoline group of the drug. In HRV-2, Val188 and Val191 have both been changed to leucine which would sterically hinder insertion of the isoxazole groups and aliphatic chain into the hydrophobic pocket. Concomitantly, the MIC value of WIN 51711 for HRV-2 increased compared with that for HRV-14. This classic study is now taken as an example of a future direction required for the selection of antiviral drugs. Nevertheless, the virological and pharmacological aspects and correlation are still very important since, for example, the X-ray study could not give an indication of whether a compound selected for very tight binding to the canyon would penetrate the cell plasma membrane to reach the intracellular virus or would be absorbed after oral administration and reach the tissues of the upper respiratory tract.

A compound known as SCH 38057 (I [6-(2-chloro-4-methoxy)-hexyl]imidazole hydrochloride) is a water-soluble antiviral agent that binds irreversibly to enterovirus and rhinovirus particles [74] and has also been subjected to X-ray crystallographic analysis. The structure of rhinovirus 14 complexed with SCH 38057 shows that this compound binds at the innermost end of the hydrophobic pocket of VP1, leaving the entrance unoccupied. This interaction induces conformational changes involving a stretch of at least 36 amino acids of VP1 and VP3.

A number of flavan derivatives have been synthesized and assayed against rhinovirus type 1B and 4,6-dichloroflavan blocked the replication of this rhinovirus in cultured cells. Clinical trials carried out at the CCU with the compound given orally or intranasally, failed to show antiviral effects. However, the compound could not be found in nasal secretions [56] which may provide a simple explanation for the failure.

The Roche Group have synthesized Ro 09-0410 (4'-ethoxy-2'-hydroxy-4,6'dimethoxy-chalcone), a compound active against rhinoviruses and which, like dichloroflavan, SCH 38057 and WIN 51711, binds to the viral capsid. Radioactively labelled compound binds specifically to rhinovirus particles and this binding is inhibited by dichloroflavan or RMI15,731, suggesting a similar mode of binding. Of 53 rhinovirus serotypes tested, 46 were inhibited by Ro 09-0410. Mutant rhinoviruses resistant to chalcone were also cross-resistant to dichloroflavan [73]. These results indicate that the three compounds had a similar mode of action based on their binding to virion particles. Several analogues of Ro-09-0410 have been synthesized, some of them being ten times more active than the parent compound. These analogues bind to the same site on the virus particles as Ro 09-0410 and have a similar mode of action [55]; however, the parent compound was not active clinically [2].

Finally, enviroxime (2-amino-1-(isopropyl-sulphonyl)-6-benzimidazole phenyl ketone oxime) is a benzimidazole derivative with high activity against rhinoviruses inhibiting RNA replication. Several human trials using enviroxime in an attempt to prevent colds induced by rhinoviruses have been conducted, but without any clear clinical benefit being observed [28,53].

21.6.2 VIRION-BINDING DRUGS ACTING AGAINST RHINOVIRUS INFECTIONS

Although the results of using dichloroflavan [3,56], Ro 09-0410 [2] and 44,081 RP [75] and other molecules in this group of virion-binding drugs clinically have been negative, recent trials with the drug R61837 have provided some striking evidence of efficacy. It is a truism that one of the major problems in the treatment of common colds is to attain drug levels in the nasal epithelium that are sufficiently high to block viral growth. When R61837 was given to volunteers as a nasal spray before they were inoculated with rhinovirus 9, the compound showed clinical efficacy, but failed to show effects when used therapeutically [1,6]. A significant factor may have been the use of a new vehicle which produced a soluble drug administered by intranasal spray. Indeed, the vital importance of the administration route of drugs with problems of solubility has been noted above with the new influenza anti-neuraminidase compound.

21.7 CONCLUSIONS

A series of crucial studies at the MRC Common Cold Unit signposted developments in virology and antiviral chemotherapy of the respiratory viruses over the past four decades. The discovery of methods for cultivating rhinoviruses and the use of organ cultures for growing coronaviruses have been key technical developments at the Unit and both discoveries resulted from keen attention to technical detail in the laboratory. The first demonstration of the clinical activity of interferon [42] and most recently the description of clear clinical effects of an anti-rhinovirus drug [I] have also shown the importance of extremely close monitoring of the clinical parameters of viral respiratory disease. At the heart of the Unit at Salisbury was the concept of the interaction of laboratory science and clinical medicine. Now, for the first time, the techniques of the molecular biologist (particularly X-ray crystallography) are being focused on the redesign or sensible alteration of existing antivirals and the last few years have witnessed the publication of some landmark papers [64,74]. The future will hold the design of an antiviral *de novo.* Concomitant with the latest discoveries in chemistry, there is now a requirement for the construction of a European facility for the clinical evaluation of these and hopefully the succession of new molecules over the next years. Antiviral chemotherapists certainly do not believe that these small molecules can be used to *eradicate* viral diseases. Compared with

those of vaccinologists, the goals are smaller but nevertheless well defined, namely to search for and to discover small molecules that will interrupt the replication of respiratory and other viruses and hence alleviate human suffering. Viruses, and in particular the two great pandemic RNA genome viruses, influenza A and HIV, are continually evolving, and it must be appreciated that they pose a tremendous threat, ever capricious and unpredictable in nature to our well-being.

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