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Clinical and Epidemiological Importance of Influenza A Viruses Resistant to Amantadine and Rimantadine

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INTRODUCTION

During more than two decades of amantadine and rimantadine use, naturally occurring strains of influenza A viruses have remained sensitive to the antiviral action of these drugs.[1,2] However, drug-resistant variants can be readily selected by growing virus in the presence of either drug in cell culture or in animals.[3–9] Drug-resistance is also transferable by genetic reassortment where RNA segment 7 coding (M gene) for the M1 (membrane) and M2 matrix proteins is transferred from resistant to sensitive influenza viruses during dual infections.[6–9] Amantadine-resistant strains are cross-resistant to rimantadine,[1,2,9] an observation consistent with their shared mechanism of anti-influenza action (see reviews[10–12]). These findings are explained by the central role of the M2 protein in mediating susceptibility of human influenza A viruses to these drugs. RNA sequencing of many resistant influenza A viruses has identified the genetic basis of resistance as single nucleotide changes leading to single amino acid substitutions in the membrane-spanning portion of M2 (see reviews[10–13]).

Not unexpectedly, isolations of drug-resistant virus have been recently documented in certain clinical situations involving the use of rimantadine or amantadine. However, the development of drug-resistant virus presents a medical problem, only when it interferes with the clinical usefulness of the drugs or is associated with greater transmissibility or severity of infection. This paper will expand on earlier reviews[13,14] of published information regarding the characteristics of drug-resistant influenza viruses and comment on the potential clinical and epidemiological importance of resistant variants, on possible strategies to limit their impact, and on areas for future investigation.

EXPERIMENTAL AVIAN INFLUENZA

Considerable work relating to drug-resistance has been performed with experimental avian influenza induced by infection with one of the avian influenza viruses. The model described by Webster and co-workers[15–17] utilised intranasal inoculation of a virulent influenza A/chicken/Penn/83(H5N2) virus that caused lethal infection in most untreated birds. Infections with this virus differ in pathogenesis and severity from human influenza infections, in that the virulent avian strains replicate to high titres in the gastrointestinal tract of infected birds and spread to multiple internal organs. The conditions of the experiments, analogous to those of flocks with numerous birds housed in close contact, were optimal for the efficient transmission of virus among birds. Importantly, the mechanism of drug resistance that developed in avian strains appeared to be similar to that found in human isolates; resistance was conferred by amino acid substitutions at critical positions of the M2 protein.[16]

Prophylaxis

Initial studies showed that prophylactic administration of high doses of amantadine or rimantadine in drinking water prevented infection and illness in birds challenged with virus.[15] Because such birds did not develop protective immune responses, they remained susceptible to later infection by the same strain. The extent of protection from the drug was related to both the virus inoculum and the drug dose, in that a 10-fold higher inoculum resulted in infection and death and a 5-fold lower drug dose usually resulted in a nonlethal illness. Whether sublethally infected birds shed resistant virus was not assessed in these studies. However, in one experiment where drug treatment was started at the time of virus challenge, early onset illness was prevented, but mortality occurred during the second and third weeks after infection in nearly 40% of birds given amantadine.[17] Drug-resistant virus was recovered from one bird, so it is possible that this occurrence represented both acquisition of resistant virus and failure of host immune responses in the affected birds.[17]

Therapy

Therapeutic administration of amantadine *up to 3 days* after viral inoculation resulted in reduced mortality compared with no treatment.[15–17] However, treated birds shed high titres of virus in their faeces for up to 10 days.

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Drug-resistant variants were detectable in tracheal samples as early as the second day of treatment and were consistently present in the faeces by the third day of treatment.[16] Although resistant virus continued to be shed throughout the duration of therapy, the treated birds recovered from their illnesses, contrasting with the high mortality observed in untreated birds. Thus, the drug remained therapeutically effective in birds despite the emergence of drug-resistant virus during therapy, perhaps because the treatment delayed progression of the infections and permitted host immune responses to develop and control the extent of infection. In contrast, both murine[5] and avian[16,17] model studies have found that amantadine administration is ineffective in modifying disease in animals infected initially with drug-resistant virus.

Transmission

Under conditions simulating natural transmission in flocks, healthy birds receiving amantadine prophylaxis and remaining in close contact with amantadine-treated, infected birds developed infections due to drug-resistant virus. These infections caused severe illness and mortality, although at lower rates than the 100% mortality rate in untreated contacts of untreated birds infected with the parental virus.[15] Similar results were reported with rimantadine.[15] In later studies with amantadine, drug-resistant virus was recovered from all contact birds receiving prophylaxis.[16]

To determine the ability of resistant variants to compete with the drug-sensitive parental strain, drug administration was stopped after 4 days and the birds shedding resistant virus were mixed with birds shedding wild-type virus and with uninfected contact birds.[16] The contact birds were removed after 4 days and exposed to another group of uninfected contacts. After this process was repeated for a total of four passages, resistant virus could still be recovered from some or all of the previously uninfected contacts in the absence of selective drug pressure in the majority of experiments. Thus, resistant avian influenza virus showed no reversion to wild-type after multiple passages extending over 20 days.

Pathogenicity

The virulence of resistant influenza viruses was assessed by direct inoculation of passaged sensitive or resistant strains into untreated birds. All passages of resistant or sensitive strains caused illness in infected birds. Mortality rates were variable (17–83%) for both passaged resistant and sensitive strains compared with the parental virus, but some resistant strains were fully virulent.[16] In addition, resistant strains caused similar mortality rates in infected birds whether given amantadine prophylaxis or no treatment.

Implications

In summary, amantadine and rimantadine were effective for prophylaxis and treatment in this model of lethal influenza A/H5N2 infection, but drug-resistant strains emerged rapidly during treatment, were transmissible to contacts, were genetically stable and capable of competing with wild-type virus for transmission in the absence of selective drug pressure, and remained virulent.[16]

Beard *et al.*[17] have suggested that a possible strategy for using these drugs in valuable flocks would be prophylactic administration for the duration of an outbreak without treatment of infected flocks. However, the results to date and the knowledge that avian influenza viruses rarely may be the source of new genes for human strains and that their gene segments coding for the internal proteins will permit growth in humans[17a] would argue against extensive veterinary use of amantadine or rimantadine in attempts to control outbreaks of avian influenza. Similarly, although no direct data exist in regard to swine influenza strains, the occasional transmissions of H1N1 subtype viruses from swine to humans would caution against use of these drugs for outbreak control in swine.

HUMAN INFLUENZA

Susceptibility testing of pandemic strains and multiple epidemic isolates has not found evidence of drug resistance in viruses recovered from untreated patients.[1,2] Although the degree of *in vitro* sensitivity varies with the assay method and the virus strain, the reported resistance of several incompletely characterised isolates[18,19] has not been substantiated by RNA sequencing. In an extensive study of isolates recovered during one decade of community surveillance, Belshe *et al.*[2] found that 100% of 65 H1N1 subtype and 97% of 181 H3N2 subtype influenza A viruses were drug-sensitive. The five resistant isolates were recovered from three members of one family treated with rimantadine during a clinical trial to be described below.

The failure to document naturally occurring drug-resistant strains has led to the speculation that resistant viruses may be at some biological disadvantage in the absence of selective drug pressure.[9,11] However, no disadvantage has thus far been identified. The exact functions of the M2 protein and the mechanisms of action of these drugs are not fully defined (see reviews[10–12]), and the effect of the single amino acid changes found in the M2 protein of resistant viruses on properties of the virus are not clear.

Prophylaxis

Seasonal prophylaxis with either amantadine or rimantadine is highly efficacious in reducing the risk of influenza A virus-related illness (see reviews[20–22, 22a]). However, some recipients develop illness, while more experience subclinical infections during prophylaxis. It is currently unknown whether some of these persons develop infections due to drug-resistant virus. Resistant virus has been recovered during amantadine prophylaxis following experimental challenge with a high titred viral inoculum of influenza A/H3N2.[23] In this trial six of 19 (32%) tested subjects had resistant virus recovered following challenge (F. Hayden and G. Schiff, unpublished observations). However, virus isolates from relatively small numbers of long-term failures of prophylaxis in a previously described outpatient study in Vermont[24] and in elderly residents of nursing homes in Rochester, New York[20] have been drug sensitive (R. Dolin and R. Betts, unpublished observations). It is unclear whether the failures of prophylaxis in the presence of

sensitive viruses could have related to poor compliance or inadequate drug levels. Because of concerns regarding drug side effects, clinical trials have been conducted to establish the prophylactic efficacy of lower doses of amantadine[23,25] and rimantadine[26] (100 mg per day). Whether such reduced doses will influence the likelihood of resistant virus emerging in populations during prophylaxis is unclear.

Therapy

Drug-resistant viruses can emerge during treatment with rimantadine in both children and adults.[9,27–30] In two paediatric studies comparing 5 days of rimantadine to acetaminophen (paracetamol) therapy, significant reductions in the fraction of children shedding virus occurred early but not later during rimantadine therapy.[27,28] One study of children infected with the H3N2 subtype found that approximately one-half of rimantadine recipients remained positive for virus following cessation of therapy and that the duration of shedding averaged one day longer in the rimantadine group.[27] In this study resistant virus was recovered from 27% of all rimantadine recipients, generally beginning on days 4–6 after starting treatment, and from 46% of those shedding virus late in therapy. A family-based study found that drug-resistant A/H3N2 virus could be recovered during therapy from approximately 30% of rimantadine-treated children or adults.[29] Of ten rimantadine recipients who shed virus on the fifth treatment day, 80% yielded drug-resistant virus. A recent study of young adults with uncomplicated influenza A/H3N2 illness found that resistant virus was recovered from three of six rimantadine recipients by the third day of therapy.[30] Finally, in a study of elderly nursing home residents treated with rimantadine, resistant virus was recovered from 11% of 26 overall and from 27% of those who shed virus for 4 days or longer (R. Betts, unpublished observations).

The studies cited have generally found that once shedding of resistant virus develops, subsequent isolates are also resistant. The limited data available suggest that the duration of shedding resistant virus extends for as long as 7 days, but usually less than 10 days, from the initiation of treatment.[27,30]

The possible clinical significance of shedding drug-resistant virus during treatment is not fully resolved. In the paediatric study by Hall *et al.*,[27] the rimantadine group experienced significantly greater reductions in fever and severity of illness during the first 3 days of treatment compared with acetaminophen. The illness severity scores later in therapy tended to be higher in rimantadine-treated children who shed resistant virus compared with those who did not, but the differences were felt to be too small to be clinically detectable.[27] A family-based study found that the rimantadine-treated patients, the majority of whom were children or teenagers, also had significant reductions in duration of fever and illness compared with those receiving placebo.[30] However, the subgroup of rimantadine recipients shedding resistant virus appeared to have somewhat slower resolution of illness than rimantadine recipients who did not shed resistant virus. No worsening of illness was observed in association with recovery of resistant virus, and the overall duration of illness and fever tended to be approximately one day less in the resistant subgroup compared with placebo. Importantly, no differences in illness severity or demographic characteristics were evident at the time of initiating treatment, so that it was not possible to predict which rimantadine-treated patients would shed resistant virus. In elderly subjects treated with rimantadine for acute influenza, no differences in the clinical course were apparent between those shedding resistant virus and those who did not (R. Betts, unpublished observations). Based on an open-label family study, Kubar *et al.*[31] also concluded that the resolution of illness was independent of whether sensitive or resistant virus was recovered from ill index cases during rimantadine treatment. Whether treated immunocompromised patients develop progressive disease due to emergence of drug-resistant virus is currently unknown.

Thus, it is unclear whether a causal relationship exists between recovery of resistant virus and prolongation of illness. Recovery of resistant virus could be more likely in patients who experience more prolonged or quantitatively greater viral replication for reasons other than the development of resistance. Unfortunately, no demographic characteristics have been identified that would allow clinicians to determine who is at increased likelihood of shedding resistant virus during therapy.[30] Also, none of the available evidence suggests that illness related to drug-resistant virus is more severe than that due to drug-sensitive virus, and several studies have found that rimantadine retains a net therapeutic benefit compared with placebo despite recovery of resistant virus from a portion of treated patients.[27,30] This conclusion is also supported by the observations described earlier for experimental avian influenza.

Transmission

Several family-based studies have assessed whether amantadine or rimantadine given as post-exposure prophylaxis for 10 days would prevent development of influenza A infection and illness in household contacts exposed to ill family members.[29,31–33] In a multi-centre trial in the United States during an influenza A/H3N2 epidemic in 1987–8, all the eligible members in a family, including the ill index cases and healthy contacts, were assigned as a block to receive either rimantadine or placebo.[29] This study found similar rates of secondary illnesses and infections in the contacts receiving rimantadine or placebo, and the calculated efficacy of post-exposure prophylaxis was only 3% for proven influenza A illness. Secondary illnesses apparently due to transmission of drug-resistant virus were found in five of 27 rimantadine-treated families with an influenza A virus introduction, and in four of these five households the implicated index case was a young child below the age of 6 years.

In contrast, a study in France during an influenza A/H1N1 epidemic in 1988–9 found 69% protective efficacy against clinical influenza in household contacts receiving rimantadine, when the index cases were not given concurrent therapy.[32] An earlier study during an influenza A/H2N2 epidemic in 1967–8, in which index cases were not treated, found that amantadine was also effective for post-exposure prophylaxis of documented influenza A illness with an

efficacy of 100%.[33] In contrast, another study[34] by the same investigators during the following year's influenza A/Hong Kong/H3N2 pandemic, in which the index cases were treated, found that amantadine prophylaxis did not significantly reduce the risk of influenza A illness in the household contacts (23% efficacy). Although viral isolates were not available for susceptibility testing from the later three studies, the differing results may relate to the possibility that the treated index cases could have transmitted resistant virus in the unsuccessful trials. However, the effect of treatment of index cases on post-exposure prophylactic efficacy has not been directly assessed in trials involving random assignment of index cases and separate random assignment of contacts to receive drug or placebo.

Transmission of resistant influenza viruses has also been implicated by the recovery of resistant virus from prophylaxis failures in nursing home outbreaks in which amantadine was used for both prophylaxis and treatment.[35,36,36a] In one outbreak, Mast *et al.*[35] documented the development of influenza over a 5 day period in three patients receiving prophylaxis who were residing in contiguous rooms; each of the three prophylaxis failures had an infection with a resistant virus having a relatively uncommon amino acid substitution at position 27 (see reviews[10,12]). Breakthrough illness associated with recovery of resistant virus has been documented as late as the second week after initiating prophylaxis. In another recent nursing home outbreak,[36a] amantadine treatment without case isolation was associated with three late prophylaxis failures, including one death, due to resistant virus. Thus, selection and transmission of drug-resistant influenza virus appears to occur in certain epidemiological situations, in which close contact occurs between treated ill persons and those receiving prophylaxis.

Pathogenicity

Patients who have developed influenza associated with the recovery of resistant virus despite receiving drug prophylaxis have generally had typical influenzal illness, including fever and functional impairment.[2,29] Such prophylaxis failures have been recognised with viral isolates containing five different M2 protein substitutions (see review[13]), although the limited data available do not allow for determination of whether a particular M2 mutation is associated with lower virulence or transmissibility. No direct inoculation studies have been done in humans with resistant isolates. However, when human influenza A/H3N2 viruses with one of three different M2 changes were inoculated intranasally into ferrets, the resistant variants replicated and induced febrile and nasal inflammatory responses similar to the corresponding drug-sensitive, parental isolates.[37]

EPIDEMIOLOGICAL AND CLINICAL IMPLICATIONS OF DRUG RESISTANCE

The findings of recent clinical trials indicate that drug-resistant strains of influenza A virus can develop in persons treated with rimantadine or amantadine and that these strains are transmissible and capable of causing disease in contacts receiving the drug. No obvious difference in viral pathogenicity has been found for drug-resistant human or avian influenza viruses to date; moreover, no other biological differences between sensitive and resistant viruses have been recognised. These viruses appear to possess the biological properties that are associated with clinically important influenza viruses (see Table 1).

Table 1. Biological characteristics of drug-resistant influenza viruses.[a]

Feature	Avian influenza	Human influenza
Rapid emergence	+	+
Genetic stability	+	+[b]
Transmissibility	+	+
Competition with wild-type	+	?
Pathogenicity	+	+

[a]Adapted from Bean *et al.*[16]
[b]Based on *in vitro* passage.

Epidemiological significance

The impact of drug-resistant influenza viruses is likely to depend on a number of factors including the specific epidemiological situation, the nature of the epidemic strain, the extent of selective drug pressure, and the transmissibility of resistant variants relative to wild-type strains. For example, most of the clinical observations regarding drug resistance have come from studies of H3N2 subtype infections. Perhaps, the higher levels of replication observed during infection with such subtypes relative to H1N1 subtypes[28] may increase the likelihood of selecting resistant variants.

Others have pointed out that the epidemiology of influenza infections, which includes immune selection of antigenic variants and disappearance of previously circulating strains, will reduce the likelihood of resistant epidemic strains emerging.[9–12] The influenza A viruses cause global epidemics (pandemics) when viruses with new antigenically distinct haemaglutinin (H) and neuraminidase (N) surface proteins arise.[38] The pandemic of 'swine' influenza in 1918, Asian influenza in 1957, and Hong Kong influenza in 1968 are recent instances of this occurrence. Currently available evidence indicates that these new viruses arise from a reassortment of gene segments between a human and an animal strain whereby the distinctive animal H and N gene segments are incorporated into viruses containing gene segments coding for internal proteins facilitating infection in humans.[38] An analysis of the RNA segment 7 coding for the M1 and M2 proteins indicated that the 'human' segment was retained over a period of 43 years.[39] Viruses containing gene segments coding for internal 'avian' proteins can infect man although attenuation is likely.[40] Acquisition of a 'resistant' M gene from avians is an extremely remote possibility (see earlier), since the resistant gene would have to be predominant among the

avians when reassortment with other internal genes from human strains occurred and the resulting virus would have to be a human-like virus with regard to pathogenicity and transmissibility. Similarly, the acquisition of new H and N genes from an animal virus by a resistant human virus is highly unlikely. Since previous recombination events leading to pandemic strains are thought to have occurred in China, the resistant gene would also have to be dominant in this part of the world to ensure transfer to the new subtype virus.

The influenza A viruses also cause epidemics yearly among the various populations throughout the world. These epidemics are primarily attributable to the minor antigenic changes in the H and N surface antigens that result from point mutations in the genes coding for the H and N proteins.[38] These changes occur frequently and lead to renewed susceptibility among human populations. These new viruses may arise anywhere, but a single strain (new variant) becomes dominant and spreads around the world as a new virus. Since these viruses apparently arise at a single location, a new variant that carries drug resistance would have to arise from mutations in the H gene of a virus containing a resistant M gene. For this to be likely, amantadine or rimantadine would have to have had extensive use in that part of the world, so that most circulating viruses were resistant. The likelihood that a new subtype or a new variant of type A influenza viruses that contains the resistant M2 protein would emerge and produce epidemic influenza is extremely unlikely considering the present circumstances of use of amantadine and rimantadine.

Moreover, it is unknown whether resistant human influenza viruses would be biologically stable and able to compete with wild-type virus for transmission in the absence of selective drug pressure, although this feature has been documented in short-term experiments in the avian influenza model. It is also unknown what degree of selective drug pressure during an epidemic would be necessary to cause substantial transmission of resistant viruses at the community level. Nevertheless, the emergence of drug-resistant influenza viruses within an epidemic could pose clinical problems under circumstances when drug resistant virus could transmit to persons at increased risk of complications.

Restrictions on drug use

Some experts have suggested that the use of amantadine and rimantadine be sharply curtailed to reduce the chance of emergence of a resistant epidemic strain. The proposal would be that the drugs be reserved for use during a pandemic or major epidemic for which vaccines were not available in a timely fashion. Other restrictions on drug use, such as limitation to prophylactic use or to use in high-risk individuals only, have also been proposed. Such marked restrictions would obviate the well-established prophylactic and therapeutic value of these drugs[20–22, 22a] and would not guarantee that resistant viruses would not emerge. Moreover, as discussed above, the appearance of a resistant epidemic strain is highly improbable. Since the current data do not allow for clear conclusions about the clinical importance of drug-resistance in influenza viruses, broad guidelines for a general restriction of drug use are not appropriate at this time. Restrictions would be appropriate only in circumstances in which drug treatment may be clearly associated with potential harm.

The available data allow for consideration of alternative strategies for drug use in certain settings. In families the maximal risk of infection relates to exposure to infected children. This conclusion is supported directly by the finding that prevention of influenza A infection in children by seasonal prophylaxis with rimantadine is associated with a significant reduction in the risk of infection (73% efficacy compared to placebo) in families.[41] Recovery of resistant virus from some patients treated with rimantadine suggests that avoiding treatment of ill index cases, in particular young children, would markedly reduce the risk of transmission of resistant virus to contacts.[29] Although no higher risk of illness is present in households where the drugs are used for both prophylaxis and therapy, the apparent limited effectiveness of post-exposure prophylaxis in this setting, when considered in the context of possible drug side effects and costs, argues against the routine use of concurrent treatment of young children and prophylaxis of contacts in families.

Rimantadine[32] and amantadine[33] appear to be effective for post-exposure prophylaxis in families when index cases are not treated. To avoid the possibility of illness due to resistant virus in high-risk susceptible household contacts, it would be prudent to give them prophylaxis and not give concurrent therapy to ill family members who were previously healthy.

An alternative approach is to treat the ill index case without providing prophylaxis to the contacts. This is based on several considerations, including the therapeutic benefits provided to the treated individual, the relatively low risk of secondary transmission events (approximately 20–30% in the case of H3N2 subtype viruses), avoidance of drug side effects in contacts, and a possible reduction in transmission due to the antiviral effects of the drugs. Couch *et al.*[42] have studied the effect of treating index cases without prophylaxis of contacts over a series of influenza outbreaks in Houston. Although different epidemic strains and treatment regimens made the outcomes not strictly comparable, the overall frequency of laboratory proven infections was 32% among 142 household contacts receiving amantadine or rimantadine compared to 47% among 146 placebo recipients (32% efficacy). Whether some of the contact illnesses were due to resistant virus was not determined. Because this strategy could be associated with the development of infections due to resistant virus, illnesses in some contacts might not be amenable to therapy with these drugs.

The effects of treatment on virus shedding appear to be age-related and may account in part for the higher risk of transmission from treated children. The reasons for the apparent prolongation of shedding in young children treated with rimantadine is uncertain but may relate to initial antiviral effects and subsequent delay in specific immune responses. In experimental murine influenza, early treatment is associated with reductions in specific cytotoxic T-cell and antibody responses.[43] In experimentally

induced influenza, amantadine prophylaxis has been associated with reduced virus titres and lower serum and nasal antibody titres compared with placebo.[44] Likewise, diminished influenza-specific IgA and IgG responses in nasal secretions have been found in rimantadine-treated individuals with natural influenza.[45] Whether such alterations in local immune responses contribute to the selection of resistant variants is unknown, but the failure of current treatment regimens to curtail viral shedding in young children is an additional reason to consider other approaches.

The possible transmission of drug-resistant virus is also a concern in the conditions of close contact found on many hospital wards and chronic care facilities. Nosocomial transmission of resistant virus might occur to susceptible high-risk contacts of drug-treated patients, if isolation procedures were not effective. Similarly, if an ongoing influenza outbreak were managed by both mass chemoprophylaxis and treatment of ill patients and staff, transmission of drug-resistant virus could result in failure of prophylaxis.[35,36,36a,46] This potential problem underlines the need for appropriate isolation of influenza patients, whether treated or not, and the restriction of ill health care workers from direct patient contact. If possible, persons with influenza who are receiving drug treatment should be separated from those receiving drug prophylaxis in such closed populations.[46] Although restriction of drug use to prophylaxis only might theoretically reduce the magnitude of the problem, this is impractical to implement and does not address the circumstance of incubating infection. Furthermore, treatment attenuates the severity of acute illness in the affected patient,[20–22,22a,47,48] although the magnitude of the therapeutic effect has been debated, in part because past studies have not determined whether therapy will prevent complications in high-risk patients.

Surveillance

One clear implication of the findings to date is that continued surveillance of the drug susceptibility of isolates needs to be maintained on a sustained basis. Enzyme-linked immunoassays which measure expression of viral haemagglutinin or type-specific antigens after infection of susceptible cells provide sensitive bioassays for resistance that have been correlated with the presence of M2 substitutions.[9,49] Such assays can be adapted for rapid screening of isolates in reference laboratories. The manufacturers of these drugs have an obligation to provide for monitoring of viral susceptibility.

DIRECTIONS FOR FUTURE INVESTIGATION

Prevention of development of resistance

Several different approaches aimed at reducing selection of resistant virus have been proposed. Drug-resistant viruses show cross-resistance to amantadine and rimantadine,[1,2,9] *cyclooctylamine*,[5] *ICI 130,685* (F. Hayden, unpublished observations), and related compounds with a similar antiviral mechanism of action. No published data are available to determine whether a particular compound within this group is less likely to be associated with the selection of drug resistant viruses. For example, if amantadine were to allow more viral replication than rimantadine in humans, as suggested by its less potent antiviral effects *in vitro*[2] but not in clinical trials,[24,48] resistance might occur more readily with amantadine treatment. This theoretical possibility has not been studied directly in human trials. Higher doses of single drugs are not well tolerated in humans and, because of their incomplete inhibitory effects, could serve to provide greater selective pressure for development of resistance, as suggested by the results of one murine model study.[4]

Because the clinical and virologic effects of rimantadine are most pronounced during the first two days of treatment in children[27,28,30] and adults,[48] the possibility that an abbreviated course of therapy (e.g. 1 or 2 days) might provide clinical benefit and reduce the likelihood of selecting resistant virus[27] warrants testing. However, the prolonged plasma elimination half-lives of these compounds[21] suggests that even short-course therapy could provide sustained drug effects, which might be sufficient to decrease virus shedding, blunt host responses, and possibly exert selective drug pressure. Because this approach might also be associated with an increased risk of rebound in symptoms, it should be studied in the context of controlled trials before being embraced as an accepted clinical practice.

Interventions that could markedly inhibit the replication of progeny virus might reduce the likelihood of resistant virus emerging. Combined use of agents having different mechanisms of action (e.g. rimantadine and ribavirin) provides more potent antiviral effects than single agents in cell culture and animals (see review[50]). A possible associated reduction in developing resistant virus has not been adequately explored.

Prevention of spread of resistance

The protective efficacy of antivirals appears to be higher in the presence of specific anti-influenza antibody.[20] In the avian model, combined use of drug prophylaxis and immunisation with inactivated vaccine at the time of virus exposure protects contact birds from lethal infection.[15] This approach warrants study in clinical settings and offers the clinical advantages of long-term immunity to infection and reduced duration of drug administration. In general, the potential problem of drug resistance underlines the continuing importance of preseason immunisation for prevention of influenza.

SUMMARY

Drug-resistant H3N2 subtype viruses have emerged during rimantadine treatment of uncomplicated influenza in children or adults. Illness due to transmission of drug-resistant virus has occurred in household contacts receiving rimantadine prophylaxis, and amantadine-treated patients may serve as a source of resistant viruses in nursing home settings. Rimantadine therapy is accompanied by clinical benefits, despite the recovery of resistant virus from

some treated patients. Further studies are needed to understand the clinical significance of drug-resistant influenza viruses and to develop methods to reduce the likelihood of their selection and spread. The available data indicate that the risks of emergence of resistant virus do not outweigh the documented benefits of drug prophylaxis and therapy in most circumstances.

REFERENCES

1. Hayden, F. G., Cote, K. M. and Douglas, R. G. Jr. (1980). Plaque inhibition assay for drug susceptibility testing of influenza viruses. *Antimicrob. Agents Chemother.*, **17**, 865–870.
2. Belshe, R. B., Burk, B., Newman, F., Cerruti, R. L. and Sim, I. S. (1989). Resistance of influenza A virus to amantadine and rimantadine: results of one decade of surveillance. *J. Infect. Dis.*, **159**, 430–435.
3. Oxford, J. S., Logan, I. S. and Potter, C. W. (1970). *In vivo* selection of an influenza A2 strain resistant to amantadine. *Nature*, **226**, 82–83.
4. Oxford, J. S. and Potter, C. W. (1973). Amino-amantadine-resistant strains of influenza A2 virus. *J. Hyg. (Cambr.)*, **71**, 227–236.
5. Appleyard, G. and Maber, H. B. (1975). A plaque assay for the study of influenza virus inhibitors. *J. Antimicrob. Chemother.*, **1** (Suppl), 49–53.
6. Appleyard, G. (1977). Amantadine-resistance as a genetic marker for influenza viruses. *J. Gen. Virol.*, **36**, 249–255.
7. Lubeck, M. D., Schulman, J. L. and Palese, P. (1978). Susceptibility of influenza A viruses to amantadine is influenced by the gene coding for M protein. *J. Virol.*, **28**, 710–716.
8. Hay, A. J., Kennedy, N. C. T., Skehel, J. J. *et al.* (1979). The matrix protein gene determines the amantadine sensitivity of influenza viruses. *J. Gen. Virol.*, **42**, 3021–3024.
9. Belshe, R. B., Smith, M. H., Hall, C. B., Betts, R. and Hay, A. J. (1988). Genetic basis of resistance to rimantadine emerging during treatment of influenza virus infection. *J. Virol.*, **62**, 1508–1512.
10. Hay, A. J. (1989). The mechanism of action of amantadine and rimantadine against influenza viruses. In, *Concepts in Viral Pathogenesis, Volume III*, ed. by A. L. Notkins and M. B. A. Oldstone, pp. 561–567. Springer-Verlag, Berlin.
11. Belshe, R. B. and Hay, A. J. (1989). Drug resistance and mechanisms of action on influenza A viruses. *J. Resp. Dis.*, **10** (Suppl 12A), S52–S61.
12. Hay, A. J., Grambas, S. and Bennett, M. S. (1991). Resistance of influenza A viruses to amantadine and rimantadine. In, *Advances in Molecular Biology and Targeted Treatment for AIDS*, ed. by A. Kumar, pp. 345–353. Plenum, New York.
13. Hayden, F. G. and Hay, A. J. (1992). Emergence and transmission of influenza A viruses resistant to amantadine and rimantadine. In, *Current Topics in Microbiology and Immunology, Vol. 176: Genetic Diversity of RNA Viruses*, ed. by J. Holland, pp. 119–130. Springer-Verlag, Berlin.
14. Hayden, F. G. (1989). Significance of rimantadine resistance in influenza A viruses. *J. Resp. Dis.*, **10** (Suppl 12A), S62–S67.
15. Webster, R. G., Kawaoka, Y., Bean, W. J., Beard, C. W. and Brugh, M. (1985). Chemotherapy and vaccination: a possible strategy for the control of highly virulent influenza virus. *J. Virol.*, **55**, 173–176.
16. Bean, W. J., Threlkeld, S. C. and Webster, R. G. (1989). Biologic potential of amantadine-resistant influenza A virus in an avian model. *J. Infect. Dis.*, **159**, 1050–1056.
17. Beard, C. W., Brugh, M. and Webster, R. G. (1987). Emergence of amantadine-resistant H5N2 avian influenza virus during a simulated layer flock treatment program. *Avian Dis.*, **31**, 533–537.

17a Sears, S. D., Clements, M. L., Betts, R. F., Maassab, H. V., Murphy, B. R. and Synder, M. H. (1988). Comparison of live attenuated H1N1 and H3N2 cold-adapted and avian-human influenza A reassortant viruses and inactivated virus vaccine in adults. *J. Infect. Dis.*, **158**, 1209–1219.

18. Heider, H., Adamczyk, B., Presber, H. W., Schroeder, C., Feldblum, R. and Indulen, M. K. (1981). Occurrence of amantadine- and rimantadine-resistant influenza A virus strains during the 1980 epidemic. *Acta Virol.*, **25**, 395–400.
19. Pemberton, R. M., Jennings, R., Potter, C. W. and Oxford, J. S. (1986). Amantadine resistance in clinical influenza A (H3N2) and (H1N1) virus isolates. *J. Antimicrob. Chemother.*, **18** (Suppl. B), 135–140.
20. Bektimirov, T. A., Douglas, R. G. Jr., Dolin, R., Galasso, G. J., Krylov, V. F. and Oxford, J. (1985). Current status of amantadine and rimantadine as anti-influenza-A agents: Memorandum from a WHO meeting. *Bull. WHO*, **63**, 51–56.
21. Tominack, R. L. and Hayden, F. G. (1987). Rimantadine hydrochloride and amantadine hydrochloride use in influenza A virus infections. In, *Infectious Disease Clinics of North America*, ed. by R. C. Moellering, pp. 459–478. W. B. Saunders, Philadelphia.
22. Douglas, R. G. Jr. (1990). Drug therapy. Prophylaxis and treatment of influenza. *N. Engl. J. Med.*, **322**, 443–450.

22a. Couch, R. B. (1990). Respiratory diseases. In, *Antiviral Agents and Viral Diseases of Man*, ed. by G. J. Galasso *et al.*, pp. 327–372. Raven Press, New York.

23. Reuman, P. D., Bernstein, D. I., Keefer, M. C., Young, E. C., Sherwood, J. R. and Schiff, G. M. (1989). Efficacy and safety of low dosage amantadine hydrochloride as prophylaxis for influenza A. *Antiviral Res.*, **11**, 27–40.
24. Dolin, R., Reichman, R. C., Madore, H. P., Maynard, R., Linton, P. N. and Webber-Jones, J. (1982). A controlled trial of amantadine and rimantadine in the prophylaxis of influenza A infection. *N. Engl. J. Med.*, **307**, 580–584.
25. Sears, S. D. and Clements, M. L. (1987). Protective efficacy of low-dose amantadine in adults challenged with wild-type influenza A virus. *Antimicrob. Agents Chemother.*, **31**, 1470–1473.

26. Brady, M. T., Sears, S. D., Clements, M. L., Pacini, D. L., Samorodin, R., DePamphilis, J., Oakes, M., Soo, W. and Clements, M. L. (1990). Safety and prophylactic efficacy of low-dose rimantadine in adults during an influenza A epidemic. *Antimicrob. Agents Chemother.*, **34**, 1633–1636.
27. Hall, C. B., Dolin, R., Gala, C. L., Markovitz, D. M., Zhang, Y. Q., Madore, P. H., Disney, F. A., Talpey, W. B., Green, J. L., Francis, A. B. and Pichichero, M. E. (1987). Children with influenza A infection: treatment with rimantadine. *Pediatrics*, **80**, 275–282.
28. Thompson, J., Fleet, W., Lawrence, E., Pierce, E., Morris, L. and Wright, P. (1987). A comparison of acetaminophen and rimantadine in the treatment of influenza A infection in children. *J. Med. Virol.*, **21**, 249–255.
29. Hayden, F. G., Belshe, R. B., Clover, R. D., Hay, A. J., Oakes, M. G. and Soo, W. (1989). Emergence and apparent transmission of rimantadine-resistant influenza A virus in families. *N. Engl. J. Med.*, **321**, 1696–1702.
30. Hayden, F. G., Sperber, S. J., Belshe, R. B., Clover, R. D., Hay, A. J. and Pyke, S. (1991). Recovery of drug-resistant influenza A virus during therapeutic use of rimantadine. *Antimicrob. Agents Chemother.*, **35**, 1741–1747.
31. Kubar, O. I., Brjantseva, E. A., Nikitina, L. E. and Zlydnikov, D. M. (1989). The importance of virus drug-resistance in the treatment of influenza with rimantadine. *Antiviral Res.*, **11**, 313–316.
32. Bricaire, F., Hannoun, C. and Boissel, J. P. (1990). Prevention de la grippe A. Efficacite et tolerance du chlorhydrate de rimantadine. *Presse Med.*, **19**, 69–72.
33. Galbraith, A. W., Oxford, J. S., Schild, G. C. and Watson, G. I. (1969). Protective effect of 1-adamantanamine hydrochloride on influenza A2 in the family environment. *Lancet*, **2**, 1026–1028.
34. Galbraith, A. W., Oxford, J. S., Schild, G. C. and Watson, G. I. (1969). Study of 1-adamantanamine hydrochloride used prophylactically during the Hong Kong influenza epidemic in the family environment. *Bull. WHO*, **41**, 677–682.
35. Mast, E. E., Harmon, M. W., Gravenstein, S., Wu, S. P., Arden, N. H., Circo, R., Tyszka, G., Kendal, A. P. and Davis, J. P. (1991). Emergence and possible transmission of amantadine-resistant viruses during nursing home outbreaks of influenza A (H3N2). *Am. J. Epidemiol.*, **134**, 988–997.
36. Roumillat, F., Rocha, E., Regnery, H., Wells, D. and Cox, N. (1990). Emergence of amantadine-resistant influenza A viruses in nursing homes during the 1989–90 influenza season (Abstr P34–42). *8th International Congress of Virology, Berlin*, p. 321.
36a. Degelau, J., Somani, S. K., Cooper, S. L., Guay, D. R. P. and Crossley, K. B. (1992). Amantadine-resistant influenza A in a nursing facility. *Arch. Intern. Med.*, **152**, 390–392.
37. Sweet, C., Hayden, F. G., Jakeman, K. J., Grambas, S. and Hay, A. J. (1991). Virulence of rimantadine-resistant human influenza A (H3N2) viruses in ferrets. *J. Infect. Dis.*, **164**, 969–972.
38. Murphy, B. R. and Webster, R. G. (1990). Orthomyxoviruses. In, *Virology*, ed. by B. N. Fields and D. M. Knipe, pp. 1091–1152. Raven Press, New York.
39. Hall, R. M. and Air, G. M. (1981). Variation in nucleotide sequences coding for the N-terminal regions of the matrix and nonstructural proteins of influenza A viruses. *J. Virol.*, **38**, 1–7.
40. Murphy, B. R., Sly, D. L., Tierney, E. L. *et al.* (1982). Reassortant virus derived from avian and human influenza A viruses is attenuated and immunogenic in monkeys. *Science*, **218**, 1330–1332.
41. Crawford, S. A., Clover, R. D., Abell, T. D., Ramsey, C. N. Jr., Glezen, W. P. and Couch, R. B. (1988). Rimantadine prophylaxis in children: a follow-up study. *Pediatr. Infect. Dis.*, **7**, 379–383.
42. Couch, R. B., Kasel, J. A., Glezen, W. P., Cate, T. R., Six, H. R., Taber, L. H., Frank, A. L., Greenberg, S. B., Zahradnik, J. M. and Keitel, W. A. (1986). Influenza: Its control in persons and populations. *J. Infect. Dis.*, **153**, 431–440.
43. Herrmann, J. E., West, K., Bruns, M. and Ennis, F. A. (1990). Effect of rimantadine on cytotoxic T lymphocyte responses and immunity to reinfection in mice infected with influenza A virus. *J. Infect. Dis.*, **161**, 180–184.
44. Reuman, P. D., Bernstein, D. I., Keely, S. P., Young, E. C., Sherwood, J. R. and Schiff, G. M. (1989). Differential effect of amantadine hydrochloride on the systemic and local immune response to influenza A. *J. Med. Virol.*, **27**, 137–141.
45. Clover, R. D., Waner, J. L., Becker, L. and Davis, A. (1991). Effect of rimantadine on the immune response to influenza A infections. *J. Med. Virol.*, **34**, 68–73.
46. ACIP (1991). Prevention and control of influenza: recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR*, **40** (no. RR-6).
47. Betts, R. F., Treanor, J. J., Graman, P. S., Bentley, D. W. and Dolin, R. (1987). Antiviral agents to prevent or treat influenza in the elderly. *J. Respir. Dis.*, **8**, S56–S59.
48. Van Voris, L. P., Betts, R. F., Hayden, F. G., Christmas, W. A. and Douglas, R. G. Jr. (1981). Successful treatment of naturally occurring influenza A/USSR/77 H1N1. *J. Am. Med. Assoc.*, **245**, 1128–1131.
49. Kendal, A. P. (1991). Short communication. Reference viruses for use in amantadine sensitivity testing of human influenza A viruses. *Antiviral Chem. Chemother.*, **2**, 115–118.
50. Hayden, F. G. (1986). Combinations of antiviral agents for treatment of influenza virus infections. *J. Antimicrob. Chemother.*, **18** (Suppl. B), 177–183.