Treatment of influenza with neuraminidase inhibitors: virological implications

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Evaluation of the emergence of influenza virus resistance to neuraminidase inhibitors (NAIs) is now demanded following experience with amantadinamines. Preliminary data have indicated that NAI-resistant virus is unlikely to emerge readily in the clinic and this is consistent with the difficulty experienced in selecting resistant virus *in vitro*. Resistance mutations can occur in both neuraminidase and haemagglutinin genes. The neuraminidase mutations are viral subtype specific and, therefore, clinically relevant subtypes must be employed for *in vitro* studies if pre-clinical data are to have predictive value. Haemagglutinin mutations generated *in vitro* are probably both subtype and cell culture system specific and, therefore, may not be predictive of clinical findings. Analysis of influenza-positive samples from NAI-treated patients in the clinical setting must include samples from late treatment time-points (day 4 and later) in order for resistant virus to be detected as *in vitro* studies and current clinical experience have indicated that resistant virus is slow to emerge and is transient.

Keywords: influenza; neuraminidase inhibitors; amantadinamines; cross-resistance

1. INTRODUCTION

Clinical experience with the anti-influenza A drugs amantadine and rimantadine over many years has shown (Hayden 1992) that viral drug resistance emerges rapidly, often within 48 h of treatment initiation and to a high incidence (20–30%). Moreover, the resistant mutant viruses are essentially equi-infective and equipathogenic to the corresponding wild-type viruses. The resultant transmission of amantadinamine-resistant virus limits the usefulness of these drugs, particularly in circumstances such as outbreak control in nursing homes. The mutants are cross-resistant to the two drugs.

The advent of the influenza neuraminidase inhibitors (NAIs), that are active against both influenza A and B viruses (Mendel & Roberts 1998), has raised the question as to the potential for drug resistance with these agents as a class.

Progeny influenza viruses emerging from an infected cell remain attached to that cell and/or to each other by the viral haemagglutinin binding to terminal sialyl residues on glycoproteins on the cell/virus surface. The role of the viral neuraminidase is to cleave these terminal sialyl residues, thereby releasing the virus for spreading the infection. NAIs block this virus release step. From this mode of action, resistance to NAIs might arise in two ways (Mendel & Sidwell 1998). The obvious and direct mechanism would be mutation of the viral neuraminidase in order to prevent the drug binding to its target. However, by design NAIs bind to the highly conserved active site of the neuraminidase and this is expected to make resistance selection difficult. The alternative mechanism for resistance would be via mutation of the viral haemagglutinin in order to reduce its binding affinity to sialyl residues. This would facilitate viral release

without the necessity for neuraminidase activity and, hence, produce virus resistant to NAIs (as a class).

2. SELECTION OF RESISTANT VIRUS *IN VITRO*

In vitro selection of influenza viruses that are resistant to the NAIs has proved difficult, requiring many rounds of virus replication under increasing drug pressure (Mendel & Sidwell 1998). This contrasts with the only one or two rounds that are required for selecting resistance to amantadinamines. To a large degree, resistance mutations that are selected in the viral neuraminidases have been both drug and virus subtype specific. One common mutation in neuraminidase A/N2, namely mutation R292K, has been selected by zanamivir, oseltamivir carboxylate and RWJ 270201 (Bantia *et al.* 2000; McKimm-Breschkin 2000). The degree of loss of drug sensitivity with this mutant varies from drug to drug, but this mutant would give cross-resistance to the class. The R292K mutation did not give resistance to oseltamivir carboxylate when it was engineered into neuraminidase A/N1, thereby confirming the subtype-specific nature of resistance mutations. Mutations E119G and E119A have only been reported for zanamivir (in the A/N2, A/N9 and B viruses). They have not been selected by nor give resistance to oseltamivir carboxylate, and E119G in A/turkey/Minnesota (H4N2) does not give resistance to RWJ 270201 (McKimm-Breschkin 2000). Oseltamivir carboxylate selects an H274Y resistance mutation in A/N1 virus (Wang *et al.* 2000). This is not resistant to zanamivir. There are, to our knowledge, no reported data on the selection of resistance to zanamivir or RWJ 270201 in A/N1 virus.

Attempts to select influenza B virus that is resistant to oseltamivir carboxylate *in vitro* have been without success, again suggesting a difference between the viral subtypes.

There are, to our knowledge, no data on the selection of resistance to RWJ 270201 in influenza B.

In the light of the different mutational responses of the neuraminidase subtypes, one must call into question the value of studies that employ N9 viruses in terms of the predictability of potential clinically selected mutants.

There is reassurance from *in vitro* experience that not only is resistance in neuraminidases selected with difficulty, but also that the mutant viruses are compromised in some way when compared with their corresponding wild-types. The common mutant R292K is particularly well characterized in this regard, with markedly reduced infectivity being seen for mutant virus selected by either zanamivir or oseltamivir carboxylate (Gubareva *et al.* 1997; Tai *et al.* 1998).

In vitro resistance selection often results in mutations in the viral haemagglutinin preceding any neuraminidase mutation. Most haemagglutinin mutants are described as arising with zanamivir (McKimm-Breschkin 2000). A very large number of different mutations have been found, with those giving the most marked decrease in drug sensitivity being near or in the sialic acid binding site. These mutations will decrease binding to the receptors of the cells in which they were selected (often Madin-Darby canine kidney (MDCK) cells with α 2,3-linked sialic acid). Sialic acid linkage in MDCK cells may not reflect the chemistry of the receptors in human respiratory epithelial cells (which is predominantly α 2,6). Hence, there is doubt as to the predictive value of these findings to the clinical situation.

3. SELECTION OF RESISTANT VIRUS IN THE CLINIC

There is a practical challenge in the assessment of resistance to NAIs in clinical samples by conventional assessment of antiviral phenotype (Tisdale 2000). (This, again, is probably due to differences in cell receptor chemistry.) Thus, resistance is best assessed primarily by neuraminidase enzyme inhibition phenotype and haemagglutinin genotype (Zambon & Hayden 2001).

In a comparative clinical study of zanamivir and rimantadine in the prevention of naturally acquired influenza outbreaks in a nursing home environment, the incidence of resistant virus in each group was assessed on study day 3 (Daly *et al.* 2000). While the incidence of resistance to rimantadine was 38% (in line with previous findings), there was no evidence of resistance to zanamivir in 93 isolates by neuraminidase phenotyping. Similarly, in a clinical trial (M76001) of the treatment of naturally acquired influenza infection in adults with oseltamivir phosphate, no resistance was seen by neuraminidase phenotyping in any culture-positive day 3 sample from the 594 oseltamivir-treated subjects.

However, the difficulty in selecting NAI resistance *in vitro* suggests that, if resistance is going to arise in the clinic, it will probably be late in the treatment period. For the clinical trials WV15670/1, again studying the treatment of influenza infection in adults with oseltamivir phosphate, resistance was examined more thoroughly (Covington *et al.* 2000). Nose and throat swabs were taken for culture on study days 1, 2, 4, 6 and 8. Neuraminidase phenotyping was carried out on all day 1 and last day positive samples.

Neuraminidase and haemagglutinin genotyping was carried out on all patients' samples that were culture positive on day 4 or beyond. This in-depth investigation revealed that, by neuraminidase phenotyping, resistant virus was selected in the clinic, but at a very low incidence (four out of 418 cases). In only one case was an additional sample, a mixed culture containing a neuraminidase mutant, identified by genotyping. The mutations were R292K in A/N2 in four cases, consistent with *in vitro* findings and a novel mutant, namely E119V, was identified in A/N2 in one case.

Subsequent studies on virus samples from over 2500 oseltamivir-treated subjects with naturally acquired influenza infection have confirmed the low incidence of resistance (current estimate 0.4% in adults and 4% in children). Most mutations are R292K in A/N2 virus (Jackson *et al.* 2000). One example of H274Y in an H1N1 virus has been identified. No resistance has been seen in influenza B. These findings are again consistent with *in vitro* data. In contrast to *in vitro* findings, there was no evidence for selection of a haemagglutinin mutation in any of the viruses with a neuraminidase resistance mutation (or, indeed, in any other of *ca.* 100 virus samples tested). This contrasts with the selection of an influenza B zanamivir-resistant mutant in an immunocompromised child where both haemagglutinin (T189I) and neuraminidase (R152K) mutations were found. Neither mutation had been predicted by *in vitro* studies (Gubareva *et al.* 1998).

The important outcome of these studies is that mutant clinical isolates have been available for evaluation. All mutant viruses (R292K, H274Y and E119V selected by oseltamivir and R152K selected by zanamivir) showed reduced infectivity/replicative ability in animal models compared to their corresponding wild-types. In addition, the common R292K mutant does not transmit and replicate from ferret to ferret under conditions where wild-type transmission and replication is 100% (Herlocher *et al.* 2002).

Thus, virus resistant to NAIs appears to be infrequently selected in clinical trials and the resistant virus is compromised. In addition, study of individual patients carrying resistant virus in oseltamivir phosphate trials shows that they carry resistant virus only transiently; resistant virus is cleared normally and is often in a mixed population with excess wild-type. Importantly, patients show no clinical deterioration following selection of resistant virus and have symptom scores comparable with similar patients in their treatment group who carry only wild-type virus (Jackson *et al.* 2000).

4. CONCLUSIONS

In conclusion, the strategy of NAI design as a class in targeting drug binding to a highly conserved part of the viral enzyme appears to be justified. Resistance mutations in the viral neuraminidases have been selected with difficulty *in vitro* and at a low frequency and transiently in the clinic. Mutations in the haemagglutinins seen with *in vitro* selection do not appear to be so readily selected in the clinic, possibly as reduced receptor affinity will also probably reduce infectivity. Neuraminidase mutants, where selected, are compromised and do not affect patient recovery.

REFERENCES

- Bantia, S., Ananth, S., Horn, L., Parker, C., Gulati, U., Chand, P., Babu, Y. & Air, G. 2000 Generation and characterisation of a mutant of influenza A virus selected with the neuraminidase inhibitor RWJ 270201. *Antiviral Res.* **46**, A60.
- Covington, E., Mendel, D. B., Escarpe, P., Tai, C. Y., Soderbarg, K. & Roberts, N. A. 2000 Phenotypic and genotypic assay of influenza virus neuraminidase indicates a low incidence of viral drug resistance during treatment with oseltamivir. *J. Clin. Virol.* **18**, 253–254.
- Daly, J., Cadman, A., Tisdale, M., Flack, N., Shult, P., Drinka, P. & Gravenstein, S. 2000 Zanamivir and rimantadine susceptibility of virus isolates from a clinical trial comparing the use of zanamivir or rimantadine in the control of nursing home influenza outbreaks. In *Proceedings of the 40th Interscience conference on antimicrobial agents and chemotherapy*, p. 260. Washington, DC; American Society for Microbiology.
- Gubareva, L. V., Robinson, M. J., Bethell, R. C. & Webster, R. G. 1997 Catalytic and framework mutations in the neuraminidase active site of influenza viruses that are resistant to 4-guanidino-Neu5Ac2en. *J. Virol.* **71**, 3385–3390.
- Gubareva, L. V., Matrosovich, M. N., Brenner, M. K., Bethell, R. C. & Webster, R. G. 1998 Evidence for zanamivir resistance in an immunocompromised child infected with influenza B virus. *J. Infect. Dis.* **178**, 1257–1262.
- Hayden, F. G. 1992 Emergence and transmission of influenza A viruses resistant to amantadine and rimantadine. *Curr. Topics Microbiol. Immunol.* **176**, 120–130.
- Herlocher, M. L., Carr, J., Ives, J., Elias, S., Truscon, R., Roberts, N. & Monto, A. S. 2002 Influenza virus carrying an R292K mutation in the neuraminidase gene is not transmitted in ferrets. *Antiviral Res.* (In the press.)
- Jackson, H. C., Roberts, N. A., Wang, M. Z. & Belshe, R. 2000 Management of influenza: use of new antivirals and resistance in perspective. *Clin. Drug Invest.* **6**, 447–454.
- McKimm-Breschkin, J. L. 2000 Resistance of influenza viruses to neuraminidase inhibitors—a review. *Antiviral Res.* **47**, 1–17.
- Mendel, D. B. & Roberts, N. A. 1998 *In-vitro* and *in-vivo* efficacy of influenza neuraminidase inhibitors. *Curr. Opin. Infect. Dis.* **11**, 727–732.
- Mendel, D. B. & Sidwell, R. W. 1998 Influenza virus resistance to neuraminidase inhibitors. *Drug Resist. Updates* **1**, 184–189.
- Tai, C. Y., Escarpe, P. A., Sidwell, R. W., Williams, M. A., Lew, W., Wu, H., Kim, C. U. & Mendel, D. B. 1998 Characterization of human influenza virus variants selected *in vitro* in the presence of the neuraminidase inhibitor GS 4071. *Antimicrob. Agents Chemother.* **42**, 3234–3241.
- Tisdale, M. 2000 Monitoring of viral susceptibility: new challenges with the development of influenza NA inhibitors. *Rev. Med. Virol.* **10**, 45–55.
- Wang, Z. M., Tai, C. Y. & Mendel, D. B. 2000 Characterization of an influenza A virus variant selected *in vitro* in the presence of the neuraminidase inhibitor GS4071. *Antiviral Res.* **46**, A60.
- Zambon, M. & Hayden, F. G. 2001 Position statement: global neuraminidase inhibitor susceptibility network. *Antiviral Res.* **49**, 147–156.