Antivirals for influenza infection

An international conference entitled *Options for the Control of Influenza III* was held in Cairns, Australia in May, 1996. Most of the discussion of antiviral agents at this meeting was concerned with GG 167, a potent and selective inhibitor of influenza virus neuraminidase, which is currently undergoing clinical evaluation. Dr M. von Itzstein (Monash University, Parkville, Australia) discussed the design and synthesis of influenza-virus-sialidase inhibitors that are novel anti-influenza drugs. Von Itzstein and his coworkers used information obtained from the X-ray crystallographic structure of influenza A neuraminidase to design and synthesize the sialic acid derivative 4-guanidino-2-deoxy-2,3-didehydro-*N*-acetyl neuraminic acid (GG 167, **1**), in which the hydroxyl group at the C-4 position has been replaced with a guanidinium moiety. Importantly, GG 167 inhibits influenza virus replication in tissue culture, in animal models of infection, and in human clinical trials. Considering that the neuraminidase active site is highly conserved and structurally very similar for all influenza viruses examined so far, GG 167 is expected to be effective against all influenza viruses, including those that have not yet appeared in human populations.

In vivo activity of GG 167

Dr L.M.R. Cass and her coworkers (Glaxo Wellcome, Stevenage, UK) reported that GG 167, when given by intravenous infusion (up to 16 mg, single dose), by intranasal delivery (up to 96 mg daily for 5 days) or by inhalation (up to 64 mg daily for 7 days using a nebulizer, and up to 40 mg daily for 7 days as a dry powder), was safe and well tolerated. This group, along with Dr E.K. Hussey and her coworkers (GlaxoWellcome, Research Triangle Park, NC, USA), also provided an overview of the pharmacokinetics of GG 167 in animals and in healthy individuals who received the compound by intravenous infusion, intranasal delivery or inhalation. Dr F.G. Hayden (University of Virginia, Charlottesville, VA, USA) reported that, in challenge trials using human volunteers, GG 167 was effective in preventing viral shedding when given as an intranasal spray (1.8 mg per nostril given twice daily for 5 days). Dr M. Matsumoto and his coworkers (Institute of Tropical Medicine, Nagasaki, Japan and Nippon Glaxo, Tokyo, Japan) reported results from a field study in which patients who had shown influenza-like symptoms for less than 36 hours were treated with placebo or GG 167, which was administered by inhalation, intranasally, or both. The clinical parameter measured was the time to alleviation of major symptoms of influenza including axillary temperature of less than 37°C, mild or absent headache, myalgia, cough and sore throat. Among influenza virus-infected individuals, the time to alleviation of these symptoms was significantly shorter in patients treated with 10 mg inhaled GG 167 (three treatments twice daily for 5 days) than in patients treated with placebo. Importantly, GG 167 was found to be safe and well tolerated. This group concluded that GG 167 is effective in the treatment of influenza.

Resistance to GG 167

Some reports were concerned with the emergence of resistance to GG 167. Dr J.L. McKimm-Breschkin and her coworkers (Biomedical Research Institute, Victoria, Australia) along with C. Penn and his coworkers (GlaxoWellcome, Stevenage, UK) discussed two types of NWS/G70c influenza virus variants that were selected for resistance to GG 167 after six passages in tissue culture. The predominant type was 1,000-fold more resistant to GG 167 than was the parent virus, and contained changes which mapped to residues associated with the receptor binding site of the hemagglutinin. It was hypothesized that changes in hemagglutinin, resulting in alterations in the receptor binding site, may compensate for inhibition of neuraminidase. The second type of variant revealed a change in glutamate 119 of the viral neuraminidase to glycine; this results in the generation of a neuraminidase that is relatively resistant to inhibition by GG 167. Previous work by Drs M. von Itzstein, J. Varghese and coworkers demonstrated that glutamate 119 participates in a strong electrostatic interaction with the C-4 guanidinium moiety of GG 167.

Dr J.M. Colacino and his coworkers (Lilly Research Laboratories, Indianapolis, IN, USA) also reported the development of resistance to GG 167 in tissue culture. This group passaged influenza A/NWS/G70c and influenza B/HK/8/73 (HG) viruses in increasing concentrations of GG 167 and obtained plaque-purified viruses that were resistant to the inhibitor. Both resistant A and B viruses contained the single amino acid change of glutamate 119 to glycine in the active site of the viral neuraminidase, leading to the formation of neuraminidase that is resistant to inhibition by GG 167. The resistant B virus, but not the A virus, also had two amino acid changes near the receptor binding site of the hemagglutinin. The resistant viruses were capable of multicycle replication in MDCK cells and were able to induce pyrexia in the ferret model of infection, even though these viruses displayed much less neuraminidase activity than did parent viruses.

Drs L. Gubareva, R. Webster and their coworkers (St Jude Children's Research Hospital, Memphis, TN, USA) reported that serial passage of influenza A/Turkey/Minnesota/833/80 (H4N2) in MDCK cells in the presence of GG 167 resulted in the emergence of inhibitor-resistant mutants. The first mutants detected in this study had substitutions in the hemagglutinin that appeared to reduce the affinity of the hemagglutinin for the host-cell receptor. However, after additional passages in the presence of GG 167, resistant mutant viruses demonstrated substitutions in the active site of the neuraminidase at conserved residues 119 and 292. These mutations were found

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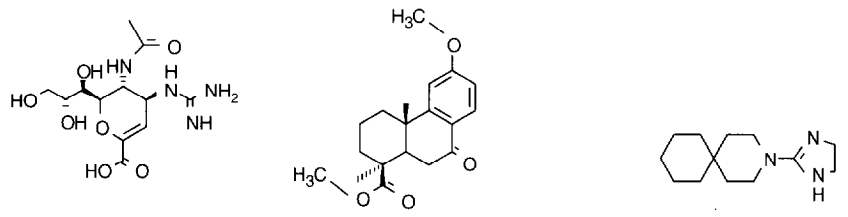

1 (GG 167) 2 (LY 180299) 3 (BL 1743)

Antiviral agents discussed at Options for the Control of Influenza III.

to reduce the level of neuraminidase activity per virion.

Although the results presented by these groups demonstrate the potential for the development of resistance to GG 167, through changes either in the hemagglutinin or in the highly conserved active site region of the viral neuraminidase, the clinical relevance of these studies is not clear. Indeed, Dr F.G. Hayden and his coworkers (University of Virginia, Charlottesville and GlaxoWellcome, Research Triangle Park) reported results of clinical studies in which GG 167 did not readily select for resistant viruses in volunteers experimentally infected with influenza A (H1N1), or in patients with naturally acquired influenza A (H3N2) and treated with GG 167. Furthermore, Hayden and his group confirmed earlier observations that clinical isolates of influenza A virus, particularly some recent H3N2 strains, differ widely in susceptibility *in vitro* to GG 167.

Other influenza-virus-neuraminidase inhibitors

Dr M. Luo and his coworkers (University of Alabama, Birmingham, AL, USA) discussed the design of aromatic inhibitors of influenza virus neuraminidase. This group initiated a new approach using the three-dimensional structure of influenza neuraminidase complexed with various inhibitors, and proposed that the catalytic mechanism of neuraminidase requires a tight binding of the transition-state intermediate, an oxocarbonium ion. This led to the design of benzoic acid derivatives that could mimic such a transition state intermediate. The advantage of such compounds, according to Luo, is chemical stability coupled with a simplicity in the chemical synthesis. The most potent benzoic-acid-based neuraminidase inhibitor displays an IC_{50} of 10 μM in enzyme-inhibition assays and reduces influenza HA titer in cell culture with an IC_{50} of 1–10 μM.

Dr G. Air and coworkers (University of Alabama, Birmingham, AL, USA) presented studies that have important implications for approaches to the chemotherapeutic intervention of influenza virus infections. Mutant influenza viruses were selected by passage in medium containing bacterial neuraminidase (Mvi), and such viruses contained no neuraminidase activity or neuraminidase protein because of extensive deletions of the neuraminidase gene. Virions of one of these mutants (NWS-Mvi) replicated in the absence of exogenously added neuraminidase, and assembled normally. However, large aggregates of this virus were found in infected cell cultures, as a result of virus–virus interactions rather than virus–cell interactions. Disruption of these aggregates resulted in the release of infectious virus particles, indicating that viral neuraminidase is not required for entry of the virus into the cell, virus replication, assembly or budding. Furthermore, this observation raised the question of which receptor is used by NWS-Mvi. This virus is able to undergo multicycle replication in the presence of sufficient Mvi neuraminidase to strip all sialic acid receptors from the surface of the cells indicating that an alternative receptor, not cleaved by Mvi neuraminidase, is used by NWS-Mvi. This group is now studying the properties of NWS-Mvi hemagglutinin and its potential role in the entry of this virus into the host cell.

Targeting other functions in the influenza virus

While the emphasis of the meeting centered on the inhibition of influenza virus neuraminidase, there were a number of reports concerning antivirals that target other functions of the influenza virus. Dr D. Bucher and her coworkers (New York Medical College, Valhalla, NY, USA and the Beckman Center, Stanford Medical School, CA, USA) presented data demonstrating that a peptide (Peptide 6), synthesized to the Zn^{2+}-finger region of the influenza matrix protein (M1), was shown to inhibit transcriptase activity to a 1000-fold greater extent than did M1 itself on a molar basis. Importantly, Peptide 6 had greater antiviral activity against influenza A/PR/8/34 than did either ribavirin or amantadine.

Drs L.R. Hoffman and I. Kuntz (University of California, San Francisco, CA, USA) with Dr J. White (University of Virginia, Charlottesville, VA, USA) described recent work concerning *t*-butyl hydroquinone (TBHQ), which inhibits both the low-pH-induced conformational change of HA and viral replication in tissue culture in the micromolar range. Currently, this group is investigating the binding of TBHQ and related compounds to hemagglutinin, as well as the mechanism of antiviral activity of this interesting class of compounds.

Dr K.A. Staschke and his coworkers (Lilly Research Laboratories, Indianapolis, IN, USA) reported the anti-influenza A (H1N1) activity of a compound derived from podocarpic acid, LY 180299 (**2**). Data presented by this group indicated that this compound targets the fusogenic function of hemagglutinin, because viral mutants resistant to the inhibition of this compound contained mutations that clustered near the fusion domain of hemagglutinin, and LY 180299 was found to inhibit the low-pH-induced fusion of human erythrocytes to influenza virus infected MDCK cells.

Dr G.G. Brownlee and his coworkers (University of Oxford, UK) presented a

new phosphorylation method for synthesizing 5′-diphosphorylated oligoribonucleotides. This group found that 2′-*O*-methylated oligonucleotides were approximately 10 times more efficiently used as primers for transcription catalyzed by influenza-virus-RNA polymerase than were the equivalent oligonucleotides lacking the 2′-*O*-methyl group.

Dr L. Pinto, Dr D. Brassard and their co-workers (Northwestern University, Chicago, IL, USA) working with scientists at Bristol-Myers Squibb (Wallingford, CT, USA) characterized the inhibition of the influenza M2 ion channel activity by BL 1743. BL 1743 (**3**), a spirene containing compound, is a novel inhibitor of influenza A virus. However, mutant viruses that are resistant to BL 1743 are also resistant to amantadine, and all known amino acid changes that result in resistance to amantadine also confer resistance to BL 1743. But some data indicate that BL 1743 and amantadine may interact differently with the M2 transmembrane pore region because inhibition by BL 1743 is complete and reversible, while that by amantadine is irreversible within the time frame of the experiment.

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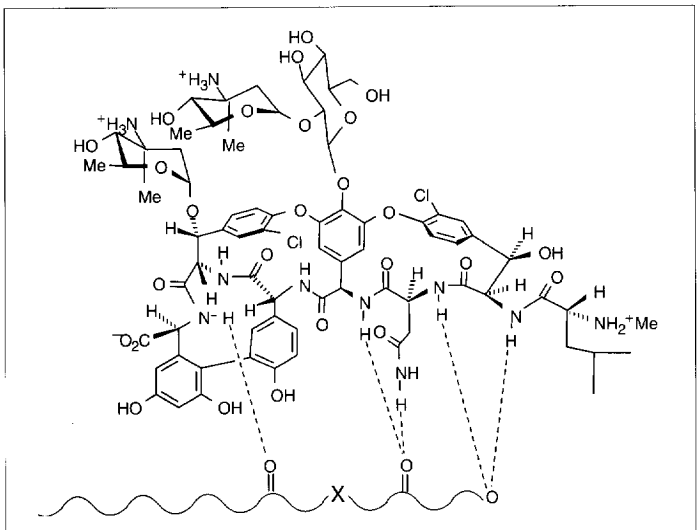
Beating superbugs with the Gulliver effect

UK researchers think they have puzzled out why an antibiotic related to vancomycin is so powerful, even though it should not bind to the vancomycin sites in bacteria any more strongly than the parent drug. According to Dr Dudley Williams of Cambridge University's Centre for Molecular Recognition the solution lies in what he calls the 'Gulliver effect'.

The glycopeptide vancomycin is often the last line of defence in a hospital's chemical weaponry against virulent strains of *Staphylococcus aureus*, which can cause lethal septicaemia. However, the increasing use of this drug in recent years has led to some bugs developing resistance to it. Williams notes that resistance follows the replacement of one D-alanine residue in the bacterial peptide overcoat with D-lactate, thus substituting a main-chain nitrogen with a oxygen. This change prevents the formation of a hydrogen bond and instead results in the repulsion of vancomycin through an unfavourable O–O interaction (see figure). Consequently, the vancomycin cannot undertake its task of killing the bacteria.

One new ally in the war against resistant bacteria is a chemical cousin of vancomycin, known as LY 264826B or chloroeremomycin. Researchers at Eli Lilly in Indianapolis have shown that a simple derivative of this compound, which contains a hydrocarbon tail, has activity against vancomycin-resistant bacteria. Williams and his team set out to elucidate the mechanism of action of this drug and find out how the new drug gets around the problem of repulsion by the altered amino acid.

In the latest issue of *Chemical Communications* (1996, 589 and 1445), Williams and his team describe evidence showing that chloroeremomycin carrying the hydrocarbon tail is likely to bind in almost exactly the same way to the bacterial cell wall, but with a clever twist that helps it avoid the effect of the repulsion. They found that vancomycin has one strong binding interaction with the bacterial cell-wall peptide, which is prevented by the amino acid swap, while the chloroeremomycin derivative is binding through surrounding groups. Many weaker adjacent bonds give rise to, what Williams calls, the 'Gulliver effect', where the hero was tied down by many weak bindings. 'An array of weak interactions is an effective way to restrict motion and ensure a strong net binding,' he explains.



Chloroeremomycin binding to the bacterial cell wall. When X is a nitrogen, a hydrogen bond is formed. When X is an oxygen, as in the resistant bacteria, repulsion results.

Whether or not bacteria develop resistance to the promising chloroeremomycin derivative, which after all works in the same way as vancomcyin but for the exact mode of binding, remains to be seen.

Williams does not wish to speculate on that point, but alludes to the fact that the new analogue would have to involve the natural selection of a new resistance mechanism – something which in thirty years of vancomycin therapy has not yet emerged.

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