to the inhibition of bacterial growth—enzyme inhibition generally and urease inhibition particularly is surely a growth industry.

References


New biochemical and clinical approaches to the control of epidemic influenza virus?

Genetic engineering and rapid DNA sequencing techniques are providing a wealth of new data about 'old' viruses. Influenza is no exception. In an early series of experiments Porter et al. (1979) cloned gene 4 (coding for the important haemagglutinin protein of an influenza A virus) by inserting a DNA copy into an Eschericia coli plasmid. Rapid DNA sequencing techniques then allowed a determination of the complete nucleotide sequence of the haemagglutinin (HA) molecule and this classic study has now been extended to other influenza A viruses. It was immediately apparent by inspection of the amino acid sequences of the haemagglutinins of viruses so unrelated antigenically as A/PR/8/34 (H1N1) and an H3N2 virus isolated in the 1970s that certain amino acid stretches had been conserved over this 45 year period, whereas most of the rest of the molecule was completely different (reviewed by Laver & Air, 1980). This might imply a common and important function of this short common sequence, and a resemblance to the...
sequence in the fusion (F) protein of a paramyxovirus led to the suggestion that the sequence at the N terminus of the HA2 polypeptide of influenza haemagglutinin had a similar function (namely, fusion with cell membranes, allowing exit of nucleic acid from the virus). Quite separate biological experiments by White, Matlin & Helenius (1981) meanwhile established that under low pH conditions influenza viruses could lyse red blood cells and cause fusion of tissue culture cells. How would this new data fit into existing theories of the entry of influenza virus by pinocytosis and what is the relevance of the observations to the life cycle of the virus and to chemoprophylaxis? Could the data be applied to a new concept of prevention or antiviral therapy by inhibitors of fusion? Independently, a group at Mount Sinai Hospital synthesized short peptides Z-gly-L-Leu-L-Phe-Gly and Z-Gly-L-Phe-L-Phe-Gly which mimicked the sequences at the N terminus of the HA2 polypeptide described above (Richardson, Scheid & Choppin, 1980). Relatively low concentrations (20-50 μM) of the peptides inhibited influenza virus replication in tissue culture studies. Tremendous problems loom when one considers the possibility of short peptides acting in vivo as antivirals in a milieu of proteolytic enzymes, but nevertheless it may be possible to synthesize analogues or add groupings to prevent rapid enzyme digestion in vivo. Hopefully such studies will not occupy the same lengthy time span as the two decades during which amantadine has been investigated. These biochemical studies have raised basic questions about the mode of action of the only existing anti-influenza A virus compound namely, amantadine. The compound acts early in infection. Does it affect fusion? The situation is even more complicated by a recent study (Richman, Yazaki & Hostetler, 1981) which demonstrated quite clearly that although amantadine is taken up rapidly by tissue culture cells, it is less rapidly lost when the cells are incubated in amantadine-free medium. On the other hand, the antiviral action is lost almost immediately the cells are placed in the drug free medium. This, of course, suggests that intracellular amantadine has little effect on the antiviral state and that we should shift our interest to the interaction of amantadine with the external surface of the plasma membrane to explain the antiviral action. These considerations may appear rather academic, but if they lead to a precise understanding of the point of action of amantadine, then a whole new series of compounds could be synthesized to act at the same stage. To confuse matters more, amantadine belongs to a class of compounds termed ‘lysosomotropic’ which can increase intralysosomal pH. If fusion is important in influenza virus replication, and if such a fusion occurs only at low pH in cytoplasmic vacuoles, then it is still possible that amantadine could act by raising the intravacular pH, stopping fusion and so blocking virus replication at this stage.

New clinical data have also been appearing at a greater rate recently in the areas of anti-influenza chemotherapy. Amantadine has been shown to have a clear therapeutic effect against recently circulating H1N1 viruses (Van Voris et al., 1981) thus confirming earlier controlled trials with H2N2 and H3N2 viruses (reviewed by Oxford & Galbraith, 1980). At present in spite of the enthusiasm for the alternative related molecule rimantadine in the U.S.S.R. (reviewed by Zlydnikov et al., 1981) most would agree that the two compounds have a very similar overall degree of activity and neither compound has a striking advantage or disadvantage compared to the other (La Montagne & Galasso, 1978; Quarles et al., 1981). A recent consensus meeting in the U.S.A. (summarized by Elliott, 1979) concluded that amantadine and rimantadine had been under-utilized clinically, and that more attention should be given to their use prophylactically and therapeutically. In spite of contention by some, most general practitioners have no difficulty in accurately diagnosing a case of influenza during an epidemic! The early studies in the U.K. with amantadine established quite clearly that treatment of families with amantadine prevented the spread of influenza within the group. Following suggestions at the NIH consensus meeting, investigators have used amantadine to treat cases of primary virus pneumonia. More heroic clinical efforts include the use of amantadine aerosols which deposit the compound in relatively high concentration in the low respiratory tract where it can act immediately (Knight et al., 1979). A similar trial has been carried out with the nucleoside analogue ribavirin. This compound has been well investigated in the laboratory as a broad-spectrum anti-viral agent, but in contrast to the in-vitro data, results from clinical studies have been generally disappointing (reviewed by Smith & Kirkpatrick, 1980). In the most recent trial 14 patients ill with A/England/333/80
(H1N1) virus were given an aerosol of ribavirin for relatively long periods and retained an average of 1.15 g of compound in 23 h of treatment given over 3 days. A highly significant reduction in height and duration of fever, reduction in systemic illness and, perhaps of most importance from the point of view of preventing virus outbreaks, disappearance of virus from respiratory secretions (Knight et al., 1981) was noted.

The techniques of molecular biology mentioned above can also be applied to the prevention of influenza virus by immunoprophylaxis. It should be possible to produce quantities of relevant haemagglutinin or neuraminidase proteins in E. coli although many hurdles remain including the immunogenicity and safety of such material compared to the easily produced virus in embryonated hens eggs. Perhaps more interesting is the use of the monkey virus SV40 as a vector to transfer gene 4 of influenza into a susceptible mammalian cell where influenza haemagglutinin was synthesized and expressed on the cell surface (Gething & Sambrook, 1981). It should be possible to ‘rescue’ relevant influenza HA and NA genes in these systems and if specific mutations can be made in the HA gene, then we have a new approach to producing live attenuated vaccine strains with defined lesions of attenuation. Influenza remains a formidable public health challenge worldwide (reviewed by Stuart-Harris, 1981) and compared to other virus infections such as measles or polio, serious and planned preventative measures involving both chemo and immunoprophylaxis may have to be invoked to combat the disease (Lennette, 1981).

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References


