ANTIRHINOVIRUS DRUGS

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INTRODUCTION

Common colds are among the mildest of diseases affecting man, but they are also very frequent. Indeed, adults are said to have from 2 to 5 episodes a year on the average and certain predisposed children may have 12 attacks a year. The disease is often sufficiently severe to cause absence from work or education. In predisposed patients the colds may go on to bronchitis or may precipitate heart failure or upset the control of diabetes.

It is now over 20 years since rhinoviruses were first cultivated and they were found in cases of typical common colds in adults. It was also found that they caused typical colds when administered intranasally to human volunteers. Thus it was proved that they cause the disease. Later studies showed that they could be cultured from children as well as adults and also from relapses in chronic bronchitis and from acute exacerbations of the wheezy bronchitis syndrome in children.(1) We are still not clear how large their contribution is to the whole clinical problem. Accurate virus diagnosis is not easy. Using a sensitive tissue culture system a virus may be cultured from nasal secretions in about $\frac{1}{4}$ of cases of colds. However some viruses can be cultured in organ cultures of nasal or tracheal epithelium, or in other specially sensitive cell cultures although they do not grow in the usual tissue cultures. There are so many serotypes that serological diagnosis is impractical. Therefore the best estimate of the proportion of colds caused by rhinoviruses is that obtained

E. De Clercq et al. (eds.), *Targets for the Design of Antiviral Agents*

from studies in which ample specimens were collected from cases and a range of highly sensitive test cultures were used. We recently reported such a study from which we conclude that approaching half of typical common colds are caused by rhinoviruses.(2) Although other viruses are involved, particularly in children, the results of tissue culture based studies suggest that a similar proportion of colds in children are also due to rhinoviruses. Since there are over one hundred serotypes of rhinoviruses and all seem to cause colds to a similar extent it is not likely that a useful antirhinovirus vaccine will be developed, but it is plausible that an antirhinovirus drug could be effective although, as we shall see later, rhinoviruses may differ greatly in their response to antivirals as well as to specific antibodies.

Pathology and pathogenesis

Full pathological studies on subjects infected with rhinoviruses have not been done but some general facts have been established. The viruses are specially well adapted to growth in the nasal mucosa, in that they grow best in cultures of the nasal and tracheal epithelial cells and do not attack other cells such as squamous epithelia. They also replicate best at the reduced temperatures of the nose, about 33°. Thus virus replication is apparently confined to the superficial cells of respiratory mucosa - there is no evidence that virus spreads into submucosal tissue or the bloodstream, though it can spread along the mucosal surface and affect the trachea and bronchi.

Virus is not usually detected in the nose on the day after infection, but appears on the second day and it is late on that date or some time on the next that symptoms develop, such as sneezing or a running nose. As we shall see later all these facts are important when deciding how to use an antiviral drug to influence the course of the disease.

The structure and replication of rhinoviruses

Rhinoviruses are in most respects typical members of the picornavirus family(3) and the main features are set out in Table 1. The characteristic 27nm particle has icosahedral symmetry and contains no lipid. The capsid is formed of 4 structural proteins VP1, VP2, VP3 and VP4 and contains a single strand of positive sense RNA. The RNA is translated to form a polymerase as the first step in the replication of the RNA molecule. The whole molecule is however translated and subsequently cleaved by a protease and gives rise to the capsid proteins also. This process is best understood in poliovirus and foot-and-mouth disease virus but something similar certainly happens with rhinoviruses. The synthesis of RNA and protein, cleaving of the peptides and the formation of the particle are all highly integrated and occur at the internal membranes of

Table 1. Some features of the biology of rhinovirus

Particle	- diameter 27nm. Lipid absent. Cubic symmetry. Peptides VP1, VP2, VP3, VP4.
Nucleic acid	- RNA single stranded, positive sense
Replication	- cytoplasmic single translation product, cleaved to provide polymerase and capsid protein

the cell. Virus is then released and passes through the medium to attach to enter another cell through interactions with cell receptors. All these virus specific effects are potential points in replication which an antiviral might block. In addition it is known that the initial attachment and uncoating step is quite specific - at least it appears to involve sites which are quite distinct from those used by other picornaviruses and might in theory be a site of antiviral action.

The search for antirhinovirus drugs

Most of the work in developing antirhinovirus drugs has begun by screening substantial numbers of organic compounds. This was started soon after it became possible to grow the viruses in readily available cells, such as sensitive strains of human fibroblasts and Hela cells. It is still not realised that antirhinovirus tests can be done as easily as, say, antiherpesvirus tests.

A fairly standard array of tests can be used as shown in Table 2. Inhibition of CPE is convenient and can be made relatively quantitative by using serial dilutions of drug in the medium of replicate cultures challenged with a standard amount of virus. Drug sensitivity can also be assessed by adding serial dilutions of virus to cultures maintained in medium alone or in medium containing a dilution of drug; the effect detected is the reduction in the end point of virus titration and the test is particularly useful for comparing the susceptibility of different viruses. It is also valuable to supplement these tests by yield experiments in which virus is grown in drug treated cultures and the amount produced is estimated by titrating in a second system, using end point or plaque assays.

Since some antiviral substances, like interferon, have different effects in different cells it has been found to be important to test at least selected antivirals in cultures of several different susceptible cells, e.g. human lung fibroblasts, and Hela cells. It would be desirable to test them in the cells in which we desire them to act in man, namely respiratory epithelium. This can be done by using organ cultures of human foetal, nasal or tracheal epithelium, and enviroxime was shown to be highly active in such cultures before it was tested in human volunteers.

Table 2. Tests used to search for antirhinovirus activity

A. Inhibition of cytopathic effect a) Roller tubes
b) Microtiter plates
c) Plaque reduction
e.g. 1. titration in presence or absence of drug - single concentration.
2. titration of dilutions of drug with single dose of virus.
B. Reduction of yield
a) Tissue culture
b) Organ cultures of respiratory epithelium

It is also important to test any candidate compounds for activity against a substantial number of different rhinoviruses. This is needed for two reasons. Firstly to find if possible a drug which has high activity against a wide range of virus isolates and serotypes: unless it is effective against most viruses it will have no impact on the clinical problem. Secondly, in planning the first volunteer trials it is important to select a virus with maximum sensitivity to the substance - the tests are often negative and are cumbersome and expensive to repeat so when investigating a new group of compounds it is desirable to do the test with the most favourable virus available.

Mode of action and pharmacokinetics

Once a substance which is potent in vitro has been found it is desirable to acquire a general picture of what effects it may have before giving it to man. Of course appropriate toxicological studies in animals are needed but they will not be discussed in detail here.

It is sometimes thought that the mode of action of the drug should be worked out before it is used. However it is my view that this is not essential, at least not in basic or molecular detail. On the other hand, some general aspects of its effect at the cellular level may be useful in planning its evaluation. For instance there are likely to be advantages in a drug which stops virus replication when added to a cell in which virus infection is already under way if one wishes to try to treat a cold rather than to prevent one. On the other hand the effects of many drugs are readily reversed by removing them from the culture medium. Interferon has the important property of inducing virus resistance that lasts for hours after it has been in contact with the cell and so makes it possible to produce an effect with long intervals between successive intranasal administrations. We therefore would like to have synthetic antivirals whose effect is not annulled by simply removing them from the tissue culture medium.

Finally it can be crucially important to consider factors such as solubility, metabolism and drug distribution. Some drugs e.g. a chalcone, may not be absorbed when given by mouth, and so a prodrug was designed which is absorbed and then hydrolysed to provide the active antiviral in the circulation. Other highly insoluble drugs may not be absorbed in an aqueous formulation but can be absorbed in a non aqueous solution e.g. dichloroflavan in maize oil. However even if it is absorbed tissue distribution may not be satisfactory - it is remarkable that amantadine seems to be concentrated in lung and probably other respiratory tissues. However the dichloroflavan just mentioned is a recent example of a drug which is found in inhibitory concentration in the circulation but at very low levels in the secretions and presumably at very low levels in the nasal epithelial cells, since it is not effective in volunteers against rhinovirus infection. On the other hand if a drug is to be given as a local spray or aerosol its solubility may not matter so much, though insoluble substances may be difficult to formulate.

Background and general considerations

There has been interest in discovering drugs against rhinoviruses ever since it became clear that the organisms were responsible for a substantial proportion of colds about 20 years ago. However the first synthetic drug that was shown to be effective against a respiratory virus was amantadine. It will be recalled that it was shown that oral administration prevented influenza virus infection, first in mice and then in men. Later it was shown to be effective if given therapeutically after the disease had commenced. These observations were important because there was a good deal of pessimism in some quarters about whether antiviral drugs could be effective in any respiratory diseases. It was postulated that the drug might not reach the respiratory epithelium or that once the disease was under way virus replication would already be largely over and so antiviral treatment would have no effect. Yet the fact that amantadine prevented and treated infection in mice and man indicated that these problems could be solved. On the other hand work on rhinoviruses was more difficult as there are many more virus types, no satisfactory animal models have been found and, as we shall see later, antiviral substances are often not effective given by mouth.

There have been various approaches to finding antirhinovirus drugs. Interferon turned out to be a powerful inhibitor of rhinovirus replication in vitro and indeed when, after many failures, it was found over 10 years ago that sufficient doses of human leucocyte interferon, given as repeated nasal sprays, prevented rhinovirus colds. (see 4) This encouraged investigators to look for synthetic antirhinovirus substances and to use them by intranasal administration. However for editorial reasons interferon is excluded from this chapter. Nevertheless we should mention that a good deal of

work was done on interferon inducers during the 1970's as a way of using the interferon mechanism even though interferon itself was in short supply. The idea was to overcome the difficulty of making exogenous infection by stimulating the respiratory tract to make its own. Polyinosinic polycyidylic acid (poly I.C.) is well known and both it and natural double stranded RNA, such as that extracted from the mycophage of Penicillium inhibits virus replication in vitro at very low concentrations. Both were given by intranasal spray to volunteers who were challenged with rhinoviruses.(5, 6) Poly I.C. produced a slight clinical effect but ds RNA not only produced little effect but caused nasal irritation. Understandably little further work was done. However low molecular weight molecules can also act as inducers and were therefore studied. Tilorone was unacceptably toxic but substituted propanediamines were more promising. Indeed two were sufficiently active in vitro and non toxic to be evaluated in volunteers. Little interferon was apparently produced when CP20961 was adminstered intranasally and although some effects against rhinovirus infections were reported this could not be replicated elsewhere and there seem to have been no recent studies. (7, 8)

We should also mention one early study done with a strain of coxsackievirus type A21. This organism is a definite enterovirus by laboratory tests but behaves like a rhinovirus as it multiplies preferentially in the respiratory tract and produces common colds. It was found that it could be inhibited in vitro by low concentrations of fusidic acid and some other related 'steroidal' antibiotics. Sodium fusidate by mouth was therefore used in a trial to prevent infection and disease with coxsackie A21.(9) Although virus inhibitory concentrations were reached in the volunteers' serum no antiviral effect or clinical benefit was seen. The reason appeared to be that the drug was excluded from the respiratory tract; at least it could not be found in nasal secretions collected from treated volunteers who developed colds.

It was also possible to test compounds which were known to be effective against influenza. One case was that of spiramantadine, a derivative of amantadine, which was effective in preventing experimental influenza in volunteers. It was found to be active against rhinoviruses in vitro and so it was then tested as an oral drug in volunteers.(10) However it had no effect - presumably it reached the respiratory epithelium but not in sufficient concentrations to prevent the growth of rhinoviruses which were less sensitive in vitro than influenza A viruses.

A number of isoquinoline compounds have been studied as antivirals and it was shown that two UK2054 and 2371 were active against influenza virus in vitro and subsequently when given orally to volunteers. The fact that they were effective suggested that they were distributed to respiratory epithelium. It was found that they

also had *in vitro* activity against rhinoviruses so it was logical to test them against these organisms in volunteers. However no effect was detected and again it was thought that this was probably because these viruses were significantly less sensitive to inhibition by the compounds than were the influenza viruses.[11] Another isoquinoline (DIQA) obviously was active against a range of viruses in mice although it had no such activity in tissue cultures. It was nevertheless tested in volunteers using rhinovirus 24 as a challenge. There was apparently a reduction of symptoms and virus excretion that was just statistically significant,[12] but I know of no follow up work.

Isoprinosine has been extensively studied as an antiviral - it is a p-aminobenzoic acid salt of inosinedimethyl aminoisopropanol. It has been tested in a number of animal and *in vitro* models. There is little evidence that it is effective though it does seem to be remarkably non toxic. Nevertheless two groups have tested it as an antirhinovirus drug in volunteers, giving it by mouth. RV9 and RV31 were used as challenge in one study[13] and RV44 or RV32 in the other but there was no evidence of significant protection. There was slight enhancement of the antibody response but this did not reach statistical significance either, though the substance is now being promoted under another name as an immune enhancer; and if it does have an effect of this sort it might provide benefit to patients.[14] Indeed Professor S. M. Chu of Beijing tells me that this group is investigating the possibility that some traditional Chinese herbal remedies, (such as *Radix Astragalus membranacea*, which is taken regularly as an aqueous extract to reduce the incidence of colds, and may indeed be effective) may work rather by enhancing immune responses than by directly impairing virus replication.

In contrast there have been extensive studies on the extent and mechanism of the antiviral effects of a number of substituted benzimidazoles. These are active against a number of RNA viruses, including influenza, entero- and rhino- viruses.[15] They have been valuable tools in understanding the mechanisms of virus replication. One of them α hydroxy benzyl, ribofuranosyl benzimidazole (HBB) is active against rhinoviruses *in vitro* and not cytotoxic. However substances arising from this work have not been brought to trial for the prevention of colds. Likewise although zinc ions are powerful inhibitors of rhinoviruses no one seems to have applied these to preventing or treating disease.[16]

This chapter does not give details of all the compounds that have been found at one time or another to have antirhinovirus activity. Instead it concentrates on substances that have, in addition, been found to be sufficiently non toxic, well tolerated and metabolically stable to be used for attempts to prevent infection in volunteers, the side of the work with which I have been particularly associated.

The methods of recruiting, housing and handling the volunteers have already been described in some detail[17] so it is unnecessary to repeat them here. One point is worth making however. In spite of all the efforts in finding potent antivirals it is still true that trials in man usually show no effect, so it has been our policy when investigating a new drug to confirm first that it is well tolerated and then to aim to do an experiment that will give an acceptable negative result, which will not need to be followed by further more stringent trials at the cost of more time and effort. We therefore aim to give the drug by as intensive a regime as seems possible and reasonable. We always give it for about a day before we give virus and continue it for 4 to 6 days. We always use a virus which has been shown in vitro to be the most sensitive to the drug that is available and we always give a small inoculum. The hypothesis we are testing is that with everything in its favour, the drug might reduce the frequency of colds substantially, e.g. by 50% and we want to exclude that possibility at conventional levels of probability, though calculating the power of our trials is not all that easy.

Some specific antirhinovirus compounds

Several substituted isoquinoline compounds were found to be active in vitro against rhinoviruses at concentrations lower than those of substances mentioned above. They are however absorbed and non toxic. A test was done in gibbons which can be infected with certain human rhinoviruses, although they do not show symptoms of respiratory disease, and some activity was detected.[18] Tests in volunteers given drug by mouth (SKF21687 and SKF30097) and challenged with rhinoviruses showed no significant effect.[19] Later a drug (SKF40491) was given by the intranasal route and again no statistically significant activity was found.[20]

At about the same time tests were done with a compound produced by Glaxo laboratories GL R9-338 - it again produced little effect but a reduction in virus shedding that was not statistically significant. Another isoquinoline derivative RP 19326 was given intranasally and produced benefits in clinical symptoms that were just significant. It is important that all these experiments were done using viruses which were found by in vitro experiments to have maximal sensitivity to the drugs. The drugs were also given frequently and the studies were designed to give as clear a negative result as the resources available would allow. However the numbers of volunteers used were quite small and only a strong effect could be excluded with any great certainty. Concentrations of about 4 μg were required for marked antiviral activity in vitro so the results implied that more potent compounds were needed.

Table 3. Trials of prophylaxis at Common Cold Unit

Compound	Route	In vitro inhibitory conc. µg/ml.	Serotype of challenge virus	Number of colds Treated	Placebo
SKF 40491	i.n.	4	3	7/10	4/9
GL R9-338	i.n.	4	9	4/11	5/13
RP 19326	i.n.	4	9	5/11	4/8
Enviroxime LY 122772	oral & i.n.	0.2	9	4/18	6/18*
4'6 Dichloroflavan 683.C.	oral	0.003	9	9/26	10/23
Chalcone Ro 09-0415	oral	0.01+	9	8/28	10/29

*statistically significant reduction in clinical scores and nasal secretion
+of active metabolite

RECENT STUDIES

Recently drugs have become available that are active at concentrations of </μg, a good deal lower than those just mentioned. example is enviroxime, which happens to be a benzimidazole derivative and was selected from a long series of compounds synthesized at the Eli Lilly laboratories. It was not tested for antirhinovirus effects in animals, but because we have experience of antirhinovirus drugs which are active in one virus-susceptible cell and not in another it was tested for its antiviral activity in organ cultures of human respiratory epithelium.(21) It was shown that it inhibited virus growth but that the effect was readily reversed on withdrawing it from the medium. It may act by inhibiting polymerase activity but whatever the mechanism it has similar potency when tested against a wide range of serotypes of virus.

In the trials it was given by both oral and intranasal routes(22) on the hypothesis that if it were active we would be willing to do further experiments to decide which was the effective route. It reduced the symptoms produced by rhinoviruses but it was less effective than interferon; it also had some irritant effects on the nasal mucosa, at least in the original formulation and induced nausea in some subjects. Later experiments indicated that if only the intranasal route was used some effect could be produced though not on every occasion(23, 24) Drug was given intranasally by an intensified regime which was delayed until after virus had been given.(25) Colds were apparently modified if treatment started before symptoms appeared. The final conclusion had to be that although enviroxime is active against a wide range of serotypes it is of limited efficacy in man. It is probably not active if given by mouth since it does not enter secretions after oral administration. It would be reasonable to survey further members of the series to look for some that are secreted after oral dosage and do not produce central nausea or vomiting.

Another compound has been developed in Wellcome Research laboratories, namely a dichloroflavan. This is believed to be the most active antirhinovirus substance yet described, although the exact degree of activity varies from serotype to serotype.(26) It is unusual in that it can inactivate the infectivity of virus particles, probably by interacting with the viral peptides - indeed virus can be reactivated by extraction with chloroform. It is lipophilic but well absorbed as a solution in oil. The blood levels are substantial but when given by the oral route it did not prevent infection with or symptoms of rhinovirus 9 which was particularly sensitive to it.(27) However if an intranasal formulation can be developed it would be worthwhile reinvestigating such a strikingly active substance.

An antiviral chalcone has been developed by the study of an oriental herbal remedy for colds which was found to contain an antiviral activity. Chemically it is not too far from the structure of dichloroflavan and seems to also have the effect of inactivating virus particles.(28) It was too irritant to give by nose and was not well absorbed. However a prodrug, which was inactive but absorbed and then converted to the active form by loss of a phosphate group has been developed. It apparently has no effect in our volunteers when given by mouth. We suspect that this is because it does not reach the respiratory mucosa.(29)

It thus seems that though these substances are more active they are still not potent prophylactics in man. The reasons are complex but it is worth remembering that they are still not as active as interferon and so we are not able to introduce as much antiviral activity into the nose with a synthetic antiviral as is possible when using interferons.

Comment

In spite of the title of this meeting I have referred mainly to drugs found by screening and not by design and I do not think we know enough about virus structure and replication to define our target in molecular terms, as may perhaps be possible with herpesviruses. I have emphasized rather that the drug must be sufficiently active, non toxic and appropriately distributed if it is to show any effect in human volunteers. There are hopes that the faults of enviroxime may be rectified by searching among related compounds for one which, in addition to being active, does not induce nausea and is excreted via the respiratory mucosa. Such a molecule might well be a useful orally active antirhinovirus drug. If a drug is to be useful it must act on many serotypes and it is likely to do this if it acts on the well conserved parts of the particle. It may be significant that enviroxime apparently acts on the polymerase while dichloroflavan apparently affects capsid peptides which are clearly variable, and affects different serotypes to a very different extent.

We can look further ahead and ask whether we might find an antirhinovirus drug that could be used therapeutically, i.e. to modify a cold that has already shown at least some initial symptoms. Preliminary unpublished experiments using interferons suggest that this is not easy to do. It has been suggested by pessimists that this will always be impossible, but amantadine apparently modifies clinically apparent influenza and enviroxime has a slight effect on colds in the incubation period. Thus to affect a cold it might be necessary to have a highly active substance or mixture of substances, which could interupt a virus infection which was already underway. It might be easier to produce an effect in a longer lasting infection like an attack of bronchitis. This would be a worthwhile target for

later work and we should continue to keep it in mind but meantime we should confirm and then apply in the community the successful prophylaxis of colds in inoculated volunteers. For instance, if enviroxime prophylaxis can be shown to work with normal subjects it might be acceptable to patients, such as those with chronic cardio-respiratory disease, to take it regularly, at least for part of the year, in order to reduce the chance of a relapse induced by a common cold. However I suspect that this stage will be reached with interferon before it becomes practical with any of the low molecular weight synthetic antivirals discussed here.

It may also be important to explore better ways of delivering drugs to the affected mucosa for instance by fine particle aerosols. Amantadine and ribavirin are being studied in this way and a recent paper suggests that aerosolized ribavirin can improve the course of bronchitis of infants due to respiratory syncytial virus.(30)

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