The efficacy and tolerance of intranasal interferons: studies at the Common Cold Unit

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Intranasal sprays of interferons (IFNs) given one day before and for three days after virus challenge can protect human volunteers from infection with rhinoviruses, coronavirus, and influenza. Longer dosage of IFN gives rise to nasal symptoms and signs such as bloodstained nasal discharge. More effective IFNs and regimes are therefore needed. IFN β is active but the degree to which it will irritate the nose is unknown. Combining IFNs with synthetic antiviral drugs can produce synergistic increases in antiviral activity. It is suggested that these increases may be exploited in future experiments.

Early investigations in human volunteers

Soon after interferons were discovered it was suggested that they might be used as antiviral drugs and this hope inspired much early work on the production and purification of interferons. It was realized early on that interferons (IFNs), produced by human or similar cells would be needed but early attempts to produce and use them were unsuccessful or only partially successful. However advantage was taken of Cantell's ability to make IFN from human buffy coat cells and enough IFN was administered to show that repeated intranasal sprays would prevent rhinovirus infections of volunteers and also the common colds they cause (Merigan *et al.*, 1973). (Table I). The material was very impure, but later when the NK2 monoclonal antibody became available it was possible to produce the same prophylactic effect with similar IFN freed of all detectable impurities (Scott *et al.*, 1982*b*).

About this time the first IFN genes were cloned and as a result IFN α 2 produced in *Escherichia coli* became available. This was important first because it protected against rhinoviruses, proving that the antiviral effect was due to IFN and not to some unrecognized contaminant (Scott *et al.*, 1982*a*), and second because experiments were no longer limited by lack of IFN. Later it was shown that IFN α also protected against corona-virus 'colds' (Higgins *et al.*, 1983) and that lymphoblastoid IFN protected against rhinovirus 'colds' and also against influenza A infections, though less well (Phillipotts *et al.*, 1984). The observations on rhinovirus infections of volunteers and recombinant IFN α 2 have been confirmed in other centres, and it is also confirmed that influenza infections are less susceptible. Attempts to treat colds by administering IFN sprays after symptoms developed have so far been unsuccessful. These results have been confirmed by a number of volunteer studies in the U.S.A.

Dosage and response to interferon

Little was known about the minimum dose of IFN required to produce an antiviral effect and it therefore seemed reasonable to try to prevent naturally acquired colds by giving

Table I. Results of some typical trials at the Common Cold Unit using interferons (IFNs) against respiratory viruses

Virus given	IFN	Doses per day	No. of days	Dose (MU)	Subjects ill		Reference
					Treated	Placebo	
RV4	Leucocyte	9	4	0.36	1/16	5/16	Merigan <i>et al.</i> (1973)
RV9	Leucocyte immuno purified	9	4	5	0/18	8/11	Scott <i>et al.</i> (1982 <i>b</i>)
RV9	Rec α 2	9	4	5	0/19	8/22	Scott <i>et al.</i> (1982 <i>a</i>)
Coronavirus	Rec α A	3	4	2.7	2/35	13/35	Higgins <i>et al.</i> (1983)

small doses of IFN, a few megaunits, or about 0.01 mg, daily. Several independent studies have been carried out with both leucocyte and recombinant IFN α and from them all the same conclusions can be drawn (see Scott & Tyrrell, 1985). A sufficient dose of IFN does indeed prevent infection, but that dose also produces, after two weeks or so, unacceptable nasal stuffiness, irritation and discharge, and sometimes epithelial ulcers and spotting with blood. This is understandable since IFNs are all apparently pleiotropic substances with many of the effects of lymphokines and they induce inflammation when injected intradermally. However, the unwelcome conclusion is that although they are nontoxic *in vitro* in clinical use against mild upper respiratory infections they have a therapeutic index of about one.

Synthetic and modified interferons

Thus after a period of irrational optimism about the possible application of IFNs in colds we now have to check against an equally irrational pessimism. The first point to make is that so far we have tested only natural mixtures of IFN α or cloned IFN α 2. There might well be another natural IFN that produces less inflammation at the dose required to prevent infection. Since a reduction of only two- or three-fold in the dose of IFN almost eliminates the local adverse effects the ratio of another molecular species may need to be increased only a few fold in order for it to be acceptable in clinical use. We have now found that IFN β_{ser} is about as active an antiviral as IFN α s (unpublished results) and so it should now be tested to see if it produces less adverse effects. We propose to examine IFN γ in the same way. Of course, by generating hybrid IFN molecules, or modified proteins, following site-directed mutations in the genes, or by chemical synthesis of alternative molecules it might be possible to produce a wide range of IFN-like molecules, some of which could have the desired combination of properties. The problem is that chemistry and genetics have advanced very rapidly but we still have no validated biological test for the characteristics of the IFN molecule which are responsible for the undesirable effects. Until suitable tests are found it will not be possible to select rationally from the many molecules now available, or which could be generated, the one or two which should be investigated clinically.

Combinations of interferon with specific antivirals

There is another approach to the problem and that is to combine IFNs with other drugs. One might add an anti-inflammatory drug but this could create as many problems as it solved. We have investigated the idea of combining antivirals, including IFNs, in order to find pairs which show a synergistic enhancement of their antiviral activity without a corresponding increase of their unwanted effects; such combinations might also circumvent a potential problem which is the subject of this supplement, drug resistance. Incidentally it is interesting that although viruses do vary in their sensitivity to IFNs I know of no reports that IFN-resistant mutants have appeared, though they might have been overlooked and it was at one time suggested that one of the factors selected for in the development of attenuated or virulent strains might be sensitivity or resistance to the effect of IFN.

L. Ahmed (personal communication) has found that synergy between antirhinovirus drugs can often be detected *in vitro* and among the most striking examples are the enhancement of both activities which is produced by combining IFNs and dichloroflavan, the chalcone Ro 09-0410 or enviroxime. The enhancement is shown by reduction of

cytopathic effect and decrease in virus yield both from tissue cultures and from organ cultures of human respiratory epithelial cells. The effect is seen over a wide range of concentrations of both drugs and can be large in size – for instance there is at least as much reduction in virus yield on combining 1/64 MIC of enviroxime with 1/64 of IFN α as there is with one MIC of each alone. There is no evidence of enhanced toxicity in cells treated with the combination.

There is clearly a need to find out whether this phenomenon of synergy can be demonstrated in man by preventing rhinovirus infections in volunteers. If it can then there will be great scope for finding out what regimens provide the most benefits, minimize the amount of drug used and any unwanted effects, and also reduce the risk that drug-resistant mutants will appear.

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