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Interferon prophylaxis of the common cold

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Interferon is a potential prophylactic agent for the common cold. But there are problems. The present levels of side-effects that have been observed don't justify its use in the long term. Robert Phillpotts describes the mechanism of interferon action and the future hopes and developments for its use in preventing colds.

Interferon appears to be the ideal antiviral drug for use in preventing colds; it is extremely potent, and active against a wide range of viruses. However, it is too toxic for systemic use in minor respiratory illnesses, and when taken in adequate dose, unusually it causes a transient inflammation of the mucosa. Current research is directed towards overcoming this problem.

For most people the common cold is a mild, self-limiting illness. However, the high incidence of colds, and their ability to cause an exacerbation of chronic underlying respiratory disease leads to considerable morbidity. Research has established that over 200 serologically distinct viruses can cause a cold, and there is little hope that infection can be controlled by vaccination. Therefore control by means of a broad-spectrum antiviral agent is the most rational approach, and interferon is without doubt the most potent, and broadly active antiviral agent yet discovered. Of course there are limitations upon the kind of medication which could be used to prevent a cold. For example it should be cheap and easy to manufacture, as well as being highly effective, and virtually free from side-effects.

What is interferon?

Human interferons (HuIFN) are proteins or glycoproteins of which there are 3 principal types, α, β, and γ. HuIFN α and β are both produced by cells after exposure to a virus; IFN α by leucocytes and IFN β by fibroblasts. HuIFN β is also produced by fibroblasts exposed to double-stranded RNA, while IFN γ is produced by lymphocytes only in response to an antigenic or mitogenic stimulus. Interferons do not directly inhibit virus growth, but exposure of uninfected cells to interferon makes them resistant to attack by viruses. Interferon is not internalized by cells but binds to cell surface receptors (there is one receptor for IFN α and β, and another receptor for IFN γ) triggering a series of events within the cell. Multiple effects have been observed in cells, including the inhibition of penetration and maturation of certain viruses. However, the principal effect of interferon treatment seems to be to inhibit viral protein synthesis. A system of interferon-induced enzymes has been described, some of which are activated by double-stranded RNA molecules such as those produced during the replication of RNA viruses. The effect of these enzymes is to inhibit the initiation of viral protein synthesis, and to increase the rate at which viral mRNA is degraded within the cell (see Fig. 1). Future research will undoubtedly disclose other mechanisms by which virus growth is prevented. There is already evidence to suggest that IFN γ has a different mechanism of action from IFNs α and β, as combinations of IFN γ with IFN α or IFN β (but not IFN α with IFN β) exhibit synergy.

The potency of various interferon preparations may therefore be compared in terms of units of antiviral activity. There is species specificity in this process v. while not absolute, means in practice that human interferon must be used to treat human cells.

Human interferon may be produced from cells cultured in vitro. A partially purified human leucocyte (α) interferon is produced by the Finnish Red Cross from buffy coat cells (derived from blood donations) which have been stimulated with Sendai virus. However, this procedure is expensive, and the amount of interferon which can be produced is limited by the availability of buffy coat cells. More recently, DNA copies of the mRNAs coding of all 3 types of HuIFN have been synthesized, and inserted into plasmid vectors downstream from a suitable prokaryotic promoter. Such plasmids are introduced into bacteria by transfection, where they multiply, and high levels of expression of the interferon gene sequence can be induced (Ref. 4 provides an excellent introduction to this subject). Bacteria may be cultured relatively easily in large-scale fermenters and so, potentially at least, all 3 types of HuIFN can be produced in large amounts at a much lower cost. Modern interferon preparations are of very high purity, and often have a specific activity in excess of 100 mega units (MU) per mg of protein. Although production of these interferons is still not
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cheap, costs will undoubtedly be further reduced.

**Intranasal interferon prevents colds**

In an initial experiment at the Common Cold Unit in Wiltshire, UK, intranasal administration of partially purified human leucocyte interferon to volunteers was shown to prevent colds caused by rhinovirus type 4 (Ref. 5). Rhinoviruses cause approximately 50% of colds. However, leucocytes from 30-50 donations of blood were required to produce the 14 MU of interferon used to treat each volunteer. Furthermore, the use of partially purified material in which only about 1% of the protein was interferon itself was responsible for preventing rhinovirus infection. Could the activity have been due to contaminating, biologically active molecules also derived from human leucocytes? This question was answered in a further experiment carried out in 1982, in which HuIFN α, purified to virtual homogeneity on a monoclonal antibody affinity column was shown to prevent colds caused by another rhinovirus, type 9 (Ref. 6). A total dose of 90 MU was given intranasally to each volunteer over a period of 4 days (12 doses, 3 doses daily). Four doses were given before, and 8 after virus challenge. There was a dramatic reduction in the frequency of colds in the interferon treated group (Table 1). This was accompanied by lesser reductions in the number of volunteers who shed virus, or who had an increase in serum neutralizing antibody to the challenge virus. In a further experiment conducted under an identical protocol, closely similar results were achieved using highly purified, HuIFN α-2, produced in *Escherichia coli* (manufactured by Schering Plough). This experiment clearly demonstrated that interferons produced in bacteria by recombinant DNA techniques could be as active as those produced by human cells.

**How can interferon be used in practice?**

In these experiments, interferon was given intranasally in large doses by a physician. Therefore if interferon is to find application as a prophylactic against the common cold, two questions have to be answered. Firstly, could people treat themselves with interferon using a simple design of nasal spray? Secondly, what is the minimum quantity of interferon necessary to protect against infection with cold viruses? Answers to these questions were sought in a dose-ranging study, in which volunteers gave themselves various doses of HuIFN α-2 using a finger-actuated nasal spray. Interferon was given for one day before, and 3 days after, challenge with virulent rhinoviruses. Not only was the dose of interferon varied, but also the time of virus challenge in relation to a dose, so that the period of maximum vulnerability to virus infection could be identified. The results of this study suggest that at least 3-4 MU of IFN α-2 self-administered intranasally 3 times daily are necessary to protect against experimental rhinovirus infection. Subsequent experiments have shown that 3-4 MU of HuIFN α-2 (a similar molecule to HuIFN α-1, produced by Roche) can protect against experimental infection with a human respiratory coronavirus (see Fig. 2). Coronaviruses are the second most frequently encountered cause of a cold, and are responsible for 15-20% of cases.

**Problems**

However, one more requirement must be met by a prophylactic against the common cold - almost complete freedom from unpleasant side-effects. It is here that research has run into difficulties. Further studies have shown that while regimes of IFN α similar to that proposed seem to be necessary for protection against natural colds, such doses are also toxic; and produce a mild inflammation of the nasal mucosa. In a recent study from the University of Virginia, prolonged intranasal administration of the HuIFN α-2 was associated with mucosal irritation in 23% of recipients. Histologically, marked epithelial acute inflammation with ulceration occurred in 19%, and 58% had pronounced submucosal lymphocytic and mononuclear cell infiltrates. Although these abnormalities resolved within 8 weeks after stopping treatment, long-term administration of IFN α would not be acceptable. However, there has been little sign of irritation of volunteers given interferon for 4 days. Therefore interferon prophylaxis could be used when the time of exposure to virus (or fear of exposure to virus) can be predicted. Examples of this situation would be contact with a cold sufferer, or before an examination or some other important event.

**Future research trends**

There is every reason to believe that the problem of poor tolerance to intranasal interferon will be overcome. Only a very small number of interferons have been tested for antiviral activity and toxicity in the nose, and it is conceivable that a molecular subspecies of HuIFN α, HuIFN β or HuIFN γ, or a hybrid interferon molecule prepared from

**TABLE 1. Clinical and laboratory findings in rhinovirus type 9-challenged volunteers**

| Treatment (n) | Nil or doubtful cold | Mild, moderate or severe cold | Virus isolation<sup>a</sup> | Antibody rises<sup>b</sup> |
|--------------|----------------------|-------------------------------|---------------------------|--------------------------|
| Interferon (8) | 8                    | 0<sup>b</sup>              | 5<sup>b</sup>             | 3<sup>b</sup>          |
| Placebo (11)  | 3                    | 8<sup>a</sup>              | 10<sup>a</sup>            | 6<sup>a</sup>          |

<sup>a</sup>: a four-fold or greater rise in the titre of serum neutralizing antibody to HRV9
<sup>b</sup>: P = 0.004 (Fisher's exact test)
<sup>c</sup>: P > 0.05 (Fisher's exact test)
these, may have an improved therapeutic ratio. Interferon could then be taken for prolonged periods as a prophylactic against the common cold by patients with underlying chronic respiratory disease, such as bronchiitis or asthma. Not surprisingly therefore, the discovery of interferons with this desirable property is one of the major goals in common cold research today. The question of whether interferon can be used to treat a cold also remains open. The relatively short course of the illness suggests that virus replication in the nose occurs rapidly, and may even be essentially complete by the time symptoms have begun. However, this pessimistic view has yet to be confirmed, and there is some evidence to suggest that even a relatively weak antiviral agent, such as Enviroxime (registered trade name, produced by Eli Lilly and Company), administered locally after virus infection can affect the course of a cold. Perhaps a suitable preparation of interferon, which could be over 1000 times more potent than Enviroxime in vitro, could prove clinically useful.

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The role of peptides in feeding

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Morley, Gosnell and Levine discuss the variety of peptides that have been demonstrated to play a role in the modulation of food intake. In particular, the endogenous opioid peptide, dynorphin, represents one of the major neurotransmitters involved in the initiation of feeding. Corticotrop releasing фактор is important in the pathogenesis of stress-induced anorexia and may have an etiological role in anorexia nervosa. Calcinin gene related peptide is also a potent central satiety factor. A number of gastrointestinal peptides including cholecystokinin, bombesin, glucagon, and somastatin have been shown to provide important input as satiety signals from the gut. The integrative action of many of the neurotransmitters involved in appetite regulation can be conceived as a satiety cascade similar to the classical cascade systems regulating blood clotting and complement fixation. But the authors stress that our increased knowledge of satiety mechanisms has not simplified concepts of pharmacological appetite suppressants.

The regulation of food intake is an extremely complex process, and it is therefore not surprising to discover that it is regulated by a variety of mechanisms. Over the last decade, it has become increasingly clear that a number of peptides play an integral role in this regulatory process. In particular, the endogenous opioid peptides have been identified as major stimulators of feeding, and a variety of gastrointestinal peptides have been shown to mediate some of the satiety signals linking the gut to the brain. Most of the techniques used to elucidate the role of peptides in feeding have involved classical pharmacological approaches and as such the physiological relevance of some of the newer findings must remain in doubt. These studies, however, have opened a Pandora's box of potential pharmacological agents for the treatment of obesity and anorexia. This brief review will concentrate on the role of peptides as modulators of appetite. The influences of other neurotransmitters and the physicochemical properties of feeding will, therefore, receive less prominence.

At the outset it should be stressed that the complexities of appetite regulation make it extremely unlikely that any single agent will prove to be the Holy Grail of appetite suppressants. Future pharmacological approaches will need to take cognizance of this fact and attempt to tailor the treatment to the individual rather than the individual to the treatment. This is particularly important in view of the fact that the majority of peptides appear to have multiple effects, making it likely that the indiscriminate use of these agents, in subjects in whom a deficit or an excess has not been demonstrated, will lead to an unacceptably high incidence of side effects.

Opioid feeding systems

A large body of evidence has now accumulated supporting a role for opioid peptides in the modulation of feeding behavior. In particular, the relatively specific opioid antagonist, naloxone, has been demonstrated to decrease feeding under a variety of conditions in many species including humans. In humans, however, the major effect of opioid blockade with naloxone appears to be to reduce carbohydrate intake rather than to produce an absolute reduction in calories. This reduction is offset by an increase in fat intake. In addition, the first long term study in humans by Atkinson using the long-acting opiate antagonist, naltrexone, produced disap-