MODULATION OF ANTIVIRAL ACTION OF INTERFERONS BY AMINO ACIDS

PhD Thesis

SUMMARY

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Interferons (IFNs) have a wide range of biological activities. Some of them -- like their anti-inflammatory, antiviral and antiproliferative properties -- are utilised in the clinical practice for therapeutic purposes. Interferon treatment is used in a selected range of tumours, in inflammatory type autoimmune diseases and in chronic, recurrent or life-threatening viral infections.

Interferon therapy, however, has severe limitations. At the doses applied in clinical practice numerous side effects may occur. Some of the major dose-limiting symptoms are cardiotoxicity and provocation of exacerbations in autoimmune diseases for IFN-γ, and flu-like symptoms, alopecia and neurotoxicity for IFN-α. Both IFN-s may cause myelosuppression, toxic shock syndrome and in rare cases development of certain autoimmune diseases. Furthermore, prolonged use of recombinant interferons may lead to appearance of anti-IFN antibodies.

In the last decade serious efforts were made to reduce these unwelcome side effects, meanwhile maintaining the desired therapeutic activity. An especially promising approach to the problem is the selective suppression of side effects (e.g., compensate myelosuppression by MGCSF) or potentiation of the required aspect of the biological activity (e.g., hyperthermia, which enhances the antiproliferative, but not the antiviral effects).

The present study is focused around a similar approach to augment selectively the antiviral activity of IFNs by certain amino acids.

The author have found that amino acids can influence the antiviral activity of all types of human IFNs in a dose dependent manner. Altough the required doses are very high (5-10 mg/ml), it was clearly proven that the modulation is due to specific amino acid effects. These effects may be complete inhibition (as by Phe), reduction (e.g., Cys, Glu) or augmentation (Asp, Ser, Tyr, etc.). The type and extent of interaction is dependent upon the IFN type as much as upon the amino acid chosen. Synergically augmented IFN-α : IFN-γ mixtures may further be potentiated by proper amino acids up to 10-12-fold of the nominal titre.

Pairing of amino acids revealed further interactions among the amino acids themselves indicating that their individual mechanisms of action during the IFN-induced antiviral cascade differ considerably. Application of certain amino acid -- IFN combinations (e.g., Asp : Ser : HuIFN-α : HuIFN-γ) resulted in a 20 to 40-fold enhancement of the nominal titre.

The antiviral microplate assay (see: *Mécs & Béládi;* Proceedings of the Symposium on Preparation and Clinical Use of Interferon, Zagreb, 1977; pp. 23-26.; Ed.: D. Ikic, 1977) which was used in the majority of the experiments can be divided into three phases. These phases might be considered as three singular biochemical entities differing from

each other. In Phase I. test cells are in exponential growth and their synthetic apparatus is attuned to proliferation. In Phase II. resting cells in monolayer stage are induced by IFN to perform biochemical reactions leading to antiviral state. In Phase III. cells are infected with a challenge virus and cell-governed events compete with virus-regulated ones. As it turned out an amino acid applied in different phases might modulate the antiviral effect of IFNs altogether differently. E.g., Cys has no influence on the antiviral activity when applied in Phase I. In Phase II. it has a weak inhibitory effect, while in Phase III. it is a good enhancer. Therefore amino acid effects are also phase-dependent. Phase III. and postinfective („late Phase III.") data are especially interesting since therapeutic applications are postinfective events. From this aspect the extremely strong (80 to 150-fold) augmentation by Asp and Opr are most notable.

Successive amino acid combinations may result in an even further enhancement of the antiviral activity (up to 200-400-fold using Ser : Asp or Tyr : Asp in Phases II. : III. respectively). It is also of special interest that Ser in Phase I. : II. or Asp in Phase II. : III. successive applications are capable of self-synergism indicating phase-dependent differences in their mechanisms of action.

As a by-product of the phase-restricted application of amino acids a further observation was made. Certain amino acids (Asp, Cys, Phe, Ser) applied in Phase I. sensitized the test cells for a direct cytotoxic effect of HuIFN-γ. The full sensitization required approximately 24 hrs. Cell death occurred in 90 mins after addition of HuIFN-γ. No toxic effect was observed when the cells used were resistant to the antiviral action of IFNs (any type). Cells recovered from a sensitive line after incomplete sensitization followed by HuIFN-γ killing lost their antiviral sensitivity. These data indicate, that survival is connected to antiviral resistance possibly due to loss of some key element in the IFN-response pathway.

The mechanism of toxicity is probably based on overproduction of H_2O_2 by the sensitized cells upon HuIFN-γ induction. This theory is supported by the results showing that presence of HuIFN-α prevents cell death. HuIFN-α enhances superoxide dismutase activity of the cells thus rendering them resistant to excess amounts of H_2O_2. It also saves target cells from being killed by exogenous H_2O_2 activating the same protective mechanism: a 250-fold higher H_2O_2 concentration is required to kill HuIFN-α-pretreated cells.

Amino acid effects on antiviral activity are not restricted to human IFNs. They are also active on homologous bovine, porcine and chicken IFN - test cell systems. However, the spectra of active amino acids in different species' are also different. Thus, amino acid effects on antiviral activity of IFNs are species specific as well.

In heterologous test systems (where IFNs and test cells are from different species) a species dominance order can be observed. Bovine-specific effects rule out human or

porcine-specific ones no matter wether the IFN or the cell line has bovine origin. on the other hand, human type actions are stronger than porcine specific ones.

Amino acid modulatory effects are also not limited to antiviral action of IFNs against Vesicular stomatitis virus. Inhibitory or enhancing influences of amino acids can be observed too, when IFNs are used against Lymphocytic choriomeningitis virus, polyoviruses or human and porcine herpesviruses.

Furthermore, the effects of other antiviral compounds -- like thymidine-analogous drugs -- can also be strongly augmented by addition of Asp or Ser. An extremely strong interaction was observed between IDU and Ser resulting in at least 10^4-fold augmentation of antiherpetic activity of IDU.

Since these thymidine analogues have very similar chemical composition [5-iodo-2'-deoxyuridine (IDU), 5-ethyl-2'-deoxyuridine (EDU), 5-izopropyl-2'-deoxyuridine (iPDU), 5-bromovinyl-2'-deoxyuridine (BVDU)], and cross-resistance to them is regularly observed, it is supposed that they have a common mechanism of action. Marked differences in their responses to Asp and Ser, however, indicate that they must exert their antiviral action on at least partially different ways.

The data obtained from the experiments above pointed out some possibilities for direct practical use of the amino acid application in the interferon research. One of them is to detect IFNs in concentrations under sensitivity level of the test system by amplifying their antiviral activity. The method was applied to serum samples from uremic patients to prove the presence of subdetectable quantities of either IFN-α or IFN-γ.

Another practical application was to characterize native HuIFN-α samples for their subtype composition using differences in amino acid sensitivity spectra of individual subtypes. The method can be used to compare native HuIFN-α preparates from commercial sources for subtype composition and possibly for therapeutic values. Purification procedures can also be monitored for possible subtype losses as well as for reproducibility.

While it is clearly proven that antiviral activity of IFNs can be enhanced by addition of selected amino acids to an extent which promises good clinical results at well tolerable doses, it have also been shown that no other major IFN effects are altered by their interactions. The best candidates for therapeutic antiviral combinations were tested for their effects on antiproliferative and NK-enhancing activities of IFNs. No significant differences could be observed in either of these activities in the presence of the tested amino acids plus IFNs when compared to the controls treated by IFNs alone.