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PHARMACOKINETIC COMPARISON OF LEUKOCYTE AND *ESCHERICHIA COLI*-DERIVED HUMAN INTERFERON TYPE ALPHA

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Human alpha interferons derived from leukocytes (HuIFN-α-Le) and from an *Escherichia coli* clone (HuIFN-α_1) were compared for their relative abilities to distribute systemically in mice following intramuscular or intraperitoneal injections, and for their rate of clearance from the blood. HuIFN-α_1, which is about 30–50 times more active on bovine cells than on human cells, was as efficient as HuIFN-α-Le at entering the bloodstream following injections by either route. However, HuIFN-α-Le was still present in the circulation at about 30% of peak serum IFN levels at 8 h post-inoculation and was present at about 3–5% at 24 h, whereas HuIFN-α_1 was present at only about 3% of peak IFN serum levels at 8 h and was undetectable (< 1%) at 24 h. These data indicate the importance of evaluating the pharmacokinetic properties of each of the molecular forms of interferons obtained from genetically engineered organisms, as each molecular species of interferon may have importantly distinct pharmacologic properties.

pharmacokinetics interferon

Human interferons alpha (HuIFN-α) and beta (HuIFN-β) have been demonstrated to exhibit markedly different pharmacologic properties: HuIFN-α has been shown to be relatively efficient at entering the bloodstream following intramuscular, intraperitoneal or subcutaneous injection into mice, rabbits or man, whereas HuIFN-β is inefficient [3, 4, 15]. Also, even though several molecular forms of HuIFN-α efficiently distributed systemically following intramuscular injections, each was cleared from the bloodstream at significantly different rates [12].

The successful introduction of the human interferon genes in expression plasmids of *Escherichia coli* [2, 6, 11] and in yeast [7] has rendered possible large-scale production of interferon at low cost. Moreover, the application of this new technology has led to the identification of several distinct HuIFN-α forms [5, 9, 10, 14]. We have, therefore, undertaken to evaluate the alpha interferon subtypes for their relative pharmacological merits. This report compares native HuIFN-α derived from leukocytes (HuIFN-α-Le) and the first *E. coli*-derived human interferon (HuIFN-α_1).

The interferons were each injected into mice at specific activities of approximately 10^6 units/mg protein: HuIFN-α-Le was prepared and purified by Dr. Walz according to

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the Cantell method [1]. HuIFN-α_1 was purified by antibody chromatography as described previously [13]. Mice (three per group) were injected either intramuscularly or intraperitoneally with 0.1 ml or 0.3 ml, respectively, of the interferon suspensions. At indicated intervals, mice were bled from the orbit and sera were assayed for interferon activity on human (GM2767) and bovine (MDBK) cells [8]. Briefly, cells were incubated for 24 h with two-fold serial dilutions of serum samples or IFN standard in a humidified 5% CO_2 incubator at 37°C and then challenged with vesicular stomatitis virus (1 p.f.u./cell). The IFN titers are expressed as reciprocal of the limiting dilutions of the serum samples giving 50% protection from the viral-induced cytopathic effect and are adjusted against parallel IFN standards.

HuIFN-α_1 is approximately 30–50 times more active on bovine cells than it is on human cells [13]. When mice were injected with amounts of HuIFN-α_1 and HuIFN-α-Le that were equal in terms of activity on bovine cells (Fig. 1), the same peak serum IFN levels (as assayed on bovine cells) were attained at about 2 h with both HuIFN-α-Le and HuIFN-α_1; however, by 4 h, the levels resulting from injection of HuIFN-α_1 were much lower than those resulting from injection of HuIFN-α-Le.

Fig. 1A shows the results obtained when the same serum samples were assayed on human cells. As expected, injection of HuIFN-α_1 yielded about 30 times lower activity on human cells than on bovine cells. In contrast, injection of HuIFN-α-Le gave comparable serum activities when assayed on either of the two cell types (Fig. 1A, B).

When mice were injected with either of the two human IFNs in amounts that were equal in terms of activity on human cells (Fig. 2), again the same peak serum IFN levels (this time as assayed on human cells) were attained at 2 h with both IFNs (Fig. 2A). Again, the HuIFN-α_1 levels decreased much faster than did the HuIFN-α-Le levels. When

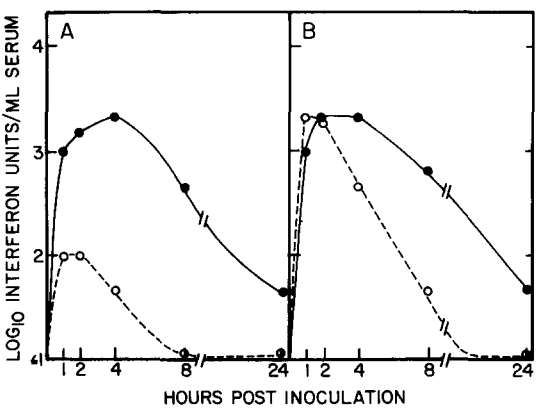

Fig. 1. Serum interferon levels in mice inoculated intramuscularly with human-alpha interferons from leukocytes or *E. coli*. Mice were inoculated intramuscularly with 0.1 ml of samples titrating about 10^5 units/ml when assayed on bovine MDBK cells. Sera collected from the orbit at indicated times post-inoculation were assayed on A) human cells or B) bovine cells: HuIFN-α-Le (●); HuIFN-α_1 (○). Plotted points represent the mean interferon levels from separate determinations from three animals.

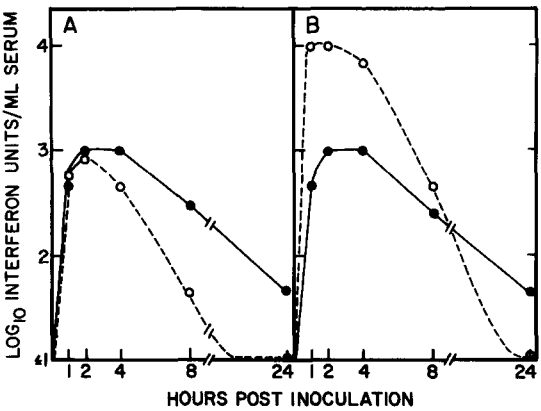

Fig. 2. Serum interferon levels in mice inoculated intraperitoneally with human-alpha interferons from leukocytes or *E. coli*. Mice were inoculated intraperitoneally with 0.3 ml of samples titrating about $10^{4.5}$ units/ml when assayed on human cells. Sera collected at indicated times post-inoculation were assayed on A) human cells or B) bovine cells: HuIFN-α-Le (●); HuIFN-α_1 (○). Plotted points represent the mean interferon levels from separate determinations from three animals.

these sera were assayed on bovine cells (Fig. 2B), the bovine cell activity in sera of mice injected with HuIFN-α_1 was initially much higher than that in sera of mice injected with HuIFN-α-Le. However, at later times these ratios reversed, with only HuIFN-α-Le being detectable at 24 h.

These data demonstrate that HuIFN-α_1 derived from *E. coli* is as efficient as native HuIFN-α derived from leukocytes at entering the bloodstream following intramuscular or intraperitoneal injections. However, HuIFN-α_1 was cleared from the bloodstream more rapidly than was the 'natural' HuIFN-α-Le. The latter is, of course, itself a mixture of various HuIFN-α forms which differ in size, cross-species activity, and in clearance rate [12]. Therefore, HuIFN-α_1 may represent a form of interferon present in natural interferon preparations which is selectively cleared more rapidly from the mixture. Moreover, it now has become known that the HuIFN-α_1 used in these studies was a 'partial-pre-IFN-α_1', as it contained six additional amino acids at the N-terminus, and should be designated HuIFN-α_1-L_6. Whether this additional peptide alters the clearance properties of HuIFN-α_1 remains to be seen. It may also be possible to obtain a particular species of HuIFN-α from an *E. coli* clone, which may be more stable in vivo than HuIFN-α_1.

Our findings demonstrate that animal studies with native and cloned IFN species are essential to understand the in vivo stability and/or pharmacological properties of cloned human interferons.

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