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Clinical Trials of a Recombinant Gamma Interferon 1. Effects of the Gamma Interferon Administered Intramuscularly or by Inhalation on Circulating Interferon and 2,5-Oligoadenylate Synthetase and Protein Kinase Activities

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The development in the CIS of recombinant human interferon preparations has been a major achievement of great medical significance [1-3,5]. A recombinant alpha-2 interferon (Reaferon) is already being widely and successfully used for the prevention and treatment of viral diseases [4]. A recombinant gamma interferon (Gammaferon) is now undergoing initial clinical trials. In order to evaluate its clinical efficacy, it is essential to know how it influences the interferon system of the body. The most reliable indicators of this influence are the interferon-dependent enzymes 2,5-oligoadenylate synthetase and protein kinase which are detectable in blood samples and which, together with circulating interferon, the interferon reaction of leukocytes, and the activity of natural killers, can provide the necessary information on the functional activity of the interferon system [7,10]. The two above-mentioned enzymes preeminently characterize the antiviral activity of interferon [11].

Previously, we showed that Reaferon and Larifane (the dsRNA of phage F2) are capable of activating the interferon system in volunteers [6]. In this communication we present the results of a study carried out to compare the effect of Gammaferon on the interferon system of volunteers after its administration intramuscularly and by inhalation.

MATERIALS AND METHODS

A Gammaferon preparation with an activity of 30,000 IU per ampoule, manufactured by the Ferment Company of the Ministry of the Biomedical Industry, was tested in the Clinical Department of the Ivanovskii Institute of Virology at the request of the Pharmacological Committee. The trials were conducted on 3 groups of volunteers - a total of 20 men recovering from influenza or acute respi-

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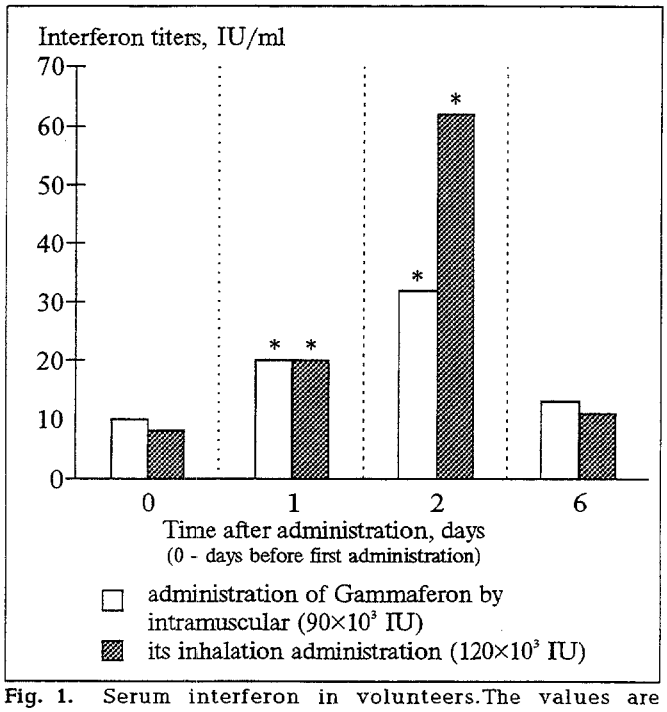

Fig. 1. Serum interferon in volunteers.The values are arithmetic means per group. Significant differences ($p<0.05$) are indicated by asterisks.

ratory infection. The first group were administered 120×10^3 IU of Gammaferon twice daily by inhalation (at 08:00 h and 17:00 h) for 5 days, while the second and third groups were given it intramuscularly at 90×10^3 and 30×10^3 IU per injection, respectively, twice daily (at 08:00 h and 20:00 h), also for 5 days. Fresh blood samples from the volunteers and from healthy donors ($n=9$) were assayed for the activities of serum interferon (IFN) and of the enzymes 2,5-oligoadenylate synthetase and protein kinase [6]. The blood samples were fractionated into serum, plasma, and lymphocytes by the standard procedure in a Ficoll density gradient. Blood was sampled into a heparin solution on the day before the start of Gammaferon treatment (day 0) and then at various times on days 1, 2, and 6 in group 1.

Interferon was titrated by a micromethod using the vesicular stomatitis virus as the test virus. Activities of the enzymes and serum IFN were determined simultaneously in each group in parallel with their determination in the healthy donors. The antigenic specificity of circulating interferon was established in a neutralization test with a purified monoclonal antibody produced to the recombinant gamma interferon by the Ferment Company. The results were treated statistically by Student's t test.

RESULTS

Administration of Gammaferon by the intramuscular and inhalation routes in doses of 90×10^3 and 120×10^3 IU, respectively, led to a 5-fold increase of serum IFN over the baseline level on day 1 and to a nearly 10-fold increase to 40-60 IU/ml on day 2. After the first administration of Gammaferon, the circulating IFN reached its highest level in 6 h, and repeated administrations stabilized its elevated levels. The used schemes of Gammaferon treatment by the intramuscular and inhalation routes thus brought about a stable rise of serum IFN activity. In some cases, the inhalation route resulted in even higher rises of serum IFN after repeated Gammaferon administration. As was indicated by the neutralization test with the monoclonal antibody, the detected circulating IFN was of the gamma type. The time course of gamma IFN accumulation in the serum of volunteers in this study was similar to that reported previously [8] from a study on monkeys and rabbits injected intramuscularly with 300×10^3 IU of natural human gamma IFN.

Figures 1, 2, and 3 present the results of assays for serum IFN and 2,5-oligoadenylate synthetase and protein kinase activities in volunteers given Gammaferon by inhalation or intramuscularly. Both these routes of administration resulted in significant rises of 2,5-oligoadenylate synthetase activity, which correlated well with the elevations of circulating IFN levels on days 1 and 2. The rise in this enzyme activity with the inhalation route was even higher than with the intramuscular. On day 6, 36 h after the last (10th) administration of Gammaferon, the IFN level and

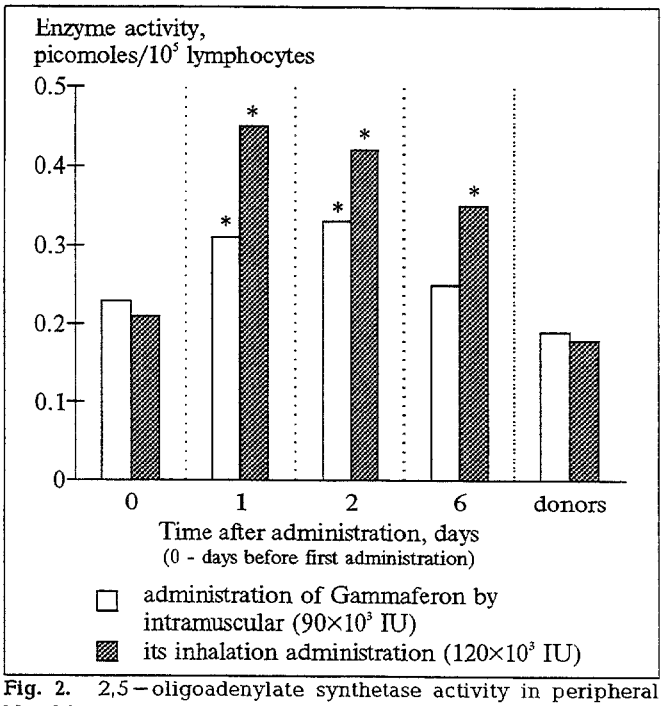

Fig. 2. 2,5-oligoadenylate synthetase activity in peripheral blood lymphocytes. The values are arithmetic means per group. Significant differences ($p<0.05$) are indicated by asterisks.

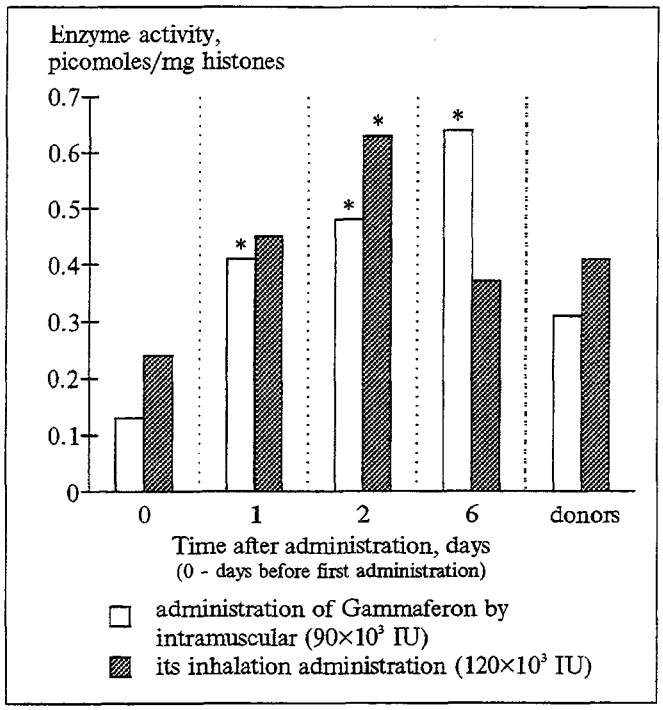

Fig. 3. Protein kinase activity in peripheral blood lymphocytes.The values are arithmetic means per group. Significant differences ($p<0.05$) are indicated by asterisks.

enzyme activity were 1.5- to 2-fold above normal, though lower than on day 2.

It should be noted that the volunteers were convalescing after influenza or acute respiratory infection and differed from the healthy donors in parameters of the IFN system. Thus, they had somewhat higher levels of serum IFN and higher 2,5-oligoadenylate activities and markedly (2- to 4-fold) reduced protein kinase activities in their lymphocytes and plasma. The intramuscular as well as inhalational administration of Gammaferon led to substantial increases in lymphocyte protein kinase activities on days 1 through 6. However, repeated intramuscular Gammaferon injections at 90×10^3 IU resulted in a marked progressive inhibition of plasma protein kinase activity, which may have been due to a reduction in the content or activity of fibrinogen, whose alpha chain is a phosphorylation substrate for interferon-dependent protein kinase [9]. This intramuscular dose of Gammaferon also elicited certain shifts in other biochemical parameters, including the composition of proteins and blood coagulability, as well as producing body temperature elevations and other clinical symptoms described for treatment with IFNs in high doses. With the lower intramuscular Gammaferon dose (30×10^3 IU), such symptoms were much less marked, but the IFN system was activated to a lesser extent. The Gammaferon dose of 120×10^3 IU given by inhalation was no less effective than its intramuscular dose of 90×10^3 IU, did not inhibit plasma protein kinase activity, and did not produce appreciable clinical symptoms. The inhalation route may therefore be recommended as one possible nontoxic method of Gammaferon application in clinical settings. The present clinical trials indicate that this new preparation of recombinant human gamma interferon can effectively activate the IFN system.

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